UNIVERSITAT DE BARCELONA

Orthopaedic device-related infections: some thoughts on management and antimicrobial efficacy from a clinical and experimental perspective

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Memòria presentada per

ALBA RIBERA PUIG

Per optar al grau de Doctor en Medicina

Barcelona, maig 2017

El Dr Javier Ariza Cardenal, Professor de la Facultat de Medicina de la Universitat de Barcelona i Sènior Docent del Servei de Malalties Infeccioses de l'Hospital Universitari de Bellvitge, i el Dr Oscar Murillo Rubio, metge adjunt del Servei de Malalties Infeccioses de l'Hospital Universitari de Bellvitge, fan constar que la tesi titulada

Orthopaedic device-related infections:
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que presenta la llicenciada Alba Ribera Puig, ha estat realitzada sota la seva direcció en el campus de Bellvitge de la Facultat de Medicina. Tesi que consideren finalitzada i autoritzen la seva presentació per la seva defensa davant del tribunal que correspongui.

A Barcelona, maig 2017

Dr Javier Ariza Cardenal

Dr Oscar Murillo Rubio

Als meus pares, per guiar-me i acompanyar-me sempre

Al Guillem, pel seu suport callat

Al Norbert, per fer que tot sigui possible

A l'Ona i al Pere, pel seu amor infinit

Em plau, d'atzar, d'errar per les muralles
Del temps antic i, a l'acost de la fosca,
Sota un llorer i al peu de la font tosca,
De remembrar, cellut, setge i batalles.

De matí em plau, amb fèrries tenalles
I claus de tub, cercar la peça llosca
A l'embragat, o al coixinet que embosca
L'eix, i engegar per l'asfalt sense falles.

I enfilar colls, seguir per valls ombroses,
Vèncer, rabent, els guals. Oh món novell!
Em plau, també, l'ombra suau d'un tell,

L'antic museu, les madones borroses,
I el pintar extrem d'avui! Càndid rampell:
M'exalta el nou i m'enamora el vell.

J. V. Foix

The research presented in this thesis has been carried out thanks to a

personal grant received from Institut d'Investigació Biomèdica de Bellvitge (IDIBELL),

and several of the mentioned studies have been supported by scientific projects promoted by the osteoarticular infection group in which I have collaborate:

FIS PI10/01573: *Estudio para determinar la presencia de microorganismos en la superficie de prótesis articulares por un aflojamiento aséptico.* Lead researcher: Oscar Murillo from Hospital Universitari de Bellvitge. Multicentre study.

FIS PI14/00511: *Alternativas terapéuticas frente a la infección in vitro de cuerpo extraño producida por bacilos gram-negativos multiresistentes: estudios farmacodinámicos en monoterapia y en combinación*. Lead researcher: Oscar Murillo from Hospital Universitari de Bellvitge.

RESUM

INTRODUCCIÓ

La infecció osteoarticular relacionada amb implants ortopèdics és un problema mèdic de primera magnitud, tant per la seva incidència creixent com per la complexitat del seu maneig. Aquestes infeccions suposen un veritable repte per a l'especialista en malalties infeccioses, principalment per les seves particularitats etiopatogèniques amb participació de bacteris en fase estacionària de creixement i formació de biopel·lícules bacterianes (biofilm) sobre la superfície de l'implant, les quals dificulten el seu diagnòstic i tractament. En l'actualitat existeixen grans àrees d'incertesa al voltant d'aquesta patologia i aspectes que generen controvèrsia entre els especialistes, i que caldria analitzar amb detall. Els treballs inclosos en aquesta tesi van dirigits a explorar alguns dels aspectes no resolts sobre el maneig i l'eficàcia antimicrobiana en el marc de la infecció osteoarticular relacionada amb implants ortopèdics, sempre des del punt de vista d'un especialista en malalties infeccioses.

Pel que fa al seu maneig, el diagnòstic d'aquestes infeccions es basa en aspectes clínics, radiològics, analítics, anatomopatològics i microbiològics. Una bona anamnesi i una adequada interpretació de les proves complementàries són essencials per arribar a un bon diagnòstic i així poder escollir el tractament òptim en cada situació, principalment en els casos d'infecció de pròtesis articulars. Segons les classificacions vigents aquestes infeccions es poden dividir en: infeccions hematògenes o postoperatòries, i en agudes, tardanes o cròniques. Però també està ben descrit un subgrup d'infeccions sense clara expressió clínica i analítica però amb cultius intraoperatoris positius concloents (≥2 mostres intraoperatòries positives). I és que, en ocasions, els cultius intraoperatoris rutinaris realitzats en casos sotmesos a recanvi protèsic per afluixament suposadament asèptic mostren resultats positius inesperats. El significat d'aquests cultius positius aïllats no és ben conegut, per la qual cosa caldria analitzar-los amb cura per poder fer una bona aproximació diagnòstica: són infeccions de baix grau?, són contaminacions que resulten del processament de les mostres?, són bacteriss adherits a l'implant sense rellevància clínica?.

Per altra banda, el maneig de les infeccions osteoarticulars relacionades amb implants ortopèdics sovint requereix una intervenció quirúrgica i una tractament antibiòtic prolongat. Aquesta intervenció depèn de les característiques de la infecció i de la situació basal de cada pacient, i inclou: el desbridament quirúrgic, l'explant de l'implant/pròtesi habitualment amb recanvi en un o dos temps o, en casos ocasionals, l'amputació de l'extremitat. En línies generals el desbridament és el procediment habitual de les infeccions agudes, amb menor

component de bacteris adherits; i l'explant protèsic el de les infeccions tardanes o cròniques que presenten biofilms més establerts i difícils d'eliminar. Tradicionalment, el tractament estàndard per a les infeccions cròniques de pròtesis articulars és el recanvi en dos temps (un primer temps que inclou un desbridament profund i la implantació d'un espaiador de ciment provisional, i un segon temps on s'implanta una pròtesi definitiva). Durant els darrers anys, s'ha anat incorporant en la pràctica clínica el recanvi en un temps: s'explanta la pròtesi, es fa un desbridament acurat i s'implanta una nova pròtesi en una única intervenció, amb el benefici que suposo per al pacient aquesta maniobra menys complexa i d'una recuperació funcional més ràpida. Falta, però, comparar les taxes de curació final d'aquestes dues estratègies per poder considerar el recanvi en un temps una estratègia igual d'eficaç.

A més a més, el tractament antibiòtic dirigit ha de tenir, idealment, activitat front als bacteris de creixement lent, freqüentment adherits a les superfícies dels implants i una bona penetració òssia. L'eficàcia dels antibiòtics β-lactàmics front a les infeccions relacionades amb biofilm ha estat molt qüestionada; mentre que altres antibiòtics com la rifampicina (front als estafilococs) o les quionolones (front als bacteris gram-negatius) tenen un millor perfil antibiofilm. Així doncs, les infeccions protèsiques per estreptococs, que es tracten habitualment (i segons les guies) amb antibiòtics β-lactàmics, però que presenten unes taxes de curació no tan bones com s'esperaria en base a les infeccions planctòniques, podrien beneficiar-se de tractaments combinats amb rifampicina. Cal tenir en compte també que durant els darrers anys, i de manera creixent, s'ha objectivat un augment dels casos d'infecció per bacteris gram negatius multiresistents. Això, traslladat a la infecció osteoarticular relacionada amb implants protèsics, suposa una dificultat més a l'hora de trobar un tractament antibiòtic eficaç. Estratègies com l'us de β-lactàmics en infusió contínua basades en les seves característiques farmacocinètiques/farmacodinàmiques o la recuperació d'antibiòtics antics (com les polimixines) i el seu ús combinat amb β-lactàmics podrien aplicar-se per tractar aquestes infeccions produïdes per microorganismes multiresistents.

OBJECTIUS

A. En el maneig de la infecció osteoarticular relacionada amb implants ortopèdics.

A.1. Aspectes diagnòstics de la infecció de pròtesis articulars.

- Objectiu 1. Analitzar les troballes microbiològiques i clíniques en pacients sotmesos a recanvi pròtesic per sospita d'afluixament asèptic, i comparació amb casos d'infeccions cròniques de pròtesis articulars.

A.2. Maneig quirúrgic de la infecció de pròtesis articulars.

- Objectiu 2. Avaluar el risc de reinfecció després del recanvi protèsic en un o dos temps en les infeccions de pròtesis de maluc.

B. En la valoració de l'eficàcia antimicrobiana per al tractament de les Infeccions osteaorticulars relacionades amb implants ortopèdics.

B.1. Infeccions per *Streptococcus spp*

- Objectiu 3. Valorar l'eficàcia d'afegir rifampicina als β-lactàmics en el tractament de la infecció de pròtesi articular estreptocòccica manejada amb retenció de l'implant, i avaluar el seu impacte en el pronòstic.

B.2. Infeccions per bacils gram negatius multiresistents

B.2.1 L´ús de β-lactàmics en infusió contínua

- Objectiu 4. Estandarditzar un procediment de mesura basat en UHPLC-MS/MS per a la determinació simultània de la concentració de β-lactàmics en el plasma humà.
- Objectiu 5. Avaluar l'eficàcia i la seguretat d'utilitzar β-lactàmics en infusió contínua per a les infeccions osteoarticulars de difícil tractament causades per bacils gram negatius, i validar un mètode senzill pel seu ús clínic.

B.2.2 L'ús de combinacions antibiòtiques amb colistina

- Objectiu 6. Avaluar els beneficis de la combinació colistina més β-lactàmics per tractar pacients amb infeccions produïdes per *Pseudomonas aeruginosa* multiresistent.
- Objectiu 7. Estudiar l'efecte d'afegir colistina als β-lactàmics enfront d'un biofilm de *klebsiella pneumonia*e BLEE, en un model experimental *in vitro*.

MÈTODES

Els estudis clínics presentats en aquesta tesi s'han desenvolupat dins del marc la Unitat d'Infecció Osteoarticular de l'Hospital Universitari de Bellvitge, reconeguda pel Ministeri de Salut com una unitat de referència nacional i on es realitza un maneig multidisciplinari de les infeccions osteoarticulars. A més a més, els estudis multicèntrics realitzats han estat possibles gràcies a l'existència de la Red Española de Investigación en Patología Infecciosa (REIPI) que consta del Grupo para el Estudio de la Patogénesis y Tratamiento Antibiótico de la Infección de Prótesis Articular i, també, gràcies al Grupo de Estudio de Infección Osteoarticular (GEIO) format recentment dins de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica. Aquests treballs han comptat amb el suport del Laboratori de Microbiologia de l'Hospital Universitari de Bellvitge per al processament de les mostres per cultiu i la realització de tècniques de sonicació i, també, amb la col·laboració del Laboratori Clínic de l'hospital per al desenvolupament i estandardització de mètodes d'UHLPC-MS/MS per a la determinació de nivells d'antibiòtic.

Durant 6 mesos em vaig traslladar al Nuffield Orthopaedic Centre de Oxford (UK) que disposa d'una Bone Infection Unit de referència nacional, on vaig poder participar en les tasques clíniques i docents diàries. Aquesta estada em va permetre incloure l'experiència d'aquest centre, en relació a la infecció protèsica estreptocòccica, en un dels treballs presentats en aquesta tesi.

Finalment, gràcies a l'equipament de què disposa el Laboratori d'Infecció Experimental ubicat a la Facultat de Medicina (Universitat de Barcelona, Campus Bellvitge), s'ha pogut dur a terme un darrer treball experimental en un model *in vitro* de formació de biofilm, el qual ha permès comparar diferents pautes terapèutiques per a la seva extrapolació a la clínica.

TROBALLES PRINCIPALS

1. En el maneig de la infecció osteoarticular relacionada amb implants ortopèdics.

- **En resposta a l'objectiu 1**

1.1 La importància d'un bon diagnòstic en el casos d'afluixament protèsic

L'afluixament protèsic pot ser el resultat d'un procés asèptic o d'una infecció. Els aspectes clínics són la principal guia inicial per fer un bon diagnòstic causal. No obstant, les troballes microbiològiques permeten fer un diagnòstic més definitiu (≥ 2 cultius positius), diagnosticant finalment d'infecció alguns casos considerats inicialment asèptics (com va succeir en 13% dels casos del nostre treball). Cal remarcar que aquests casos van presentar característiques particulars al comparar-los amb el grup control d'infecció protèsica crònica. Ocasionalment, ens trobem amb casos que presenten un únic cultiu positiu de teixit intraoperatori (de ≥ 5 cultius recollits). En aquestes situacions és difícil establir si es tracta d'una infecció de baix grau o bé es tracta de contaminacions produïdes durant el processament de les mostres. En aquestes situacions el fet de disposar de mostres de sonicació dels materials explantats pot ajudar-nos a interpretar aquests resultats. En el nostre treball 10 pacients van presentar un cultiu de teixit positiu que va ser concordant amb el cultiu de la mostra de sonicació. És probable que aquestes situacions es puguin definir com a casos d'infecció i que alguns centres que incorporen rutinàriament tècniques de sonicació tractin aquests pacients amb antibiòtics; no és el cas del nostre centre, on aquests pacients no van rebre antibiòtic (donada la condició retrospectiva del treball). Considerem que, probablement, es tracta d'infeccions de baix grau que poden ser curades simplement durant el recanvi de la pròtesi. El grup de casos amb un únic cultiu positiu de teixit o de sonicació és difícil d'interpretar. Comparant aquests casos amb les mostres discordants dels grups diagnosticats és probable que els cultius únics de teixit corresponguin més freqüentment a contaminants, i contràriament els cultius únics de les mostres de sonicació reflecteixin la presència real de microorganismes adherits a l'implant (sense gran rellevància clínica).

En l'avaluació de les troballes clíniques vam observar que aquells casos que presentaven major número de cultius positius havien requerit un recanvi protèsic més precoçment. Aquesta apreciació suggereix que els microorganismes tenen el seu paper en el procés de fracàs protèsic. No obstant, el número de cultius positius no es va correlacionar amb el grau d'afluixament protèsic, que va dependre, en gran mesura, de l'edat de la pròtesi.

- **En resposta a l'objectiu 2**

1.2 Taxa d'èxit en el recanvi protèsic de maluc en un o dos temps

Aquest estudi multicèntric pretén comprar les estratègies de recanvi protèsic articular en un o dos temps en el tractament de les infeccions de pròtesis de maluc (en general). En l'anàlisi de les característiques particulars de cada grup establert vam observar que dins del grup de recanvi en un temps hi havia més proporció de casos amb antecedents de recanvis múltiples protèsics, així com d'infeccions protèsiques prèvies. Aquests pacients semblava que tenien infeccions més greus, amb nivells de PCR més alts i signes clínics més evidents (abscés, fístula, secreció purulenta) que els casos on es va realitzar un recanvi en dos temps. Tenint en compte el moment de la presentació d'aquests infeccions (> 24 mesos després de la cirurgia) sembla que aquest grup de pacients sotmesos a recanvi en un temps correspon majoritàriament a casos d'infecció protèsica hematògena, fet que explicaria l'evident expressió clínica. En canvi, els casos manejats amb recanvi en dos temps corresponen principalment a casos d'infecció protèsica crònica amb menys expressió clínica. En els dos grups el microorganisme causal més freqüent va ser *l'Staphylococcus spp.* En l'estudi multivariant no es van objectivar diferències estadísticament significatives en els risc de reinfecció entre les dues estratègies. Tradicionalment, el recanvi en dos temps s'ha considerat el tractament d'elecció per les infeccions cròniques de pròtesis articulars; no obstant, aquesta intervenció suposa més temps d'hospitalització, una recuperació funcional més lenta, més dolor i una major mortalitat associada ja que suposa dues intervencions quirúrgiques complexes. Tot i les limitacions d'aquest treball multicèntric, on manquen algunes dades clíniques rellevants i on cada grup de treball va incloure només un tipus d'estratègia, podem concloure que el recanvi en un temps es pot considerar un procediment eficaç a tenir en compte per tractar infeccions de pròtesis de maluc.

2. En la valoració de l'eficàcia antimicrobiana pel tractament de les Infeccions osteoarticulars relacionades amb implants ortopèdics

- **En resposta a l'objectiu 3**

2.1 El paper de la combinació antibiòtica amb rifampicina en les infeccions de pròtesis articulars estreptocòcciques i el seu impacte en el pronòstic

Presentem la sèrie més llarga descrita d'infecció estreptocòccica manejada amb desbridament i retenció de l'implant. Aquesta és una causa no infreqüent d'infecció protèsica, especialment en infeccions hematògenes (que representa un 52% dels casos d'aquest estudi). En termes de

pronòstic, la nostra sèrie de casos va mostrar una taxa de curació (57%) pitjor de l'esperada en comparació amb treballs previs. Els factors predictors de mal pronòstic van ser similars als descrits en altres etiologies; i la bacterièmia i la infecció per *Streptococcus pyogenes* es van relacionar amb un fracàs precoç. Com ja s'havia observat en altres estudis el recanvi dels components mòbils durant el desbridament es va associar amb un pronòstic més favorable. No tots els casos van seguir els criteris de l'IDSA, que recomanen la realització d'un desbridament amb retenció de l'implant si la infecció es manifesta durant el primer mes després de l'implant de la pròtesi. El pacients que complien els criteris de l'IDSA van presentar millor pronòstic; tot i així aquells pacients els símptomes dels quals es van iniciar entre el primer i tercer mes després de la implantació de la pròtesis van presentar un pronòstic similar als que complien estrictament els criteris de l'IDSA.

Els antibiòtics β-lactàmics són els clàssicament recomanats per a la infecció estreptocòccica, incloent la infecció de pròtesi articular. Sabem que tenen bona activitat durant la fase planctònica inicial; però la seva activitat antibiofilm necessària per erradicar la infecció ha estat molt qüestionada. En les infeccions estafilocòcciques hi ha una forta evidència que el tractament combinat amb rifampicina és clarament superior a la monoteràpia amb β-lactàmics. En el nostre grup de pacients vam observar una tendència cap a un millor pronòstic quan s'afegia rifampicina al tractament β-lactàmic , en comparació amb la monoteràpia amb β-lactàmic (10% vs 16.8% de taxa de fracàs). A més a més, l'administració precoç de rifampicina va resultar ser un factor predictor independent de bon pronòstic.

- **En resposta a l'objectiu 4 i 5**

2.2 L'eficàcia d'utilitzar β-lactàmics en infusió contínua pel tractament de bacteris gram negatius, sempre des d'una posició segura calculant la concentració predita de β-lactàmics en el plasma dels pacients, o mesurant la seva concentració en plasma utilitzant un mètode d'UHPLC-MS/MS (si està disponible).

L'administració de β-lactàmics en infusió contínua pot optimitzar els seus paràmetres farmacocinètics/farmacodinàmics, especialment en les infeccions de difícil tractament causades per bacteris gram negatius multiresistents. El seu ús permet mantenir la concentració d'antibiòtic per sobre de la concentració mínima inhibitòria (CMI) durant més temps i, també, permet que soques inicialment resistents als β-lactàmics es converteixin en sensibles en termes farmacocinètics/farmacodinàmics. La dosi ideal de β-lactàmics en infusió contínua o estesa no està ben establerta i, per altra banda, la monitorització dels nivells de β-lactàmics en

plasma no està disponible en la majoria d'hospitals per al seu ús en la pràctica clínica diària. Conèixer els nivells en plasma de β-lactàmics és recomanable tant per poder guiar el tractament com també per anticipar possibles nivells tòxics, principalment en tractaments prolongats. A través de dos treballs, per una banda hem estandarditzat un mètode d'UHPLC-MS/MS per a la mesura i monitorització simultània dels nivells de diferents β-lactàmics en mostres plasmàtiques de diferents pacients; i, per altra banda, hem pogut validar unes equacions senzilles per estimar els nivells plasmàtics de β-lactàmics dels pacients quan no es disposa d' UHPLC-MS/MS.

La validació d'UHPLC-MS/MS per a la mesura simultània i en pocs minuts de nou β-lactàmics (amoxicil·lina, ampicil·lina, cloxacil·lina, piperacil·lina, cefepime, ceftazidima, cefuroxima, aztreonam i meropenem) i de dos inhibidors de β-lactamases (clavulànic i tazobactam) ha permès la seva utilització a nivell institucional en el nostre hospital. Aquest procediment ha permès ajustar i individualitzar les dosis de β-lactàmics durant la pràctica clínica, especialment en pacients crítics (pels seus paràmetres farmacocinètics particulars) o bé en pacients amb insuficiència renal i en els casos infecció osteoarticular que requereixen tractaments prolongats (pel potencial risc d'acumulació progressiva d'antibiòtic en plasma).

Mitjançant la comparació amb els resultats obtinguts per UHPLC-MS/MS, hem pogut validar unes equacions senzilles per a l'estimació individualitzada de la dosi òptima de β-lactàmics, en perfusió contínua o estesa, i dels seus nivells en plasma quan no es disposa de mètodes d'UHPLC-MS/MS.

- *Equació per estimar la dosi òptima de beta-lactàmics:*

  - Dosi diària (mg) = 24 (h) × TBC (L/h) × C_{ss} (mg/L) (Equació 1)

    TBC: aclariment corporal total del β-lactàmic
    C_{ss}: objectiu de concentració estable

- *Equació per estimar els nivells de beta-lactàmics en plasma, per una dosi concreta administrada :*

  - C_{pred} (mg/L) = Dosi diària (mg/24h)/ TBC (L/h) (Equació 2)

    C_{pred}: concentració predita

En global, vam poder demostrar una bona correlació entre els resultats obtinguts per a l'equació 2 i els nivells en plasma mesurats per UHPLC-MS/MS. No obstant els nivells calculats per UHPLC-MS/MS tendien a ser sempre majors als estimats, probablement perquè els valors d'aclariments dels β-lactàmics estudiats no s'ajustaven perfectament al de la nostra cohort.

L'ús de β-lactàmics en infusió contínua va ser segur i no va presentar efectes adversos greus, tot i assolir concentracions elevades durant llargs períodes de temps. Encara que no podem parlar en termes d'eficàcia, principalment per la manca d'un tractament comparatiu, vam obtenir molt bon resultats, també en aquelles infeccions produïdes per soques resistents als β-lactàmics utilitzats. I, finalment, tots els pacients menys un es van curar.

- **En resposta a l'objectiu 6**

2.3 Els beneficis de la combinació colistina més β-lactàmics per a les infeccions osteoacticulars causades per *Pseudomonas aeruginosa* multiresistent

Com ja s'ha comentat, l'increment progressiu de les infeccions osteoarticulars causades per bacteris gram negatius multiresistents i el seu tractament representa un nou repte per a l'especialista de malalties infeccioses. L'ús de β-lactàmics en les infeccions relacionades amb biofilm ha estat molt qüestionat. Quan s'han analitzat els casos d'infeccions de pròtesis articulars per bacteris gram negatius resistents a quinolones tractats amb β-lactàmics en monoteràpia s'ha vist que aquest tractament era molt inferior al tractament amb quinolones (recomanat habitualment en les infeccions per bacteris gram negatius). És per això que es necessari redescobrir antibiòtics oblidats, com la colistina, per poder dissenyar noves estratègies terapèutiques.

Presentem una sèrie de 34 casos d'infecció osteoarticular causada per *Pseudomonas aeruginosa* multiresistent (tant soques multiresistents com extremadament resistents), amb una taxa de curació després d'una primera línia de tractament de 50%, que va augmentar fins a >85% després d'una teràpia de rescat. Els casos van ser analitzats retrospectivament, casos que havien rebut tractament amb monoteràpia (β-lactàmics o colistina) o tractament combinat (principalment, β-lactàmics més colistina), a més a més d'un tractament quirúrgic concomitant en la majoria de casos.

La teràpia combinada amb β-lactàmics més colistina va ser significativament més efectiva que la monoteràpia, inclús en aquelles casos amb infeccions per soques sensibles als β-lactàmics utilitzats. Aquests beneficis es van evidenciar especialment en aquells casos considerats de més difícil tractament (casos d'infecció de pròtesis articulars o osteoartritis manejades amb retenció de l'implant), amb una taxa de fracàs del 82% (monoteràpia) vs del 29% (teràpia combinada). És difícil separar la contribució individual de cada antibiòtic (β-lactàmics o colistina) dins de la combinació; tot i així, es coneix que la colistina és efectiva front als bacteris de les capes més profundes del biofilm; i això contrasta amb la majoria d'antibiòtics (com els β-

lactàmics) que actuen principalment sobre els bacteris en fase de creixement de les capes més superficials del biofilm. A més a més, donades les propietats de la colistina com a pèptid catiònic, és probable que en la seva combinació situï al β-lactàmic en una millor posició i en faciliti la seva penetració. Així doncs, aquest tractament combinat es podria fer extensiu no només a les infeccions osteoarticulars causades per bacteris gram negatius multiresistents sinó també a les causades per bacteris gram negatius resistents a quinolones.

Està ben descrita l'heteroresistència de la colistina en diferents soques de *Pseudomonas aeruginosa*, quan s'exposen a colistina en monoteràpia. Davant d'aquesta situació, treballs realitzats en unitats de cures intensives suggereixen utilitzar dosis altes de colistina, amb el risc de toxicitat renal que això suposa. Creiem que les infeccions osteoarticulars es troben en un altre escenari, ja que no són infeccions potencialment mortals i, a més a més, requereixen tractaments prolongats. Aquesta situació, sumada a la potencial sinèrgia en la combinació amb β-lactàmics, justifica que les dosis utilitzades de colistina en la nostra sèrie de casos siguin menors (inicialment 6 MIU/dia) a les recomanades, sense una dosi de càrrega inicial. No es va objectivar aparició de resistències a la colistina. El tractament va ser molt ben tolerat; només alguns pacients van presentar deteriorament de la funció renal associat a la colistina, però la funció renal es va normalitzar en reduir les dosis

- **En resposta a l'objectiu 7**

2.4 L'efecte d'afegir colistina al meropenem enfront d'un biofilm de *Klebsiella pneumoniae* BLEE, en un model experimental *in vitro*.

Seguint en la línia de l'estudi presentat en l'apartat anterior, hem estandarditzat un model *in vitro* per a la formació de biofilm de bacteris gram negatius (en concret *Klebsiella pneumoniae* BLEE) amb l'objectiu de poder avaluar l'efecte que suposa afegir colistina al tractament amb meropenem front a bacteris del biofilm. Aquest model *in vitro* (realitzat amb el CDC Biofilm reactor) havia estat prèviament estandarditzat per altres microorganismes (principalment, estafilococs i *Pseudomonas aeruginosa*) però no per enterobacteris. Per aquest motiu va ser necessari un període inicial per tal de testar les condicions més adequades per a la formació d'un biofilm de dues soques de *Klebsiella pneumoniae* BLEE. El biofilm format es va poder visualitzar mitjançant microscopia electrònica de rastreig, i va resultar ser més abundant sota les condicions establertes en l'experiment 1 que en les de l'experiment 3. Un cop establert el biofilm, es va procedir als experiments terapèutics. Les pautes establertes van ser: 1) grup control, 2) colistina en infusió contínua (3,5mg/L, per aconseguir concentracions estables

equivalents a 2 MUI/8 hores en humans), 3) meropenem en bolus cada 8 hores (dosis equivalents a 2g/8 hores en humans, Concentració màxima de 90mg/L), 4) combinació meropenem més colistina.

Com ja s'esperava, el tractament amb colistina en monoteràpia va ser ineficaç per tractar els bacteris del biofilm i va afavorir l'aparició de soques resistents a la colistina. Tant el tractament amb meropenem en monoteràpia com la seva combinació amb colistina van assolir una taxa de mort bacteriana ràpida ja durant les primeres hores, que es va mantenir (i inclús va millorar) fins al final del tractament. El meropenem en monoteràpia va presentar una activitat no bactericida enfront de les dues soques de testades (A i B, les dues susceptibles a carbapenems), i la seva combinació amb colistina va resultar bactericida per a la soca A. Es va observar una eficàcia superior i estadísticament significativa en la combinació meropenem més colistina respecte a la monoteràpia amb meropenem enfront dels bacteris de soca A adherits al biofilm, sota les condicions que produïen més grau de biofilm (Experiment 1); però aquestes diferències no van ser tan òbvies sota les condicions de l'Experiment 3 (amb menys grau de biofilm).

En general, es van aconseguir resultats lleugerament millors a l'afegir colistina als β-lactàmics per tractar els bacteris adherits al biofilm, tot i la susceptibilitat de les soques als carbapenems, en el nostre model *in vitro* de *K. pneumoniae* BLEE. A més a més, la combinació va protegir de l'aparició de soques resistents a la colistina. No obstant això, aquests són resultats preliminars; i, per tant, són necessaris més estudis per continuar explorant l'efecte *in vitro* d'afegir colistina als β-lactams front a *K. pneumoniae* BLEE i poder determinar la seva rellevància clínica.

CONCLUSIONS

A. En el maneig de la infecció osteoarticular relacionada amb implants ortopèdics

A.1. Aspectes diagnòstics de la infecció de pròtesis articulars (sobre l'Objectiu 1):

1.1 Tot i l'ús apropiat de les guies clíniques actuals, alguns pacients amb sospita d'afluixament protèsic asèptic són realment casos no diagnosticats d'infecció de pròtesis articulars o presenten microorganismes en les seves mostres quirúrgiques.

1.2 Les mostres de la sonicació proporcionen informació microbiològica addicional que pot ajudar en el diagnòstic d'infeccions protèsiques tardanes de baix grau que mimetitzen situacions d'afluixament asèptic però que tenen un cultiu de teixit periprotèsic positiu.

1.3 Els paràmetres clínics (que determinen el recanvi protèsic) es correlacionen amb el número de cultius periprotèsics positius; i això dona suport al probable paper dels microorganismes en la taxa de fracàs protèsic.

A.2. Maneig quirúrgic de les infeccions de pròtesis articulars (sobre l'Objectiu 2):

2.1 L'estratègia de recanvi protèsic en un temps pot ser tan efectiva com l'estratègia de recanvi en dos temps, amb taxes de reinfecció similars.

B. En la valoració de l'eficàcia antimicrobiana per al tractament de les Infeccions osteaorticulars relacionades amb implants ortopèdics

B.1. Infeccions per *Streptococcus spp* (sobre l'Objectiu 3):

3.1 Dins de la sèrie de casos més llarga descrita d'infeccions de pròtesis articulars estreptocòcciques manegades amb desbridament i retenció de l'implant, aquesta patologia va presentar taxes de curació no tan bones com s'esperava.

3.2 El tractament clàssic amb β-lactàmics és probablement el més adequat per actuar sobre el component planctònic de les infeccions protèsiques estreptocòcciques; i l'addició de rifampicina uns dies/setmanes després del desbridament podria tenir un paper antibiofilm i millorar els resultats modestos d'aquesta patologia.

3.3 Es recomana un procediment quirúrgic concomitant i òptim, seguint els criteris de l'IDSA i assegurant el recanvi dels components mòbils (polietilè) durant el desbridament. Es va observar un pronòstic similar tant si els criteris de l'IDSA per

realitzar un desbridament amb retenció de l'implant s'assolien durant el primer mes com si s'assolien durant els tres primers mesos des de la intervenció.

B.2. Infeccions per bacils gram negatius multiresistents

L'ús de β-lactàmics en infusió contínua (sobre els Objectius 4 i 5):

4.1 El desenvolupament d'un mètode UHPLC-MS/MS ha permès la mesura simultània de la concentració de diferents β-lactàmics en plasma i la seva aplicabilitat en la pràctica clínica rutinària, i a la vegada la validació d'una equació senzilla pel seu ús clínic.

5.1 L'ús de β-lactàmics en infusió contínua és segur i efectiu; i permet recuperar soques prèviament resistents que es converteixen en susceptibles segons els seus paràmetres farmacodinàmics. Dosis més baixes de β-lactàmics en infusió contínua es podrien utilitzar per soques susceptibles

5.2 Una simple equació pot ajudar al clínic a estimar la dosi de β-lactàmics en infusió contínua i els seus nivells en plasma durant les primeres hores de tractament, quan el mètode d'UHPLC-MS/MS no està disponible.

L'ús de combinacions antibiòtiques amb colistina (sobre els Objectius 6 i 7):

6.1 Hi ha una evidència creixent que les actuals recomanacions haurien de considerar la combinació de baixes dosis de colistina en combinació amb β-lactàmics com un tractament optimitzat per a les infeccions osteaorticulars causades per *Pseudomonas aeruginosa* multiresistent. Calen més estudis per considerar també aquesta teràpia en casos de bacils gram negatius resistents a quinolones.

6.2 Quan s'utilitza com a part d'un tractament integral que inclou un tractament quirúrgic apropiat, la combinació antibiòtica (β-lactàmics + colistina) és essencial per aconseguir bons resultats en aquestes infeccions de difícil tractament produïdes per *Pseudomonas aeruginosa* multiresistents.

7.1 En un model *in vitro* per a la formació de biofilm de *Klensiella pneumoniae* BLEE, la colistina en monoteràpia va ser ineficaç i va donar lloc a l'aparició de soques resistents a colistina.

7.2 Tant la monoteràpia amb meropenem com la seva combinació amb colistina van aconseguir taxes de mort bacteriana ràpides, que es van mantenir fins al final del tractament. No obstant, només la combinació va mostrar activitat bactericida en una de les dues soques testades de *K. pneumoniae BLEE*, i el seu efecte es va evidenciar

principalment sota aquelles condicions amb major grau de biofilm. La combinació va protegir de l'aparició de soques resistents a la colistina.

7.3 Els nostres resultats preliminars van mostrar una lleugera superioritat global *in vitro* a l'afegir colistina als β-lactams per tractar soques de *Klesbiella pneunomiae* BLEE susceptibles a carbapenems; tot i així, estan planejats més estudis per tal d'explorar millor aquest camp i poder determinar la seva rellevància clínica.

SCIENTIFIC PRODUCTION

Most of the studies included in this thesis have been published in scientific journals and/or presented in national or international scientific congress.

Publications in scientific journals

1 *Clinical and microbiological findings in prosthetic joint replacement due to aseptic loosening*. **A. Ribera**, L. Morata, J. Moranas, JL. Agulló, JC. Martínez, Y. López, D. García, X. Cabo, S. García-Ramiro, A. Soriano, O. Murillo. Journal of Infection 2014; 69(3):235-43. doi: 10.1016/j.jinf.2014.05.003

2 *Risk of re-infection following one- and two-stage surgical revision of peri-prosthetic joint infection of the hip: A pooled individual participant data analysis of 44 observational cohort studies.* The global inform collaboration leaded by Sk. Kunutsor is listed at the end of the paper, and includes **A. Ribera** et al**.** Submitted for publication.

3 *The Not-So-Good Prognosis of Streptococcal Periprosthetic Joint Infection Managed by Implant Retention: The Results of a Large Multicenter Study*. J. Lora-Tamayo, E. Senneville, **A. Ribera**, L. Bernard, M. Dupon, V. Zeller, HK. Li, C. Arvieux, M. Clauss, I. Uçkay, D. Vigante, T. Ferry, JA. Iribarren, TN. Peel, P. Sendi, NG. Miksić, D. Rodríguez-Pardo, MD. del Toro, M. Fernández-Sampedro, U. Dapunt, K. Huotari, JS. Davis, J. Palomino, D. Neut, BM. Clark, T. Gottlieb, R. Trebše, A. Soriano, A. Bahamonde, L. Guío, A. Rico, M. JC Salles, MJ. G Pais, N. Benito, M. Riera, L. Gómez, J. Esteban, JP. Horcajada, K. O'Connell, M. Ferrari, G. Skaliczki, R. San Juan, J. Cobo, M. Sánchez-Somolinos, A. Ramos, E. Giannitsioti, A. Jover-Sáenz, J. M Baraia-Etxaburu, JM. Barbero, P. FM Choong, N. Asseray, S. Ansart, G. Le Moal, W. Zimmerli, J. Ariza. Accepted in Clinical Infectious Diseases. doi: 10.1093/cid/cix227

4 *Development and validation of a measurement procedure based on ultra-high performance liquid chromatography-tandem mass spectrometry for simultaneous measurement of beta-lactam antibiotic concentration in human plasma.* R. Rigo-Bonnin, **A. Ribera**, A. Arbiol-Roca, S. Cobo-Sacristán, A. Padullés, O. Murillo, E. Shaw, R. Granada, XL. Pérez-Fernández, F. Tubau, P. Alía. Clinica Chimica Acta 2017; 468:215-224. doi: 10.1016/j.cca.2017.03.009

5 *Beta-lactams in continuous infusion for difficult-to-treat osteoarticular infections caused by Gram-negative bacilli: validation of an easy method for clinical use*. **A. Ribera**, L. Soldevila, R. Rigo, F. Tubau, A. Padullés, J. Gómez-5 Junyent, J. Ariza, O. Murillo. Submitted for publication in Antimicrobial Agents and Chemotherapy journal, a detailed revision has been sent according to editor/reviewers comments.

6 *Osteoarticular infection caused by MDR Pseudomonas aeruginosa: the benefits of combination therapy with colistin plus beta-lactams*. **A. Ribera**, E. Benavent, J. Lora-Tamayo, F. Tubau, S. Pedrero, X. Cabo, J. Ariza, O. Murillo. Journal of Antimicrobial Chemotherapy. 2015; 70(12):3357-65. doi: 10.1093/jac/dkv281

7 *Activity of colistin combined with meropenem against ESBL-producing Klebisella pneumoniae in an in vitro dynamic model for growing biofilm*. **A. Ribera**, J. Gómez-Junyent, C. El Haj, F. Tubau, S. Martí, E. Benavent, K. Jiménez, J. Ariza, O. Murillo. Under elaboration.

Papers at scientific congress

1. *Microbiological findings in prosthetic hip or knee replacement due to aseptic loosening* **A. Ribera**, L. Morata, J. Moranas, A. Coscujuela, JC. Martinez, Y. López, D. García, S. Garcia-Ramiro, A. Soriano, O. Murillo. 23rd ECCMID. Berlin, Germany, 2013. (P2035)

2. *Análisis de las características clínicas y microbiológicas en el recambio protésico articular con sospecha de aflojamiento aséptico.* **A. Ribera**, L. Morata, J. Moranas, A. Coscujuela, JC. Martínez, Y. López, D. García, S. García-Ramiro, A. Soriano, O. Murillo. XVIII Congress SEIMC. Valencia, Spain, 2014. (Comunicación 298)

3. *Streptococcal Prosthetic Joint Infection Managed with Implant Retention.* J. Lora-Tamayo, E. Senneville, **A. Ribera**, L. Bernard, V. Zeller, H. Li, P. Tattevin, M. Clauss, I. Uçkay, D. Vigante, T. Ferry, J. Ariza. 55th ICAAC. San Diego, California, 2015. (K-221)

4. *Infección estreptocócica de prótesis articular manejada con retención del implante: influencia del tratamiento con rifampicin.* J. Lora-Tamayo, **A. Ribera**, JA. Iribarren, M. Fernández, D. Rodríguez-Pardo5, MD. del Toro, J. Palomino, A. Soriano, L. Guío, A. Bahamonde, A. Rico, J. Corredoira, M. Riera, N. Benito, L. Gómez, J. Esteban, L. Sorlí, R. San-Juan, A. Ramos, A.Jover-Sáenz, JM. Baraia-Etxaburu, J.Ariza. XIX Congress SEIMC. Sevilla, Spain, 2015. (Comunicación 488)

5. *Beta-lactams in continuous infusion for difficult-to treat osteoarticular infections caused by Gram-negative bacilli: a preliminary validation of an easy-to-use method*. **A. Ribera**, J. Gómez-Junyent, L. Soldevila, R. Rigo, F. Tubau, A. Padullés, J. Ariza, O.Murillo. 27th ECCMID. Vienna, Austria, 2017. (Abstract number 5179)

6. *Role of Combined Therapy Including beta-lactams on Intermittent or Continuous Infusion for the Treatment of Osteoarticular Infection (OI) by Extensively Drug-resistant Pseudomonas aeruginosa (PA.)* **A. Ribera**, O. Murillo, E. Benavent, G. Euba, J. Lora, S. Pedrero, F. Tubau, J. Cabo, J. Ariza. 54th ICAAC. Washington, USA, 2014. (L-416)

7. *Eficacia comparativa de meropenem versus su combinación con colistina frente a Klebsiella pneumoniae BLEE en un modelo dinámico de biofilm in vitro*. **A. Ribera**, C. El Haj, J. Gómez-Junyent, F. Tubau, E. Benavent, K. Jiménez, J. Ariza, O. Murillo. XXI Congress SEIMC. Málaga, Spain, 2017. (Comunicación 358)

- Other related scientific production not included in this thesis

Publications in scientific journals

1. *Risk factors and prognosis of vertebral compressive fracture in pyogenic vertebral osteomyelitis.* **A. Ribera**, M. Labori, J. Hernández, J. Lora‐Tamayo, L. González‐Cañas, F. Font, J. Nolla, J. Ariza, JA. Narváez, O. Murillo. Infection 2016; 44:29–37. doi: 10.1007/s15010-015-0800-z
2. *An observational retrospective multicenter study of Gram negative multi-drug and extensively drug resistant chronic osteomyelitis and prosthetic joint infections.* Co-ordinated by Dr Antonios Papadopoulos and Dr Efthymia Giannitsioti from the 4th Department of Internal Medicine at ATTIKON University General Hospital, Athens, Greece. Under elaboration.
3. *An international retrospective multicenter study of Suppressive antibiotic therapy in Prosthetic Joint Infections.* Co-ordinated by the Spanish public network (REIPI). Main investigators: Javier Cobo Reinoso from the Infectious Diseases Department at Hospital Ramón y Cajal (Madrid, Spain) and Eric Senneville from the Infectious Diseases Department at Gustave Dron Hospital (Tourcoing, France). Under elaboration.

Book chapters

1. *Osteomielitis*. O. Murillo, **A. Ribera**, J. Ariza. In: Urgencias y Cuidados Críticos en Reumatología. MR. Aragonés, FG. Jiménez (editors). Madrid: Ed. Médica Panamericana; 2012.
2. *Epidemiology of Prosthetic Joint Infections*. N. Benito, J. Esteban, JP. Horcajada, **A. Ribera**, A. Soriano, R. Sousa. In: Prosthetic Joint Infections. Trisha Peel (editor). New York: Ed. Springer; 2017.

Papers at scientific congress

1. *Espondilodiscitis infecciosa con fracturas vertebral: una complicación poco estudiada*. O. Murillo, **A. Ribera**, J. Lora-Tamayo, L. González-Cañas, D. García, JA. Narváez, J. Narvaez, J.M. Nolla, J. Ariza. XVI Congress SEIMC. Bilbao, Spain, 2012
2. *Infección Polimicrobiana de Prótesis Articular Aguda Postquirúrgica: características clínico-epidemiológicas y pronósticas.* **A. Ribera**, J. Lora-Tamayo, O. Murillo, G. Euba, X. Cabo, S. Pedrero, J. Moranas, D. García, J. Ariza. XVII Congress SEIMC. Zaragoza, Spain, 2013. (Comunicación 404).

3. *Particularities of infectious spondylodiscitis with vertebral fracture: is it time to face up a new challenge.* **A. Ribera**, M. Labori, J. Hernández, J. Lora-Tamayo, L. González-Cañas, JM. Nolla, J. Ariza, JA. Narváez, O. Murillo. 24th ECCMID. Barcelona, Spain, 2014. (ECCMI-4231).

INDEX

ABBREVIATIONS

AL: aseptic loosening

BL: β-lactams or beta-lactams

CBR: CDC Biofilm Reactor

CI: continuous infusion

CI: confidence interval

$CL_{CR:}$ creatinine clearance

C_{max}: steady-state peak concentration

C_{obs}: observed concentration

CoNS: coagulase-negative staphylococci

$C_{pred:}$ predicted concentration

DAIR: debridement antibiotic and implant retention

EI: extended infusion

GNB: gram-negative bacilli

HR: Hazard ratios

HPLC: high-performance liquid chromatography

IB: intermittent bolus

IQR: interquartile range

LCPJI: late chronic prosthetic joint infection

MBC: minimal bactericidal concentration

MBEC: minimal biofilm eradication concentration

MBIC: minimal biofilm inhibitory concentration

MDR: multidrug resistant

MIC: minimal inhibitory concentration

MS/MS: mass spectrometry

MIU: million international units

MS/MS: mass spectrometry

OA: osteoarthritis

OI: osteoarticular infection

PJI: Prosthetic joint infection

PK/PD: Pharmacokinetic/Pharmacodynamic

SEM: scanning electron microscopy

T>MIC: time over the minimum inhibitory concentration

TDM: therapeutic drug monitoring

TSB: Tryptic soy broth

UHPLC: ultrahigh performance liquid chromatography

VF: vertebral fracture

VO: vertebral osteomyelitis

Δ_{Conc}: difference between C_{obs} and C_{pred}

INTRODUCTION

1. Osteoarticular infections

Osteoarticular is one of the most difficult to treat infections, and it leads to considerable morbidity and functional sequelae. Its incidence has increased over the time and it now represents a first magnitude health-care problem. Patients with immunosuppressive conditions such as rheumatoid arthritis or other underlying comorbidities have higher risk of infection.

1.1 Orthopaedic device-related infections - Prosthetic joint infections

The extraordinary development of orthopaedic surgery in recent years explains why orthopaedic devices are increasingly used for fracture fixation, including intramedullary nails, external-fixation pins, plates, and screws; and also to replace native joints with joint prostheses or arthroplasties. Prosthetic joints are also the treatment for certain types of fractures, especially among the elderly; their most frequent indication is joint degenerative disease. In this context, prosthetic hip or knee replacement is considered a highly effective intervention that significantly improves the quality of patients' lives.

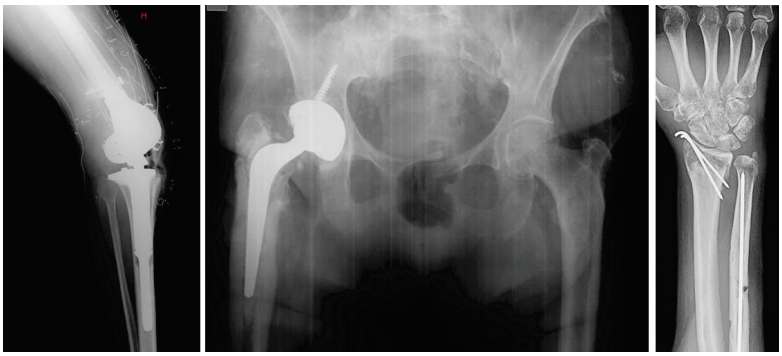


Figure 1. Plain radiographies of different orthopaedic devices

2. Epidemiology and risk factors

Approximately two million fracture-fixation devices are inserted annually in the United States (Darouiche 2004). On average, five percent of initially inserted internal fixation devices become infected. One to two percent of infections occur after internal fixation of closed fractures and more than 30% occur after fixation of open fractures (Trampuz and Zimmerli 2006). In the case of prostheses, in the United States alone, there were 332,000 total hip arthroplasties and 719,000 total knee arthroplasties performed in 2010, and the incidence of prosthesis implantation is expected to continue rising. The numbers are projected to reach 572,000 and 3.48 million by 2030 for hips and knees, respectively (Kurtz et al. 2007; Tande and

Patel 2014). Prosthetic joint infection (PJI) remains one of the most serious complications of prosthetic joint implantation, and the overall likelihood of infection is 0.5-4% (Ariza et al 2008; Kurtz et al. 2012; Osmon et al. 2013; Peel et al. 2011; Del Pozo and Patel 2009; Pulido et al. 2008; Zimmerli et al 2004).

3. Clinical aspects

3.1 Clinical presentation and classification

Infections associated with internal fixation devices are classified as early (< 2 weeks), delayed (2–10 weeks), and late (> 10 weeks). Infections with delayed and late manifestations are usually grouped together, since their clinical presentations, treatments, and prognoses are similar (Trampuz and Zimmerli 2006).

In the field of PJI, Tsukayama's and Zimmerli's classifications are both helpful for guiding medical and surgical decisions in patients with PJI. These proposed classifications are based on pathogenic aspects, the time of infection, and the diagnostic circumstances (Tsukayama et al. 1996; Zimmerli and Ochsner 2003).

Tsukayama's classification:

Positive intraoperative cultures	This group includes cases with prostheses that were presumed to have aseptic loosening, but intraoperative cultures from the surgical site reveal an unexpected PJI. The pathogenesis and etiology are assumed to be similar to those cases with late chronic infection, but with silent symptoms and signs. Thus, patients are managed with a one-stage revision procedure due to prosthetic loosening.
*Early postoperative infection**	This group includes instances when signs and symptoms of the infection appear during the first 30 days after the prosthesis replacement.
*Late chronic infection**	This group includes instances when signs and symptoms of the infection begin after the first 30 days of prosthesis replacement.
Acute hematogenous infection	This group includes instances when microorganisms reach the prosthesis via the bloodstream from distant infectious foci (e.g. skin, respiratory, or urinary tract infections) or as a primary bacteremia. In these patients, a primary site of infection is identified, and the onsets of the symptoms at that site precede the symptoms in the joint.

*In these two situations microorganisms colonize the implant during the surgery, but depend on the causative microorganisms that symptoms express before or after.

Zimmerli's classification:

Early	Symptoms of infection emerge within the first three months after the placement of the prosthesis.
Delayed	Symptoms of infection begin within three months and two years after the placement of the prosthesis.
Late	The infection occurs beyond two years after the placement of the prosthesis, as a consequence of a bloodstream infection (either suspected or proven).

Early infections are typically manifested as fever and an acute onset of joint pain, effusion, erythema and warmth at the implant site. These infections are commonly caused by virulent microorganisms, such as *S. aureus, Streptococcus spp,* and Gram-negative bacilli. Patients with delayed (low-grade) infection usually present with subtle signs and symptoms, such as implant loosening, persistent joint pain, or both, and this may be difficult to distinguish from aseptic failure. These infections are usually caused by less virulent microorganisms, such as coagulase-negative staphylococci and *Propionibacterium acnes*. During the course of infection, clinically significant cellulitis and the formation of a sinus tract with purulent discharge may occur. (Ariza et al. 2008; Cobo and Del Pozo 2011; Trampuz and Zimmerli 2008; Zimmerli et al. 2004).
Based on the classifications above, the surgical and clinical management of these infections is different; early/acute infections are linked to early diagnosis, in which the exchange of the prosthesis may be avoided, and the infection can be healed with debridement and implant retention (Zimmerli et al. 2004).

3.2 Diagnosis of prosthetic joint infection and global orthopaedic device-related infections

This section focuses on PJI, but is is applicable to all orthopaedic device-related infections. The diagnosis of PJI is based on clinical, radiological, analytical, histopathological, and microbiological findings. As described above, local inflammatory signs, wound discharge, or the presence of a sinus tract or fistula should cause a clinician to suspect a PJI. A good anamnesis and a careful examination are advised since local pain is often the only symptom in late, chronic infections and a diagnosis is difficult to established (Ariza et al. 2008; Zimmerli et al. 2004).

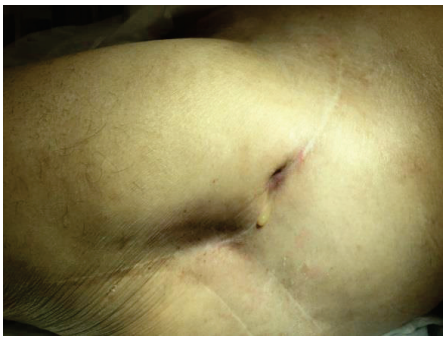


Figure 2. Picture of a discharging sinus tract from a hip PJI

Blood tests including acute-phase reactants (e.g. erytrosedimentation rate and C-reactive protein) could support the diagnosis of infection. Their sensitivity and specificity are approximately 90% when both parameters are used. However, in chronic inflammatory joint diseases, the presence of false positive values is not depreciable (Ariza et al. 2008). By contrast, negative values make the diagnosis of PJI unlikely (Spangehl et al. 1999; Della Valle et al. 2007). The blood leukocyte count and the percentage of band forms are not sufficiently discriminative to predict the presence or absence of infection (Zimmerli et al. 2004).

Plain radiographs performed six months after implantation are useful for detecting signs of infection, especially if they are studied serially over time (Tigges et al. 1994; Zimmerli et al. 2004). Peri-implant radiolucency >2mm, peri-implant osteolysis and radiological changes in implant components are indirect signs of prosthetic loosening, which could appear due to an infection or aseptic loosening. The earlier that they are observed, the more likely it is that they are related to an infection. Conversely, if this occurs after two years post implantation, it may suggest aseptic loosening. Other radiological signs such as periostic reaction are more characteristic of infection (Ariza et al. 2008; Trampuz and Zimmerli 2005).

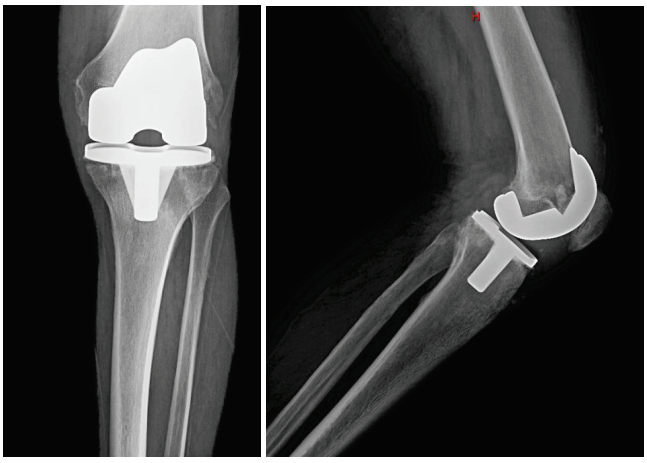


Figure 3. Peri-implant radiolucency on the tibial plateau, from a patient with a late chronic knee PJI

Gammagraphy with ^{111}In marked leukocytes is the preferred nuclear scintigraphy for the study of PJI, with a sensitivity of around 80%. However, it has high number of false-positive results for non-cemented prostheses. The specificity can be increaded to 94% by adding a ^{99m}TC with sulphur colloid BMS. Newer nuclear scintigraphy techniques include gammagraphy with anti-granulocyte antibodies or 18F-fluodeoxyglucose positron-emission tomography (Ariza et al. 2008; El Espera et al. 2004; Zimmerli et al. 2004) .

Swabs cultures from a wound or through the sinus tract have a low predictive value and may reflect a superficial colonization of patient's skin flora, rather than the infecting pathogen. However, if samples are taken early after the sinus tract reaches the skin, when the wound starts discharging, or when the isolated pathogen is *S.aureus,* the predictive value is higher (Ariza et al. 2008; Mackowiak et al. 1978).

In daily clinical practice, it is difficult to differentiate a low-grade PJI from prosthetic aseptic loosening. Aseptic loosening is the most common cause of implant failure, followed by PJI. The pathogenesis of aseptic loosening is not well known, but it includes a local inflammatory process in which several cells and cytokines activate osteoclasts involved in bone resorption (Granchi et al. 1998; Hoenders et al. 2008; Nivbrant et al. 1999). Prosthesis loosening can also be the consequence of low-grade infection that is usually produced by low-virulence microorganisms which can survive in biofilm populations on the implant surface (Costerton et al 1999; Tsukayama et al. 1996; Zimmerli et al. 2004). The synovial fluid leukocyte count and differential represent simple, rapid, and accurate testing for differentiating PJIs from aseptic failure. A synovial fluid leukocyte count of >1.7 $\times 10^9$/L and a differential of >65% neutrophils have sensitivities for diagnosing PJI of 94% and 97%, and specificities of 88% and 98%, respectively (Trampuz et al. 2004).

Microbiological deep samples include those collected from a needle joint puncture or from peri-implant tissues during surgery. In aspirated synovial fluid, the pathogen can be detected in 45-100% percent of cases.

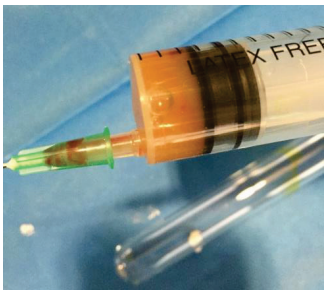


Figure 4. Pus collected from a needle joint puncture

Intraoperative cultures from peri-implant tissue provide the most reliable means of detecting a pathogen and they are frequently used as a reference standard for diagnosing infections associated with prosthetic joints. The sensitivity of these cultures ranges from 65 to 94 percent (Atkins et al. 1998; Spangehl et al. 1999; Zimmerli et al. 2004). A minimum of five intraoperative tissue specimens must be sampled for culture during a revision procedure since the cut off for a definite diagnosis of late chronic PJI is three or more operative specimens that yield an indistinguishable organism (sensitivity, 65%; specificity, 99.6%;). In cases with two or more specimens growing in the same organism, the specificity is 97%. Although these findings are specific to infected prosthetic hips and knees, they may also hold true for other low-grade chronic infections where pathogens and commensal organisms overlap. This includes infections of other prosthetic joints and implantable devices, fracture fixations and non-unions, and other forms of chronic osteomyelitis including vertebral and contiguous osteomyelitis (Atkins et al. 1998). Both aerobic and anaerobic medium must be included; samples must be incubated for at least 7-10 days, and up to 14 days for slow-growing or anaerobic cultures (Ariza et al. 2008; Atkins et al. 1998; Schäfer et al. 2008; Spangehl et al. 1999; Zimmerli et al. 2004). Additional samples of mycobacteria and fungi are also advised. Any antimicrobial therapy should be discontinued at least two weeks prior to tissue sampling for culture (Spangehl et al. 1999). Finally, perioperative prophylaxis should not be started at revision surgery until after tissue specimens have been collected for culture (Widmer 2001).

In terms of histological findings, the criteria to differentiate a PJI from aseptic loosening is the presence of >5-10 neutrophils per high-power field at a magnification of 400 in the histopathological examination of intraoperative samples (sensitivity, 67-80%) in patients without chronic inflammatory diseases. The degree of infiltration of inflammatory cells may vary considerably among specimens from the same patient. Therefore, areas with the most florid inflammatory changes should be sampled (Ariza et al. 2008; Banit et al. 2002; Trampuz and Zimmerli 2005; Zimmerli et al. 2004).

In recent years, new and sophisticated technologies that recover bacteria attached to the prosthesis have been applied in the setting of implant failure revisions. Tunney et al. used prosthesis sonication and microscopy techniques (e.g. scanning electron and immunofluorescence microscopy) to identify the presence of microorganism aggregates in sonicated fluid from the explanted prosthesis. They, as well as other authors, have postulated that PJI is underdiagnosed among cases of prosthesis loosening (Dobbins et al. 1988; Gristina and Costerton 1985; Nelson et al. 2005; Nguyen et al. 2002; Tunney et al. 1998, 1999). In contrast, other studies have identified the presence of microorganisms such as coagulase-

negative staphylococci (CoNS), which the authors interpreted as contaminants (Barrack et al. 2007). Thus, the presence of a single positive culture, either from tissue or from prosthesis sonication, still remains a matter of concern due to challenge of distinguishing infection (active or subclinical) from contamination (Atkins et al. 1998; Mirra et al. 1982).

Since most of the new technologies, except sonication, are difficult to incorporate into clinical practice, recent efforts have been made to validate the results obtained by this methodology by comparing them with results of histopathology or periprosthetic tissue cultures (Piper et al. 2009; Portillo et al. 2012; Trampuz et al. 2007; Vergidis et al. 2011). Controversy still exists regarding the universal use of sonication in clinical practice (Osmon et al. 2013), but some personal opinions recommend the inclusion of the sonication technique in evaluations of prosthesis failure to improve the etiologic diagnosis of infection (Del Pozo and Patel 2009).

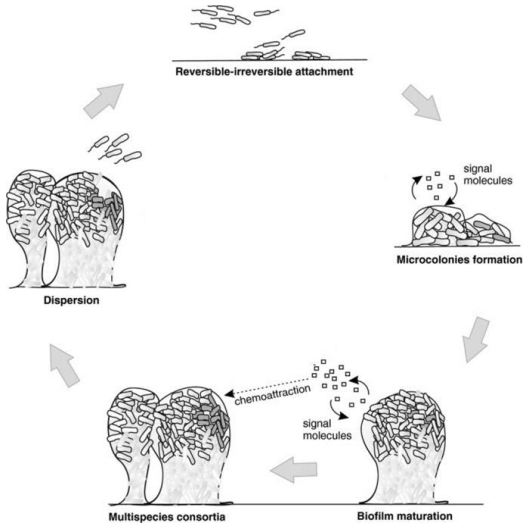
Thus, initial suspicions of implant failure etiology based on clinical and biochemical aspects, and on laboratory studies, histopathology, and microbiological findings, help physicians make an accurate diagnosis. (Osmon et al. 2013; Zimmerli et al. 2004).

4. Pathogenesis of osteoarticular infection and biofilm formation

4.1 Pathogenesis

The pathogenesis of ostearticular infection can be due to a hematogenous seeding, a contiguous spread from adjacent soft tissues and joints, or direct inoculation of microorganisms into the bone because of a trauma or surgery. When established, bacteria produce a local inflammatory reaction that promotes bone necrosis. Joint or bone destruction and the formation of sequestra are characteristics of this disease (Mandell 8th edition).

Figure 5. Biofilm formation on surfaces [image from (Wolska et al. 2016)]



4.2 Biofilm related infections

Bacteria can attach to surfaces aggregated in a hydrated polymeric matrix which results from their own synthesis. This formation called biofilm constitutes a protected mode of growth that allows bacteria to survive in a hostile environment. Inside the biofilm, there are a cell-to-cell communication and signaling molecules (*quorum sensing*) that induce biofilm microorganisms to change their patterns of gene expression. At a high population density, such signals reach sufficient concentrations to activate genes involved in biofilm differentiation. Biofilms develop preferentially on inert surfaces, or on dead tissue. They commonly occur on medical devices and fragments of dead tissue such as the sequestra of dead bone. Biofilms are dynamic systems. Their formation is a progressive process in which colonizing bacteria move or are transported to a surface, attach, and through a series of steps, produce a biofilm. Since they grow slowly, biofilm infections are often slow to produce apparent symptoms. Moreover, these sessile communities of bacteria have an inherent resistance to antimicrobial agents and are at the root of many persistent and chronic infections (Costerton et al. 1999; Pasmore and Costerton 2003; Patel 2005). It has been reported that antimicrobial MICs of bacteria embedded in biofilms can be 10 to 1,000 times higher than those in a planktonic state (Ceri et al. 1999). Biofilms decreased susceptibility to antimicrobials have been explained by three mechanisms (Costerton et al. 1999; Høiby et al. 2010; Stewart and Costerton 2001):

- the difficulty of an agent to penetrate the full depth of the biofilm, such polymeric matrix substances retard the diffusion of most of the antibiotics,
- the presence of many cells in biofilm that suffer nutrient limitation and therefore exist in a slow-growing or starved state, and

- phenotype change: some of the cells in a biofilm adopt a distinct and protected biofilm phenotype. This phenotype is not a response to nutrient limitation; it is a biologically programmed response to growth on a surface.

Numerous strategies have been proposed to remove biofilm from device-related infections in humans, including: 1) ultrasounds, 2) agents that either eradicate or penetrate the extracellular polymeric substances, 3) treatments based on disruption of *quorum-sensing* systems, 4) elucidating the genes that are activated or repressed during initial biofilm formation (before it becomes mature) since younger biofilms are more susceptible to antimicrobial agents, 5) the application of a direct electrical effect with an antimicrobial chemotherapy, and 6) identifying the best antibiotic strategies for acting against the biofilm (Donlan and Costerton 2002; Del Pozo et al. 2008).

4.3 Experimental models for growing biofilms

Several experimental studies have attempted to reproduce a biofilm infection to test different antibiotic strategies and evaluate their activity against bacteria embedded in biofilm, such that they could be applied in clinical practice. The CDC biofilm reactor (CBR) is an in vitro dynamic model designed for growing biofilms of different microorganisms under repeatable and reproducible conditions (Goeres et al. 2005). Different publications have used this model for Gram positive cocci or Gram negative bacteria (Buckingham-Meyer et al. 2007; Donlan et al. 2004; Goeres et al. 2005; Lora-Tamayo et al. 2014; Parra-Ruiz et al. 2010; Williams and Bloebaum 2010). Parra-Ruíz et al. demonstrate, in their biofilm-growing model with *Sthapylococcus aureus* (using one methicillin-susceptible and one methicillin-resistant strain), that combinations such as daptomycin at a high-dose (10mg/kg) or moxifloxacin plus clarithromycin are the most effective regimens and may represent promising options for treating persistent biofilm-embedded infections caused by methicillin-susceptible *S. aureus*. Combination therapy with daptomycin plus rifampicin significantly improved the bacterial killing effect against staphylococci methicillin-resistant strains biofilms (Parra-Ruiz et al. 2010). On the other hand, Lora-Tamayo et al. have shown that colistin (with clinical dosage regimens, 3.5mg/L) and doripenem in combination increase the bacterial killing of biofilm-embedded *Pseudomonas aeruginosa*, including carbapenem-resistant isolates, with negligible emergence of colistin resistance (Lora-Tamayo et al. 2014).

5. Management of orthopaedic device-related infections

The successful treatment of orthopaedic device-related infections requires a combination of an adequate surgical procedure and prolonged antimicrobial therapy, acting on adhering stationary-phase microorganisms that grow in biofilms. Thus, an essential component of the care of these patients is strong collaboration between all involved medical and surgical specialists (e.g. orthopaedic surgeons, plastic surgeons, infectious disease specialists, and internists) (Osmon et al. 2013; Trampuz and Zimmerli 2006).

5.1 Surgical treatment

Surgical treatments for PJI include debridement with retention of the prosthesis, one-stage or two-stage exchange, resection arthroplasty, arthrodesis, and amputation (Zimmerli et al. 2004).

- **Debridement** involves the removal of the hematoma, fibrous membranes, sinus tracts, devitalized bone and soft tissue, and the exchange of the removable components of the prosthesis (e.g. the polyethylene liner) (Byren et al. 2009; Zimmerli et al. 2004). It is a less aggressive operation than an explantation, and it is followed by a long duration of antibiotic treatment. Therefore, it is called debridement antibiotic and implant retention (DAIR). According to Zimmerli's algorithm, DAIR is the surgical option for patients with an early postoperative or acute hematogenous infection if the duration of clinical signs and symptoms is less than three weeks, the implant is stable, the soft tissue is in good condition, and an agent with activity against biofilm microorganisms is available. Intravenous treatment should be administered for about two weeks, followed by a prolonged oral therapy (Zimmerli et al. 2004).

- **One-stage revision** includes the removal of all foreign material, debridement, and the reimplantation of a new prosthesis during the same procedure. Although it is not a gold standard procedure, it is increasingly incorporated into clinical practice with a success rate of 86 to 100 percent (Callaghan et al. 1999; Raut et al. 1994). One-stage exchange provides an advantage to the patient, in that only one operation is required with a fasted functional recovery. The following prerequisites are advised: a satisfactory condition of soft tissue and the absence of difficult-to-treat microorganisms (Ariza et al. 2008; Zimmerli et al. 2004). A systematic reviewed of data and meta-analysis by Kunutsor et al. suggested that the one-stage revision strategy may be as effective as the two-stage revision strategy when treating infected knee prostheses in unselected patients (rate of re-infection of 7.7%

vs 8.8, respectively) (Kunutsor et al. 2016). Data from a detailed meta-analysis of infected hip prostheses are needed (Kunutsor et al. 2015). A trial comparing one-stage and two-stage hip revision is currently being conducted. In this study, the analysis of the outcome focuses on the following patient-reported symptoms: pain, function and long-term wellbeing. Patients state that these outcomes are more important than clinical outcomes such as re-infection, and they have been commonly used in previous non-randomised studies (Strange et al. 2016).

- **Two-stage exchange** includes the removal of the infected prosthesis (first stage) and its replacement with an antibiotic-loaded cement spacer to prevent joint space contracture between stages. Once the infection has been treated with systemic antibiotics and cured, the second-stage is performed where a new prosthesis is implanted for a variable period of time (second stage). Two-stage revision has reported success rates as high as 90% in PJI management and it is the current procedure of choice for late chronic infection (Ariza et al. 2008; Cabo et al. 2011; McDonald et al. 1989; Windsor et al. 1990; Zimmerli et al. 2004).

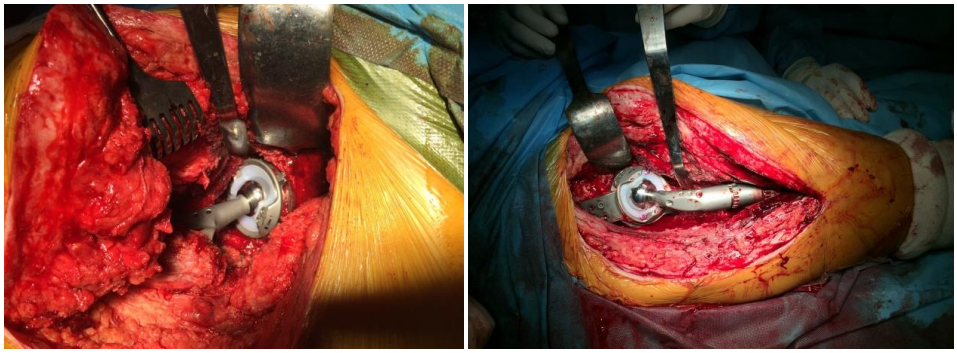


Figure 6. Hip PJI submitted to two-stage exchange procedure

- **Resection arthroplasty** consists of the permanent removal of the prosthesis and debridement without reimplantation. It is performed in patients with a poor bone stock or poor soft tissue conditions, as well as in severely immunocompromised patients and patients for whom arthroplasty will not provide any functional benefit. Orthopaedic alternatives in these cases include a two-step arthrodesis (for knee joints) or Girdlestone arthrodesis (for hip joints) (Ariza et al. 2008; Zimmerli et al. 2004).

In contrast to PJI, complete eradication of infection is not the primary goal for osteoarticular infections associated with internal fixation since the device can be removed after consolidation. The nature of the surgical intervention for this condition depends on the type of

device, the presence or absence of bone union, and the patient's underlying condition (Darouiche 2004). If the implant is stable, debridement with retention of the fracture-fixation device combined with long-term antibiotic treatment is reasonable (Trebse et al. 2005; Zimmerli et al. 1998). If there is dead tissue or abundant purulence, repeated debridement is usually required (Trampuz and Zimmerli 2006). Long-term suppressive antimicrobial therapy is reasonable if surgery is contraindicated or suboptimal because the patient has a severe coexisting illness, does not need a functional prosthesis because of immobility, or refuses further procedures. The goal of suppressive treatment is to control clinical manifestations rather than eradicate infection (Zimmerli et al. 2004).

5.2 Antimicrobial treatment

5.2.1 General principles

The antimicrobial treatment for orthopaedic device-related infections should have bactericidal activity against surface-adhering, slow-growing, and biofilm-producing microorganisms. Moreover, standard antimicrobial susceptibility tests for these infections are not appropriate to predict their outcome; since they are reliable for planktonic infections. It is well documented that minimal bactericidal concentration (MBC) increases significantly in bacteria embedded in biofilms (Costerton et al. 1999; Widmer et al. 1990). Several authors have proposed that minimal biofilm inhibitory concentration (MBIC) and minimal biofilm eradicate concentration (MBEC) are more suitable for testing the antibiotic susceptibility of bacteria in biofilms (Ceri et al. 1999). In this setting, rifampicin is considered the best antibiotic to treat osteoarticular infections caused by staphylococci as it fulfils these requirements (Ariza et al. 2008; Zimmerli et al. 2004). However, rifampicin should never be administered alone since staphylococci rapidly develop antimicrobial resistance (Kadurugamuwa et al. 2004). Quinolones are excellent combination agents because of their bioavailability, antimicrobial activity, and tolerability (Zimmerli et al. 1998). Experiments comparing other antibiotic regiments in animal experimental models, such as the tissue-cage model with rats (Garrigos et al. 2013; El Haj et al. 2014, 2015), osteomyelitis models with rats (Vergidis et al. 2011), and the PJI model with rabbits (Saleh-Mghir et al. 2011) have been performed. There is little published experience about Gram-negative bacilli and the data regarding treatment efficacy are inconsistent. *In vitro* studies and animal models show that ciprofloxacin had better efficacy than BL (Widmer et al. 1991), and a case series study showed a 79% success rate when quinolones where used to treat PJI by ciprofloxacin-susceptible Gram-negative bacilli managed with DAIR (Rodríguez-Pardo et al. 2014). New therapeutic strategies are needed for quinolone-

resistant microorganisms. In these situations, the role of β-lactams is questioned and is further complicated if microorganisms show reduced susceptibility or resistance to β-lactams. In this field, regimes with combination therapy with β-lactams should be explored.

Bone diffusion is usually poor after the administration of systemic antibiotics. Reviews have been published on the bone-to-serum ratio as a reflection of antibiotic concentration at the infection site (Boselli and Allaouchiche 1999; Landersdorfer et al. 2009; Sendi and Zimmerli 2012; Spellberg and Lipsky 2012). The mean bone-to-serum ratio concentrations for antibiotics range between 0.3 and 1.2 for quinolones, macrolides, and linezolid; between 0.15 and 0.3 for cephalosporins and glycopeptides; and between 0.1 and 0.3 for penicillins (Landersdorfer et al. 2009). It is therefore common, that high antibiotic doses (Murillo et al. 2006, 2009; Zimmerli et al. 2004) and combined therapy are needed to achieve higher concentrations in bone. Since long-term antimicrobial therapy is needed, high tolerability and oral bioavailability are advisable. However, if intravenous drugs are the only option, the use of an intravenous access device for out-patients may be considered (Osmon and Berbari 2002).

5.2.2 The use of betalactams in continuous infusion (CI)

β-lactams (BL) are time-dependent antibiotics; the longer they are present at the site of infection above the targeted pathogen's minimum inhibitory concentration (T>MIC), the more effective they are (Craig 1998; Eagle at al. 1950). While a T>MIC of 40-60% achieved by standard intermittent bolus (IB) administration has traditionally been effective (Craig 1998; Drusano 2004; Vogelman et al. 1988), higher T>MIC rates may be needed in particular scenarios to manage difficult-to-treat infections (Van Herendael et al. 2012; McKinnon at al. 2008; Mohd Hafiz et al. 2012) and reduce the risk of emerging resistant strains (Alou 2005; Cappelletty et al. 1995; Mouton and Vinks 2007). Pharmacodynamic data suggest that CI may be more effective than IB, because it maintains an antibiotic concentration above the MIC for longer (and may obtain a T>MIC≈100%), particularly for bacteria with high MIC values. However, clinical data is scarce (Dulhunty et al. 2013; Roberts et al. 2016).

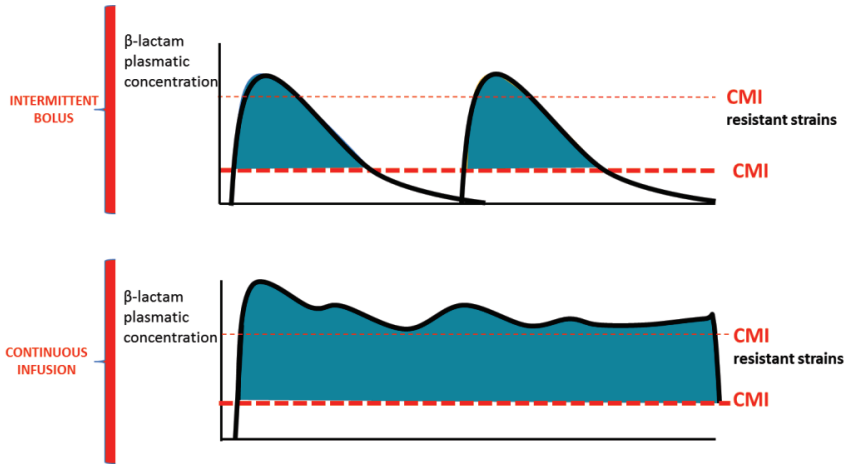


Figure 7. PK/PD features using β-lactams by intermittent bolus or continuous infusion

Pharmacokinetic and pharmacodynamic (PK/PD) studies have consistently shown that the maximum killing rate of BL occurs at concentrations that are three to four times above the MIC values (Craig 1998; Mouton and Vinks 1996, 2007). These concentrations are usually achieved after the administration of a BL bolus. When CI is used, an initial loading dose is administered immediately before the CI in order to achieve the required concentration levels (3-4 times above the MIC if possible) in patients from the intensive care unit (Karaiskos et al. 2015): However, a loading dose might not be necessary in other clinical situations.

These general approaches have been applied in the field of biofilm-related infections such as to osteoarticular infections, in which the bactericidal effectiveness of BL has been questioned (Gilbert and Brown 1998; Gilbert et al. 1990). The required levels of BL remain unclear in these difficult-to treat infections since the clinical evidence is scarce (Dulhunty et al. 2013; Roberts et al. 2016), but it seems reasonable that a higher plasma concentration at a longer T>MIC could reflect higher BL levels at the site of infection and improved clinical outcomes.

5.2.3 Therapeutic drug monitoring (TDM)

Plasma levels of antibiotics depend on multiple individual factors and scenarios such as renal failure and sepsis. Therefore, TDM is essential for individualizing antibiotic dosages and guiding therapy in different clinical situations (Huttner et al. 2015). While it is commonly used in clinical practice for some antibiotics, this is not the case or BL. Since there are no available commercial procedures for the routine measurement of BL concentration in human plasma in our clinical practice, measurement procedures should be developed and validated in-house. High-performance liquid chromatography (HPLC) procedures for the simultaneous

measurement of multiple BL concentrations in plasma using ultraviolet detection have been described (Denooz and Charlier 2008; Legrand et al. 2016; McWhinney et al. 2010; Nemutlu et al. 2009; Verdier et al. 2011; Wolff et al. 2013). These procedures usually present low detection capabilities and low selectivity due to endogenous interferences, the limited ultraviolet absorption characteristics of the BL moiety, and the low wavelengths required to measure BL concentrations.

Greater detection capabilities and more selective HPLC procedures have been developed using HPLC coupled with tandem mass spectrometry (MS/MS) (Ahsman et al. 2009; Carlier et al. 2012, 2015; Cazorla-Reyes et al. 2014; Cohen-Wolkowiez et al. 2011; Colin et al. 2013; Ohmori et al. 2011; Sime et al. 2014). To our knowledge, only some of these methods have been used to measure BL concentrations in human plasma with ultrahigh performance liquid chromatography (UHPLC)-MS/MS procedures (Ahsman et al. 2009; Carlier et al. 2012, 2015; Cazorla-Reyes et al. 2014; Colin et al. 2013). UHPLC has characteristics that provide more resolution and shorter retention times than HPLC (Churchwell et al. 2005; Gumustas et al. 2013; Nováková et al. 2006).

Among the UHPLC or HPLC-MS/MS procedures reported previously, none of them have been used for the simultaneous measurement of multiple BL concentrations in human plasma, which is essential for their routine use in the daily clinical practice of tertiary-care hospitals. These previous studies also had limitations, such as time-consuming sample extraction procedures, and a lack of investigation into performance characteristics such as carry over and dilution integrity.

Therefore, the validation of an easy-to-use UHPLC-MS/MS procedure for the simultaneous measurement of concentrations of multiple BLs in human plasma that may be used in CI or extended infusion (EI) would be desirable.

5.2.4. Specific antibiotics for specific microorganisms

5.2.4.1. *Streptococcus spp*

Streptococci are responsible for PJI in 4–12% of cases (Benito et al. 2016; Peel et al. 2012), especially in hematogenous infections (Marculescu et al. 2006; Tsukayama et al. 1996). Some studies have suggested that streptococcal PJI may have a more favorable outcome than other etiologies (Betz et al. 2015; Everts et al. 2004; Zürcher-Pfund et al. 2013), but this finding has been contested (Zeller et al. 2009). In fact, the success rate of streptococcal PJI (mostly *Streptococcus agalactiae*) treated with DAIR varies from 22–100%, presumably depending on

the selection criteria used (Corvec et al. 2011; Duggan et al. 2001; Everts et al. 2004; Meehan et al. 2003; Sendi et al. 2011; Zeller et al. 2009). Thus, the real success rate for patients managed by DAIR remains unknown. Likewise, the optimal antimicrobial treatment for streptococcal PJI is also unknown, though current guidelines recommend the use of BL (Osmon et al. 2013; Zimmerli et al. 2004). BLs have high activity for the initial planktonic phase of these infections (Baker et al. 1981). However, once this initial phase has passed, the antibiofilm profile of these antimicrobials is questionable because any antibiotic with a mechanism of action dependent on cell wall synthesis will become less effective against biofilm-embedded bacteria (Costerton et al. 1999) with a high minimal biofilm eradication concentration (García-Castillo et al. 2007; Olson et al. 2002; del Prado et al. 2010).

In the field of PJI, there is now strong evidence that BL has poor efficacy for staphylococcal and GNB, especially when contrasted with other antibiotics that have superior antibiofilm profiles, such as rifampin against staphylococci or fluoroquinolones against GNB (Lora-Tamayo et al. 2013; Martínez-Pastor et al. 2009; Rodríguez-Pardo et al. 2014; Senneville et al. 2011; Zimmerli et al. 1998). However, these findings have not yet been demonstrated in streptococcal PJI. Therefore, the role of alternative compounds with a better antibiofilm profile must be explored for subsequent application in clinical practice.

5.2.4.2. Gram-negative bacilli - The era of antibiotic multiresistance.

Gram-positive bacteria are the most frequent infective agents in osteoarticular infection, whereas Gram-negative bacteria (GNB) may be responsible for 10%–23% of cases (Murillo et al. 2015; Trampuz and Zimmerli 2008; Zimmerli et al. 2004). In particular contexts, such as with PJIs (Hsieh et al. 2009; Rodríguez-Pardo et al. 2014; Tattevin et al. 1999; Zimmerli et al. 2004), *Pseudomonas aeruginosa* may cause up to 20% of these GNB infections (Rodríguez-Pardo et al. 2014). While current antibiotic recommendations for the treatment of OIs caused by GNB are ciprofloxacin and BL (Lew and Waldvogel 2004; Osmon et al. 2013), there is no standard treatment for multidrug resistant (MDR) GNB infections. The use of BL to treat PJIs caused by quinolone-resistant GNB is associated with a poor cure rate (Rodríguez-Pardo et al. 2014), and the role of antibiotics is complicated in situations with reduced susceptibility or resistance to BL. The progressive emergence of MDR GNB represents a new challenge in the treatment of nosocomial infection. In the field of PJI, a recent study showed that the percentage of MDR GNB almost tripled from 3.3% in 2003 to 9.4% in 2012 (Benito et al 2016).

Among these pathogens, *P. aeruginosa* is particularly problematic since there are few therapeutic options (Magiorakos et al. 2012). For some strains which are resistant or not fully susceptible to BL, the only active antimicrobials are polymyxins and aminoglycosides (Suarez et al. 2011). Therefore, older antibiotics, such as the polymyxins [mostly polymyxin B and polymyxin E (colistin)] have recently gained prominence in the treatment of problematic MDR GNB (e.g. *P. aeruginosa*) and their activity against associated biofilms has been demonstrated by *in vitro* and *in vivo* experimentation (Brochmann et al. 2014; Chambers and Sauer 2013; Chiang et al. 2012; Haagensen et al. 2007; Herrmann et al. 2010; Pamp et al. 2008). Colistin was used in the 1960s and then abandoned because of its toxicity (mainly nephrotoxicity). It has a wide anti-GNB spectrum including *Pseudomonas spp* (Li et al. 2006; Nation and Li 2009) and a bactericidal effect that is concentration dependent. Colistin is administered to patients as an inactive-prodrug (colistin methasulphonate) that is mostly excreted by urine (70%), and a small component is hydrolised to colistin (Couet et al. 2011; Garonzik et al. 2011). Thus, high doses are required to reach the required colistin concentrations; heteroresistance is common and microorganisms exposed to suboptimal concentrations may amplify their resistant subpopulations, leading to clinical failure (Bergen et al. 2010; Li et al. 2006; Poudyal et al. 2008).

Colistin is effective against less active bacteria located in the deeper layers of the biofilm structure, which contrasts with the majority of antibiotics that operate solely at the upper layers (Haagensen et al. 2007; Klausen et al. 2003; Pamp et al. 2008). This observation is supported by colistin's bactericidal activity, which is independent of hydroxyl radical formation and consumption (Brochmann et al. 2014). Several publications based on pharmacokinetic, pharmacodynamic and experimental models have suggested the potential clinical benefits of systemic colistin in combination with other antimicrobials (such as BL) (Hengzhuang et al. 2012; Hengzhuang et al. 2014; Herrmann et al. 2010; Lora-Tamayo et al. 2014). This combination can kill different subpopulations or layers of the biofilm. Moreover, as a cationic peptide targets the bacterial external membrane, changes its permeability and facilitates the penetration of other antibiotics into the GNB (Hancock 1997; Hancock and Wong 1984; Lorian 1971). However, the lack of clinical studies to treat orthopaedic device-related infections necessitates future studies to propose optimized treatment guidance for these difficult-to-treat infections.

HYPOTHESIS AND JUSTIFICATION

Orthopaedic device-related infections represent a health care problem of first magnitude due to the increasing incidence, complexity of management, and elevated cost. The increasing incidence of prosthetic joint infections mainly occurs in developed countries, in part because of the increasing life expectancy of the population, which promotes prosthetic implantation and consequently, infections.

These device related infections have etio-pathogenic features that involve the participation of bacteria in the stationary growth phase as well as mature biofilms, which makes their diagnosis and treatment more challenging. Furthermore, these infections often require prolonged antibiotic therapy, concomitant surgeries, and long hospitalizations. Therefore, an accurate based on clinical symptoms, radiological changes, and the correct interpretation of microbiological findings is essential to ensure optimal treatment.

Multidisciplinary management is preferred when treating orthopaedic device-related infections. To improve the performance of diagnostic tests and design of the best antibiotic pattern for each microorganism (with the current problem of multiresistance), and to determine the most effective surgical strategy that positively impacts patient's lives (e.g. functional recovery, shorter hospitalization, and fewer surgeries) several studies have been performed that ameliorate the present guidelines. Nevertheless, there are still many points of uncertainty and many relevant clinical questions remain unanswered. Due to the challenge of collecting large cohorts of homogeneous cases, and the long therapies and follow-up periods, there is a lack of prospective studies, including clinical trials. Most problems are being resolved with multicentre, retrospective observational studies that combine many patients and reduce the inter-individual and inter-centre variations. Experimental studies are also needed to understand the basis and behaviour of the different antibiotic patterns. Several *in vitro* and *in vivo* biofilm models have been described; their results support clinical approaches and present ideas for future studies.

This thesis explores some of these unanswered questions in the field of orthopaedic device-related infections from the perspective of an infectious diseases specialist. Several questions about clinical management and antimicrobial efficacy regarding this pathology were developed and supported by experimental approaches such the *in vitro* model for growing biofilms. All the presented studies were conducted in the osteoarticular infection unit in the Hospital Universitari de Bellvitge (Barcelona), which is formed by a multidisciplinary team of specialists in infectious diseases, microbiologists, rheumatologists, orthopaedic surgeons, and radiologists. Through experience, this unit has compiled many patients with long follow-up

times and a comprehensive database, which is essential to conducting different clinical studies. Throughout the past five years, I have been involved in the management of these patients through daily clinical practice. I have also participated in developing and updating the corresponding database that was used to introduce the relevant clinical variables for analysis.

Among the clinical studies presented in this thesis, three are multicentre studies (two are international) and were performed in the setting of the Spanish Network for Research into Infectious Diseases (REIPI), which is coordinated by Prof. Javier Ariza (one of the directors of this thesis) and the Grupo de Estudio de Infección Osteoarticular (GEIO) from the Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). These studies investigate the interpretation of unexpected positive cultures in the setting of prosthetic loosening; explore the risk of prosthesis reinfection by comparing one-stage and two-stage revision; and conducting the largest study of streptococcal prosthetic joint infections managed with DAIR, to elucidate the prognosis and the best antibiotic patterns. In the latter study, clinical data from the bone infection unit in the Nuffield Orthopaedic Centre in Oxford was incorportaed, which I collected during my six-months stay at this centre. Moreover, I was involved in the daily clinical practice of patients attended in this bone infection unit in the UK. Thus, I had the opportunity to work with a group of different bone and joint infectious disease specialists and learn a new point of view on the management of osteoarticular infections. This thesis also includes a local clinical study of the antimicrobial combination therapy with colistin plus β-lactams for osteoarticular infections caused by multidrug resistant *Pseudomonas aeruginosa*, which provides original data that will be included in an international multicentre study from Greece.

Through an institutional program from theHospital Universitari de Bellvitge, a study on the use of β-lactams in continuous infusion for osteoarticular infections was developed. It was coordinated by the Antibiotics Committee (Dr Oscar Murillo, one of the directors of this thesis, was a member of this commission) and conducted with the clinical laboratory department. Plasmatic samples from a cohort of patients with osteoarticular infections were prospectively collected and analysed with UHPLC-MS/MS to measure β-lactam levels and standardize this novel method for institutional, clinical use. By comparing UHPLC-MS/MS results with the predicted plasmatic levels of β-lactams, we attempt to validate an easy to use equation to predict β-lactams plasmatic levels in patients when UHPLC-MS/MS is not available.

The laboratory of experimental infection located at the Faculty of Medicine (Universitat de Barcelona, campus Bellvitge) has a wide experience with experimental foreign-body infection

models in rats and provides an equipped platform for the Spanish Network (REIPI) to approach current clinical problems through translational research. This platform has provided me the opportunity to develop and standardise an *in vitro* model for the study of Gram-negative bacilli biofilm.

All the studies presented in this thesis provide novel information on different clinical aspects and therapeutic approaches, including laboratory and basic studies, in the field of orthopaedic device-related infections.

AIMS

A. On the management of orthopaedic device-related infections

A.1. Diagnostic aspects of PJI

- Aim 1: to analyse the microbiological and clinical findings in patients with suspected prosthetic joint aseptic loosening, and to compare them to patients with chronic PJI

A.2. Surgical management of PJI

- Aim 2: to evaluate the risk of re-infection following one-stage and to-stage surgical revision with hip PJI

B. On the assessment of antimicrobial efficacy for the treatment of orthopaedic device-related infections

B.1. Infections by *Streptococcus spp*

- Aim 3: to assess the efficacy of adding rifampicin to β-lactams for the treatment of streptococcal PJI managed with implant retention, and its impact on the prognosis

B.2. Infections by MDR Gram-negative bacilli

B.2.1 The use of β-lactams in continuous infusion

- Aim 4: to standardize a measurement procedure based on UHPLC-MS/MS for the simultaneous determination of multiple β-lactam concentrations in human plasma
- Aim 5: to evaluate the efficacy and safety of β-lactams in continuous infusion for difficult-to-treat osteoarticular infections caused by Gram-negative bacilli, and to validate an easy method for clinical use

B.2.2 The use of antibiotic combinations with colistin

- Aim 6: to evaluate the benefits of the combination of colistin and β-lactams when treating patients with MDR *Pseudomonas aeruginosa* infections
- Aim 7: to study the effect of adding colistin to β-lactams against ESBL-producing *klebsiella pneumonia*e biofilm in an *in vitro* experimental model

MATERIALS AND METHODS

1. Setting

The following entities have provided the opportunity to work in the field of osteoarticular infection diseases, perform local clinical and experimental studies, and participate in multicentre studies:

The Osteoarticular Infection Unit of the Hospital Universitari de Bellvitge

The Hospital Universitari de Bellvitge is a tertiary-care teaching hospital in Barcelona. Cases with osteartícular infection are attended in the osteoarticular infection unit located on the tenth floor of the building. This unit consists of a multidisciplinary team including specialists in infectious diseases, traumatology, microbiology, radiology, rheumatology and nurses, who have a wide range of experience in this field. The team is led by the orthopaedic surgeon Dr. Javier Cabo and the specialized team of nurses is headed by Isabel Vila. This unit is recognized by the Ministry of Health as a Reference Unit of the National Health Service for the treatment of difficult-to-treat osteoarticular infections. Inside the unit, standard sterility measures and a strict policy of hand-washing are applied. Patients' rooms have an airlock to isolate cases that are colonized by MDR microorganisms (e.g. MRSA, MDR *P.aeruginosa*, and ESBL-producing *Enterobacteriaceae*). Cases that are hospitalized in the unit have a wide variety of osteoarticular infections that include septic arthritis, osteomyelitis, diabetic food, device-related infections including PJI, and complex skin and soft tissue infections.

Figure 8. Infectious disease doctor team from the bone infection unit

Daily clinical practice on this unit primarily consists of a morning ward round with infectious disease specialists, orthopaedic surgeons, and nurses to guide every clinical case and make decisions about surgical and antibiotic management. . Every day at midday, there is a meeting of specialists in infectious disease and microbiologists to check the microbiology results of the

samples that are isolated from the attended patients. The laboratory of microbiology is situated on the ground floor of the Hospital Universitari de Bellvitge and it provides specialized support for clinical practice and for the microbiological aspects of all studies performed within the infectious diseases department. After discharge, patients are followed-up at outpatient clinics by the same orthopaedic surgeons and infectious diseases specialists. The activities related to infectious diseases are led by Prof. Javier Ariza who is one of the directors of this thesis.

The Spanish Network for Research into Infectious Diseases (REIPI)

REIPI (www.reipi.org) was recognized and funded by the Instituto de Salud Carlos III fifteen years ago, and the members of the infectious diseases and microbiology department at the Hospital Universitari de Bellvitge are part of this network. One of the primary research lines is antibiotic resistance and the work-package 'Optimizing the management of prosthetic joint infections by MDR bacteria', belongs to this line. Within the REIPI, this work-package is conducted by the *Spanish Group for the Study of Pathogenesis and Antimicrobial Treatment of PJI*, led by Prof. Javier Ariza and formed with researchers from 20 Spanish hospitals. This group has published guidelines and protocols to homogenize clinical practice among different Spanish hospitals. Moreover, REIPI has made a common on-line database for multicentre national and international studies, which is essential to recruiting larger uniform samples and drawing the best conclusions.

Grupo de Estudio de Infección Osteoarticular (GEIO) from the Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC).

The GEIO from SEIMC was created in 2015 with the aim of bringing together specialists who are interested in the area of osteoarticular infection. The main target of this group -currently headed by Prof. Javier Ariza and Dr. Javier Cobo- is to share opinions among experts, design updated protocols and guidelines, and promote the performance of common projects in the field of bone and joint infection.

Clinical Laboratory Department

Located on the first floor of the Hospital Universitari de Bellvitge, staff members from the clinical laboratory department conduct several clinical, teaching, and research activities related to clinical biochemistry, clinical molecular biology, haematology, and immunology. This clinical laboratory involves a variety of specialists, including specialists in clinical biochemistry, and its

main clinical activity is *in vitro* testing that facilitate the diagnosis, prevention, prediction, and follow up of several diseases. All of these activities must be approved by the Departament de Sanitat i Seguretat Social de la Generalitat de Catalunya (Decret 7/1995 approved on the 7th of March). Thus, there is a catalogue of services according to the specific requirements of each speciality. However, if there is an appropriate proposal from any clinical or surgical department, these services can be extended.

An institutional program about the use of BL in continuous infusion is being conducted by the Antibiotics Committee. Since the measurement of plasmatic levels of BL is highly recommended, this measurement was requested. Dr. Raül Rigo-Bonnin from the clinical laboratory is a specialist in clinical biochemistry who leads the development of a UHPLC-MS/MS method for the simultaneous measurement of multiple BL concentrations in human plasma.

The Laboratory of Experimental Infection located at the Faculty of Medicine (Universitat de Barcelona, campus Bellvitge)

This laboratory belongs to the Department of Clinical Sciences and is linked to the Department of Infectious Diseases at the Faculty of Medicine (Universitat de Barcelona, campus Bellvitge). This campus includes IDIBELL (an excellent institute from ISCIII).

Figure 9. The laboratory of experimental infection (Faculty of Medicine, Universitat de Barcelona) where the experiments with CBR were conducted

Inside this laboratory, several projects have been performed; one of the highlights was an experimental animal model with rats to reproduce a foreign body associated infection by *S. aureus*, with relevant results that have been published in recognised journals (Garrigos et al.

2010; Garrigós et al. 2013; El Haj et al. 2014, 2015, Murillo et al. 2006, 2008). Recently, preliminary static experiments of a dynamic in vitro biofilm model (CDC-reactor) with *Pseudomonas aeruginosa* were performed in this laboratory and continued at the Monash Institute of Pharmaceutical Science in Melbourne by Lora-Tamayo et al (Lora-Tamayo et al. 2014). All of this research has a close connection with clinical research that has a bench-to-bed basis since is conducted from the perspective of an infectious diseases specialist.

The Bone Infection Unit of Nuffield Orthopaedic Centre (Oxford, United Kingdom)

The Nuffield Orthopaedic Centre belongs to the Oxford University Hospitals (UK) from the National Health Services foundation trust. This centre, located in Headington (Oxford) has been treating patients with bone and joint problems for more than 80 years and has a world-wide reputation.

Figure 10. The Nuffield Orthopaedic Centre (Oxford, United Kingdom)

The hospital provides specialist services such as the treatment of bone and joint infection within the bone infection unit, which is a reference unit in the United Kingdom and throughout the world; it conducts several clinical, teaching and research activities. This unit offers a clinical multidisciplinary approach to the management of these infections with orthopaedic and plastic surgeons, microbiologists, radiologists, and specialists in infectious diseases, and patients with difficult-to treat osteoarticular infections from the throughout the country are admitted. Dr. Ivor Byren, Dr. Bridget Atkins, Dr. Matthew Scarborough, and Dr. Andrew Brent are the infectious disease specialists. They have contributed to the development of intense scientific activity around the prosthetic joint infection with leading international publications such as the

work of Dr. Bridget Atkins *Prospective Evaluation of Microbiological Criteria for Diagnosis of Infection at Joint Prosthetics-Revision Arthroplasty* (Atkins et al. 1998), which is currently used and has facilitated the interpretation of intraoperative periprosthetic cultures and the management of these infections.

During the six-month stay at this unit, an honorary contract permitted me to participate in the clinical management of patients admitted to the bone infection unit and those who visited the clinics, and to collaborate in scientific research including patients with streptococcal PJI that was managed with DAIR in an international database, which is included in this thesis.

2. Study design

The clinical studies discussed in this thesis include five observational studies, of which three are multicentre projects. The observational studies are all retrospectively analysed, though data was prospectively collected. Local data from patients with PJI who attended the osteoarticular unit have been recorded in a database since 2003, including patients' main characteristics and underlying clinical conditions, clinical presentation features, microbiological findings, surgical and antibiotic treatment, and follow-up (the protocol is annexed; Annex 1). For multicentre observational studies, common protocols and uniform databases were filled.

3. Clinical management, antimicrobial assessment, and follow-up

3.1 Clinical diagnosis and definitions

A diagnosis of presumed aseptic loosening was made when patients had joint pain and radiological signs of prosthesis loosening in the absence of signs or symptoms of infection (e.g. local inflammatory signs, the presence of a sinus tract, and systemic symptoms of infection), and the C-reactive protein and the erythrosedimentation rates were not considered clinically relevant (values lower than 15 mg/L and 40 mm/h, respectively).

Diagnosis of PJI was established according to the last recommendations (Osmon et al. 2013). It was considered based on: the presence of signs and symptoms of infections (defined above) or purulence around the prosthesis during surgery, the histopathologic findings (at least five neutrophils per high-power field -x400- found in at least five separate microscopic fields; Feldman's criterion), and the microbiological results obtained from preoperative and intraoperative cultures (two or more cultures that yielded the same organism or the growth of a virulent microorganism in a single sample).

For the streptococcal study, PJI was classified as early postoperative if the symptoms began within the first three months after the prosthesis was placed (Zimmerli et al. 2004). It was classified as late chronic infection if it started after three months. The episode was considered acute hematogenous if it occurred after an uneventful postoperative course with microbiologically confirmed or clinically suspected streptococcal bacteremia. A contiguous spread was considered if the PJI occurred in a limb with either infectious cellulitis or a soft tissue abscess.

Pseudomonas aeruginosa resistance was defined according to Magiorakos et al. as follows (Magiorakos et al. 2012): (i) MDR when *P. aeruginosa* was non-susceptible to one or more agent(s) in three or more antimicrobial categories (aminoglycosides, anti-pseudomonal carbapenems, anti-pseudomonal cephalosporins, anti-pseudomonal fluoroquinolones, anti-pseudomonal penicillins+b-lactamase inhibitors, monobactams, phosphonic acids and polymyxins); or (ii) XDR when *P. aeruginosa* was non-susceptible to one or more agent(s) in all but two or fewer antimicrobial categories.

Osteoarticular infection caused by *P. aeruginosa* was defined by positive cultures in two or more surgical samples, or by one positive culture in surgical samples, joint-aspirate, or blood cultures, in the presence of typical clinical symptoms and signs of infection.

Although all cases with osteoarticular infections caused by multi-drug resistant microorganisms were assumed to have more difficult-to-treat infections, prosthesis removal could introduce a new foreign body (e.g. a spacer) or a new cavity with liquid retention (e.g. Girdlestone resection), which could promote the persistence of infection. Thus, two groups were created according to the type of infection and the initial surgical treatment: Group A included those with OIs that were considered more difficult to treat (including patients with PJIs and OA managed with device retention), while Group B included OIs that were considered less difficult to treat (including patients with OA managed without device retention).

3.2 Surgical management

Surgical and medical management of cases with orthopaedic device-related infections (including PJI) is based on current knowledge and guidelines (Ariza et al. 2008; Cobo and Del Pozo 2011; Osmon et al. 2013; Zimmerli et al. 2004). However, since the criteria was not strictly met by all patients, each case was evaluated considering its own particularities and the final decision was made by an individual medical group. The general management process is as follows.

Patients with an early postoperative PJI (<1 month), acute hematogenous PJI with ≤3 weeks of symptoms, or osteoarthritis and devices were managed with DAIR according to the current recommendations (i.e. patients with acute infection, implant stability, and integrity of surrounding soft tissues) (Cobo and Del Pozo 2011; Trampuz and Zimmerli 2006, 2008; Zimmerli et al. 2004). Mobile parts of the device (e.g. the polyethylene liner) were exchanged if feasible. We also recommended DAIR when, in addition to the established criteria, anti-biofilm antimicrobials were not active, which departed from current recommendations.

Patients with presumed prosthetic aseptic loosening received one-step revision arthroplasty, in which one or two prosthetic components were removed according to radiological signs or surgical findings of loosening. Standard peri-operative antibiotic prophylaxis (Cefazolin 2 grams) was administered immediately after surgical samples were collected as one or two doses depending on the duration of the operation (fewer or more than six hours).

Patients with late chronic PJI were primarily managed with device removal by two-stage revision. In the first operation, the foreign material was explanted and a debridement of the surgical site was performed. In the same procedure, a cement spacer was implanted to avoid the collapse of the remaining cavity and to provide local antimicrobial therapy (with antibiotic-loaded spacers and/or antibiotic-loaded cement). In the second stage, a new prosthesis was placed after hospital discharge and an antibiotic-free period of greater than six weeks, such that the patient's normal flora could be reconstituted. New samples were taken at that time to confirm the sterility. Peri-operative antibiotic prophylaxis was designed according to the causative infection and was maintained for five to seven days. A one-stage revision procedure was occasionally performed with accurate debridement of the surgical site and complete removal of all foreign material.

3.3 Antimicrobial therapy

The antipseudomonal antimicrobial therapy was chosen from the available agents, which included colistin, aminoglycosides, and BL (used in IB or CI). Most of the patients were managed with antibiotic combinations including BL in accordance with our protocol. The combination of BL and ciprofloxacin was used in susceptible *P. aeruginosa* during the first two weeks, followed by ciprofloxacin in monotherapy until the end of treatment, and combination of colistin and BL in cases of quinolone-resistant GNB. The colistin dose generally started at two million IU (MIU) every eight hours; this value was adjusted to renal function in patients with chronic renal failure or treatment-induced renal impairment. Of the antipseudomonal BL,

the one with the lowest MIC value was chosen. Patients with quinolone-resistant GNB infections were treated with the selected intravenous antibiotic plan for six weeks.

BL in CI were administered to achieve target drug concentrations at or above the MIC, using the same intermittent total daily dose over 24 hours or by calculating individual dose regimens according to Mouton et al.'s proposed equation (Mouton and Vinks 1996). This formula considers that the required BL daily dose is directly related to the BL total body clearance (TBC) and the desired target concentration.

Equation to estimate individual BL dose regimes for CI:

- Daily dose (mg) = 24 (h) × TBC [(1)] (L/h) × C_{ss} [(2)] (mg/L) **(Equation 1)**

TBC: Total Body Clearance.
C_{ss}: The target steady-state concentration

[(1)] For ceftazidime TBC, which is cleared almost completely by glomerular filtration, its creatinine clearance was used (CL_{CR}, calculated using the Cockcroft-Gault formula) (Cockcroft and Gault 1976). For piperacillin and aztreonam, which have renal (glomerular filtration and active tubular secretion) and non-renal clearance, TBC values were used from respective CL_{CR} that were previously reported (Hayashi et al. 2010; Xu et al. 2017).

[(2)] The target steady-state concentration (C_{ss}) varied for each strain (C_{ss} = 3-4 TIMES x MIC), depending on the requirement of each strain (MIC breakpoint) and the expected number of times over the MIC (TIMES×MIC) to reach 3-4 times above the MIC.

The daily dose was calculated to reach the C_{ss}, without exceeding a potentially toxic drug concentration of 100 milligrams(mg)/L (Moriyama et al. 2009, 2010). When the calculated *theoretical daily dose* represented a significant reduction in comparison with the usual total daily dosage by intermittent boluses, a dose was administered that was considered more appropriate (called the *real dose*). This situation primarily occurred at the beginning of the study due to the researchers' lack of experience.

Equation for predicting clinical BL concentrations for a specific administered real dose:

- C_{pred} (mg/L) = Daily dose (mg/24h)/ TBC (L/h) **(Equation 2)**

C_{pred}: Predicted concentration

Our C_{pred} was correlated with the patients' observed concentrations (C_{obs}), measured by UPLC-MS/MS. The difference between C_{obs} and C_{pred} (Δ_{Conc}) was also calculated and correlated with different clinical variables.

3.4 Follow-up

After treatment, patients were clinically assessed in the outpatient clinic at months 1, 3, 6, and 12; after one year, patients were reviewed at the discretion of each researcher. Failure was

defined as: (i) death related to the infection; (ii) amputation of the affected limb; or (iii) persistence of the infection (i.e. signs/symptoms of infection and/or positive cultures) despite an appropriate initial therapy.

In the streptococcal study, several failure dynamics were studied. *Early failure* was when the failure occurred in the first 30 days after surgical debridement, *late failure* was when the failure occurred beyond the first 30 days after debridement in patients who were still under antimicrobial therapy, and *failure after therapy* was when failure occurred in patients who had finished the scheduled therapy.

4. Complementary tests

4.1 Microbiological process

a) Conventional tissue samples

All specimens (e.g. tissue samples, joint aspirates, and blood cultures) were processed in the microbiology laboratory of the Hospital Universitari de Bellvitge. In cases that received a prosthesis revision with suspected late chronic PJI, ≥5 periprosthetic tissue samples were sent and processed. Microorganisms that caused early acute PJI were isolated from needle joint punctures, blood cultures, or intraoperative samples. Cultures of tissue and joint-aspirate samples were cultured in 5% horse blood, chocolate, MacConkey agar plates, and thioglycolate medium with prolonged incubation (10 days) at 30-35ºC under aerobic and anaerobic conditions. Blood samples were processed using a Bactec 9240 (Becton-Dickinson Microbiology Systems) and the inoculated bottles were incubated for five days at 35ºC before being discharged.

b) Samples from sonication

At the time of revision surgery, the prosthetic components were removed and introduced separately into sterile air-tight containers in the operating theatre as follows: acetabular component plus polyethylene, femoral component plus femoral head, femoral component, or tibial component plus polyethylene. This process enabled the analysis of the relationship between the bone loosening of each component and the microbiological culture. Once in the Microbiology Laboratory, 150 mL of Luria-Bertani medium was added to the sterile container to cover the prosthetic material. Then, the container was introduced into an ultrasound bath (Branson 3510, Bransonic Danbury, USA) for five minutes at 40 Hz. Next, 100 µl of the sonicated fluid was inoculated in a blood-agar plate for a first colony count, and aliquots of 1

mL of this fluid were kept frozen at -80 ºC for further microbiological analyses. The container with the removed component and the remaining fluid was incubated overnight at 37ºC. A new blood agar plate and a thioglycolate medium were processed the next day and cultured for 48 hours. Finally, fluid from sonication was considered negative if there was no macroscopic bacterial growth. All the microbiological processes were performed in a laminar flow cabinet to ensure that manipulation was not a cause of contamination.

All microorganisms were identified by standard biochemical reactions or using the MALDI-TOF Biotyper® measurement system (Bruker, Billerica, MA, USA). Susceptibility was studied with commercial panels from the MicroScan automated system (Siemens Healthcare Diagnostics Ltd, West Sacramento, CA, USA) for Gram-negative bacteria, enterococci, and staphylococci (not coagulase-negative staphylococci) or using commercial panels Sensititre TM from the microdilution method (Thermo Scientific, TREK Diagnostic Systems) for the other Gram-positive bacteria, following CLSI recommendations (CLSI 2016). MIC values for each antibiotic that was administered were measured using an E-test® diffusion procedure (bioMérieux, Marcy-l'Étoile, France) on an agar plate. Criteria of susceptibility or resistance to the various antibiotics were established according to the current CLSI or EUCAST recommendations (CLSI 2016; EUCAST 2015).

4.2 Radiological evaluation

Radiological bone loosening was blindly evaluated by a senior orthopaedic surgeon. Results from acetabular and femoral bone lysis were interpreted according to the Paprosky classification (for hip arthroplasties) (Paprosky et al. 1994; Sporer and Paprosky 2003), and femoral and tibiae lysis were interpreted according to the Engh classification (for knee arthroplasties) (Engh and Ammeen 1998). Type I acetabular and femoral defects (Paprosky classification), and Type I tibia and femur bone lysis (Engh classification) were considered the minimal lysis for further analysis.

4.3 Sequential procedures for the development of the UHPLC-MS/MS method used for simultaneous measurement of multiple BL concentration in human plasma

- Define operating conditions and antibiotics

Plasma is considered the ideal biological sample for measuring BL concentrations. Nine BLs were selected including amoxicillin, ampicillin, cloxacillin, piperacillin, cefepime, ceftazidime, cefuroxime, aztreonam, and meropenem, in addition to two β-lactamase inhibitors (clavulanat

and tazobactam). For each antibiotic, the physicochemical properties of chemical structure, molar mass, polarity, solubility, and acid dissociation constant were defined to select the optimal chromatographic and mass spectrometric conditions.

- Preparation of plasma *calibration samples*, *plasma quality control samples*, and *internal standards*

Plasma calibration samples were prepared to correlate the mass spectrometer response with BL concentration (calibration curve). Plasma quality control samples were needed to ensure proper operation of the UHPLC-MS/MS and guarantee the reliability of the results. To compensate for errors related to matrix effects, the autosampler pipetting, and inconsistent values in the MS detector, labelled internal standards for each antibiotic were used.

Nine calibration and three quality control plasma samples (containing BL) were prepared using certified reference materials of pure BL (from European Pharmacopeia; European Directorate for the Quality of Medicines-Council of Europe, Strasburg, France) and a pool of drug-free human plasma. Calibrator values were 0.00, 0.50, 1.00, 5.00, 15.0, 45.0, 75.0, 125, and 175 mg/L, and the quality control values were 3.0, 30.0, and 120 mg/L. Furthermore, a working solution of internal standards was prepared using labelled internal standards of each BL from Toronto Research Chemicals (Ontario, Canada) and acetonitrile as solvent. The working solution value was 2.5 mg/L.

- Sample preparation

One hundred µL of either calibration, quality control, or plasma samples from patients were transferred to 1.50 mL-polypropylene microcentrifuge tubes and 300 µL of the internal standards working solution was added for protein precipitation. After vortexing for three minutes, the tubes were centrifuged for ten minutes. One hundred µL of the supernatant was transferred into a new 1.50 mL-polypropylene microcentrifuge tube containing 400 µL of 0.1% (v/v) formic acid in water. The tubes were vortexed for ten seconds and the full volume was transferred into specific screw-neck glass vials with silicon septa caps and placed in the autosampler for injection **(Figure 11**).

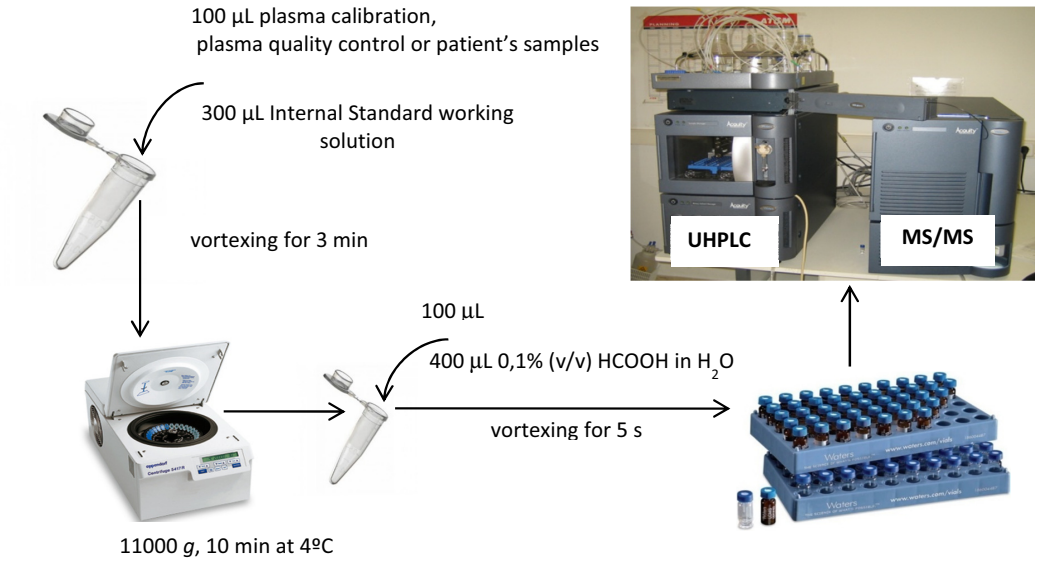


Figure 11. Sample extraction procedure scheme

- Instrumentation

Analyses were conducted using an Acquity® UPLC® integrated system (Waters, Milford, MA, USA) consisting of a thermostatic autosampler, a binary solvent delivery manager and a column over a thermostat compartment. Chromatographic separation was performed on an Acquity® UPLC® BEHTM C18 reverse-phase column, and an Acquity® UPLC® BEHTM C18 VanGuard Pre-column (Waters, Milford, MA, USA).

Detection was conducted using an Acquity® TQD® tandem-quadrupole mass spectrometer equipped with a Z-spray electrospray ionization source (Waters, Milford, MA, USA). The mass spectrometer operated in multiple reaction monitoring, and in positive and negative electrospray ionization modes. For each antibiotic, two transitions were followed: one was used for quantification (the *quantifier*) and the other was monitored for identification or confirmation (the *qualifier*).

- Validation of the method

The UHPLC/MS-MS method was validated according to the current European Medicines Agency (EMA) guideline (European Medicines Agency 2011). The developed procedure was validated in terms of selectivity, carry-over, lower limit of quantification, imprecision, bias, dilution integrity, recovery, matrix effect, and stability.

- Applicability of the method

The applicability of the UHPLC-MS/MS procedure was evaluated by processing different plasma samples collected from patients treated with BL in continuous or extended infusion. Blood samples were taken at least 24 hours after the start of therapy to ensure steady-state concentrations (Roberts et al. 2009). In prolonged therapies, monitoring samples were taken from some patients during the following days. Approximately three mL of blood were collected in a lithium-heparin tube (Vacuette, Kremsmünster, Austria) and immediately refrigerated at 2–8 ºC. Samples were then centrifuged at 2000 *g* for 10 minutes at (4 ± 1) ºC, aliquoted, and stored at (– 75 ± 3) ºC until analysis. The plasma concentrations of all patients were measured together (and double-checked) afterwards by UPLC-MS/MS

5. A dynamic *in vitro* biofilm model

- Bacterial strains.

Two ESBL-producing *Klebsiella pneumoniae* strains (A and B) were recovered from clinical isolates of patients admitted at the Hospital Universitari de Bellvitge. Among a pool of *ESBL*-producing *Klebsiella pneumoniae* strains, those that formed a greater biofilm according to crystal violet absorbance measurement (using microplate spectrophotometer) were selected.

They were then subcultured from a frozen stock on nutrient trypticase soy agar plates with 5% sheep blood (TSA; Becton Dickinson, Madrid) and preserved in cryotubs at -80ºC for subsequent use. Antibiotic susceptibility was determined by macrodilution and microdilution in Muller-Hinton broth (MHB; Becton Dickinson, Madrid). Both strains were carbapenem and colistin susceptible. The MICs (mg/L) of meropenem were 0.06 from strain A and 0.03 from strain B, and the MICs (mg/L) of colistin were 0.12 in both strains. The MBCs of meropenem from strains A and B were 1 mg/L and 2mg/L respectively; and the MBECs of meropenem were 512 µg/mL for both strains.

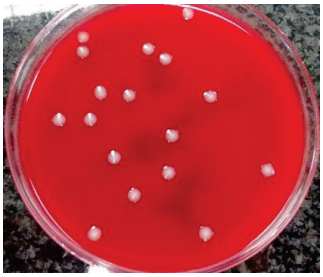


Figure 12. *Klebsiella pneumoniae* (strain A) on a nutrient Trypticase soy agar plate

- Model system design

A CDC biofilm reactor (CBR) from BioSurface Technologies Corp. (USA) was used to conduct this project. This system may operate under batch or continuous-flow configurations, and it provides a surface that can be removed and examined once it is colonized to assess biofilm formation. The model must be standardize according to each specific microorganism to provide the best operating conditions for biofilm formation. After standardization, the model is designed to test the effect of antimicrobial regimens on the biofilm.

- Components of the CBR

The CBR consists of a one-litre glass vessel with an effluent spout at 400 mL (for a final volume of 350 mL. Continuous mixing of the reactor fluid is provided by a baffled stir bar that is magnetically driven. In addition, a polyethylene top supports eight independent rods, and each road houses three removal coupons made of Teflon (biofilm growth surfaces of 1.27 cm in diameter) for a total of 24 sampling opportunities. The CBR operates as a continuous flow stirred tank reactor, meaning nutrients are continuously pumped into the reactor at the same rate that they flow out of the reactor. Autoclavable polycarbonate carboys of 10 or 20 L (Thermo Scientific-Nalgene, ref 029105-029106) were used to store the medium that was pumped into the reactor, and the same size containers were used for effluent waste. Carboy tops are equipped with three barbed fittings to accommodate tubing for nutrients, tubing for the injection of antibiotics when administered in continuous infusion, and tubing for holding an air filter. A peristaltic pump (Masterflex, Fisher Scientific SL) drives the medium into the reactor during the continuous flow-phase at a specific rate. A hot magnetic stir plate (VWR, ref 444-0629) that can operate at 100-400 rpm and provide temperatures from 20-80ºC is required to hold the CBR.

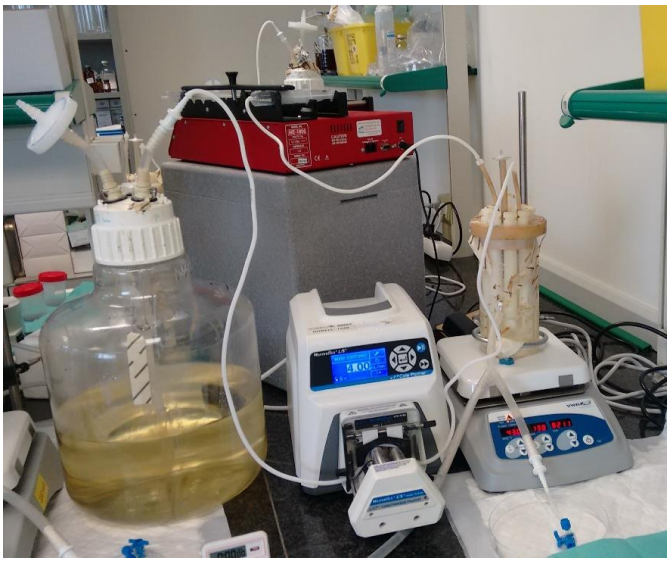


Figure 13. CBR in operation (with *K.pneumoniae*), during the therapeutic phase with meropenem

- Operating procedure

Experiments to standardize a method for growing *klebsiella pneumoniae* biofilms in a CBR were performed. Scanning electron microscopy (SEM) was conducted to assess biofilm formation, and the microbiological response to antimicrobial agents was evaluated. This protocol was based on previously published methods (Goeres et al. 2005; Lora-Tamayo et al. 2014; McLeod and Sandvik 2010; Parra-Ruiz et al. 2010)

Prior to each experiment, isolates from strain A or B were subcultured onto TSA plates incubated at 37ºC for 24 h. Several colonies were then incubated for two hours in 10 mL of tryptic soy broth (TSB) until the suspension became turbid (considering at 1×10^{8} CFU). A seven-mL aliquot of this bacterial suspension was mixed into 343 mL of 100% TSB (total 350 mL) and inoculated into the glass vessel of the model (inoculum concentration $1\times10^{7\text{-}8}$ cfu/mL).

A biofilm conditioning phase was then initiated to grow *K. pneumoniae* biofilm, consisting of a static phase followed by a dynamic phase with continuous flow. The static phase included 24 hours of incubation at 37ºC, according previous work (Goeres et al. 2005; Lora-Tamayo et al. 2014; McLeod and Sandvik 2010; Parra-Ruiz et al. 2010) and the dynamic phase was established under different conditions as described below. When the biofilm conditioning phase was completed (referred to as time 0), antimicrobial agents were injected. Evaluated regimens were colistin (colistin sulphate, C4461 obtained from Sigma-Aldrich, USA) and meropenem (meropenem trihydrate, M2574 obtained from Sigma-Aldrich, USA), alone or in

combination. Colistin was simulated as a continuous infusion at 3.5 mg/L. This was achieved by an initial bolus (1,23 mg) administration of colistin to the model to achieve the desired concentration and by spiking the medium in the carboy with colistin to achieve the same concentration (Lora-Tamayo et al. 2014). For meropenem regimens, a bolus dose was injected into the model every eight hours to achieve the desired steady-state peak concentration (C_{max}) of 90mg/L. The flow rate to the glass reactor vessel (4mL/min) was chosen to simulate a meropenem elimination half-life ($t_{1/2}$) of one hour in patients.

- Culture media and operating conditions

Based on existing theory and previous publications, different operating conditions were considered for growing *Klebsiella pneumoniae* biofilm during the biofilm conditioning phase. 'Fixed conditions' refers to conditions that are well established and globally accepted for the majority of microorganisms in CBR, and 'tested conditions' refers to conditions that are not globally defined and should be modified according to each specific microorganism to improve the capability of forming biofilm.

- *Fixed conditions*: The static phase was performed over 24 hours at 35-37ºC, with TSB at 100% that was mixed and shear generated at 130 rpm. The residence time (time for one reactor sized volume of liquid to flow through the reactor) for *K. pneumoniae* from 0 to 6 hours was calculated based on *bacteria generation time* (see below) and was 26 min for strain A and 25 min for strain B. Thus, flow rate during the dynamic phase was established at 13.5 mL/min to eliminate the growing planktonic bacteria.

* The *generation time* (g) was calculated according to the following equation:

$g = \text{Ln } 2/\mu$ (where μ is the growth rate)

$\mu = \text{Ln } N - \text{Ln } N_0 / t - t_0$ (where N is the number of bacteria at time t, and N_0 is the number of bacteria at time t_0)

- *Tested conditions*The nutrient feed during the dynamic phase was specified and adapted for each bacteria. Different nutrient conditions have been used in previous work, including very restrictive medium (1%) (Goeres et al. 2005; Lora-Tamayo et al. 2014), restrictive medium (10%) (McLeod and Sandvik 2010; Williams and Bloebaum 2010), and not restrictive medium (Parra-Ruiz et al. 2010). To standardize CBR for growing *K. pneumoniae strains,* restrictive medium conditions (TSB 20%) and not restrictive medium conditions (TSB 100%) were tested during the dynamic phase. The duration of the dynamic phase was tested at 24 h and at 72 h.

However, the latter could not be conducted at optimal residence time due to the large amount of medium required at this rapid rate.

According to fixed and tested conditions, three experiments were evaluated with ESBL-*Klebsiella pneumoniae* strain A. Static phase conditions were maintained for all experiments (defined before) and dynamic conditions are defined below.

EXPERIMENT	STATIC PHASE	DYNAMIC PHASE	
	Fixed conditions	Fixed conditions	Tested conditions
1	24 h 35-37ºC 130 rpm TSB 100%	35-37ºC 130 rpm Flow rate: 13.5 mL/min	72h, TSB 20% *
2			24h, TSB 100%
3			24h, TSB 20%

* due to the large amount of medium required for 72 hours, flow rate was established at 13.5 mL/min over six hours and at 4 mL/min for 66 hours.

- Assessing biofilm formation

To evaluate the presence and the structure of biofilm, scanning electron microscopy (SEM) was performed under different operation conditions. One coupon was recovered at time 0 and another at the end of treatment. After removal, the coupon was washed for one minute in cacodylate buffer (0.1 M, ph 7.4) to remove nonadherent cells, and then fixed in a solution of 2.5% glutaraldehyde in cacodylate buffer for 24 h. Coupons were then submerged in cacodylate buffer and sent at 4ºC to scientific and technical services (IRB, Lleida). First coupons were washed to remove the excess fixer with the same buffer, and then they were postfixed in osminum tetroxide-potassium ferrocyanide. After they were washed in MilliQ water, coupons were dehydrated in a graded ethanol series, mounted on a support, and coated with a film of amorphous carbon under the standard conditions. The coupons were imaged using a Zeiss DSM-940A SEM.

Moreover, manual enumeration of viable cells that were suspended in the CBR medium and adhered to the coupons was performed. Samples were removed (one medium and three coupons) after the static phase and after the dynamic phase (time 0), and were serially diluted and plated on TSA (24h incubation at 37ºC). Cfu/mL and $\log_{10}$ cfu/mL from coupon samples were quantified and the different tested conditions were compared.

- Pharmacokinetics analysis

Samples (200 µL) collected in duplicate from the model were placed in 1.5 mL microcentrifuge tubes and immediately stored at -80ºC. Concentrations of meropenem were measured by UHPLC-MS/MS at the Hospital Universitari de Bellvitge.

- Pharmacodynamics analysis

Samples extraction:

One medium sample and three coupons were aseptically removed at 0, 6, 24, 30, 48, and 54 h (in both experiments) and 72 h (in experiment 1). Each coupon was washed twice in sterile phosphate buffered saline (PBS) for three minutes to remove the excess planktonic cells. Then, each coupon was placed in a sterile tube containing 10 mL of PBS. Biofilm bacteria were recovered by three alternating one-minute vortexing cycles and double sonication at 43 kHz (Branson 5510).

Evaluation of microbiological response and the emergence of antibiotic resistance:

To grow an enumerate medium and coupons of viable cells, the respective samples were serially diluted with sterile saline and 100 µLs were plated onto TSA. Colonies were manually counted after 24 hours of incubation at 37ºC. Microbiological responses of monotherapy or combination regimens were examined using the log change method by comparing the change in $\log_{10}$ cfu/mL from 0 (cfu_0) h to time t (6, 24, 30, 48, 54, ±72 h; cfu_t) as shown: log change = $\log_{10}$ (cfu_t) – $\log_{10}$ (cfu_0). Treatments were considered bactericidal (99.9% kill) when they led to a ≥3 $\log_{10}$cfu/mL reduction compared to the corresponding counts at time 0. Monotherapy or combination regimens causing a reduction of ≥1 $\log_{10}$ cfu/mL at a specified time were considered active. Synergy was defined as ≥2 $\log_{10}$ cfu/mL killing for the combination relative to the most active corresponding monotherapy at a specified time, and additivity was defined as 1 to < 2 $\log_{10}$ cfu/mL greater killing for the combination

Additional plates with Muller-Hinton agar containing antibiotic (colistin or meropenem) were prepared to evaluate any change in the MIC during the treatment. For each antibiotic, two concentrations were tested: the MIC point of the strain and the standard EUCAST breakpoint (EUCAST 2015). Susceptibility testing of colonies was performed from 6h to 72h, both in the medium and in the coupons. If subpopulations grew on these plates, an Etest was performed to quantify the MIC. Resistance to colistin and meropenem in *K.pneumoniae* was defined as MIC > 2mg/L.

6. Statistical analysis

Data were analysed using the SPSS program (version 20.0, Chicago, IL). Continuous variables were preferably expressed as the median and interquartile range (IQR), and were compared by means of the *Mann-Whitney U* test or the *Kruskall-Wallis* test as appropriate. Categorical variables were expressed as counts and valid percentages, and were compared with the χ^2 test or Fisher's exact test as appropriate. Furthermore, changing trends in categorical parameters were evaluated with the *Mantel-Haenszel* χ^2 test for trends. Predictor parameters of failure were analysed by logistic regression. In addition, Kaplan–Meier curves and the log-rank test were used to compare the cumulative likelihood of failure between patients. *Spearman's* rank correlation coefficient was also calculated to correlate continuous variables. Univariate and multivariate linear regression analyses were performed to evaluate the relationships between continuous variables and statistical significance was defined as a two-tailed *p* value ≤0.05.

7. Funding and grants

The following grants and funding opportunities were received during the research process:

- A post-residence grant from Bellvitge Biomedical Research Institute (IDIBELL) at the Hospital Universitari de Bellvitge (2012-2014)
- A travel grant from the Universitat de Barcelona (2014)
- A contract for the intensification of research activity in the NHS (2015, 2017)

In addition, some of the studies in this thesis were conducted due to the:

- **FIS PI10/01573:** *Estudio para determinar la presencia de microorganismos en la superficie de prótesis articulares por un aflojamiento aséptico.* Lead researcher: Oscar Murillo from Hospital Universitari de Bellvitge. Multicentric study.

- **FIS PI14/00511:** *Alternativas terapéuticas frente a la infección in vitro de cuerpo extraño producida por bacilos gram-negativos multiresistentes: estudios farmacodinámicos en monoterapia y en combinación*. Lead researcher: Oscar Murillo from Hospital Universitari de Bellvitge.

RESULTS

A. ON THE MANAGEMENT OF ORTHOPAEDIC DEVICE-RELATED INFECTIONS

A.1. Diagnostic aspects of PJI

Aim 1: to analyse the microbiological and clinical findings in patients with suspected prosthetic joint aseptic loosening, and to compare them to patients with chronic PJI

Article 1. *Clinical and microbiological findings in prosthetic joint replacement due to aseptic loosening*. **A. Ribera**, L. Morata, J. Moranas, JL. Agulló, JC. Martínez, Y. López, D. García, X. Cabo, S. García-Ramiro, A. Soriano, O. Murillo. Journal of Infection 2014; 69(3):235-43. doi: 10.1016/j.jinf.2014.05.003

Communication 1. *Microbiological findings in prosthetic hip or knee replacement due to aseptic loosening* **A. Ribera**, L. Morata, J. Moranas, A. Coscujuela, JC. Martinez, Y. López, D. García, S. Garcia-Ramiro, A. Soriano, O. Murillo. 23rd ECCMID. Berlin, Germany, 2013. (P2035)

Communication 2. *Análisis de las características clínicas y microbiológicas en el recambio protésico articular con sospecha de aflojamiento aséptico*. **A. Ribera**, L. Morata, J. Moranas, A. Coscujuela, JC. Martínez, Y. López, D. García, S. García-Ramiro, A. Soriano, O. Murillo. XVIII Congress SEIMC. Valencia, Spain, 2014. (Comunicación 298)

The first cause of implant failure is aseptic loosening. However, it is not uncommon that one or more peri-implant operative cultures are positive after a prosthesis revision of suspected aseptic loosening. The interpretation of these cultures as clinical silent infections or as contamination of the surgery and laboratory process still remains a challenge in some instances (e.g. cases with a single positive culture). To better understand these unexpected results, the microbiological and clinical findings of patients with suspected prosthetic joint aseptic loosening were analysed.

1.1 Patients' baseline characteristics, clinical findings and features of the removed prostheses in a case series of patients with suspected prosthetic joint aseptic loosening, in comparison with a control group with diagnosis of late chronic PJI

A total of 89 patients with presumed aseptic loosening (AL) were included in the study: 60 (67%) had undergone hip replacement, and 29 (33%) knee replacement. The general characteristics of the presumed AL (group 1-4) and the control group with late chronic

prosthetic joint infection (LCPJI, n= 23) were similar, except in terms of the prosthesis location **(Table 1.1)**.

1.2 Microbiological findings within the established groups

The microbiology results of all cases included in the study are shown in **Table 1.2** According to standard and sonication cultures, AL were divided into Group 1 ("Definitive PJI"): those with ≥2 concordant positive tissue samples, disregarding the results in the sonication culture, which were treated with long-term antibiotics (n = 12); Group 2: cases with a single positive intraoperative tissue culture plus a concordant positive sonication culture with the same microorganism (defined as same species name and susceptibility pattern), which were treated with long term antibiotics or were left untreated according to the clinician criteria (n = 10); Group 3: cases with one positive culture (standard or sonication) or a non-concordant microorganism either from the tissue sample or the sonication fluid, which were treated with antibiotics or were left untreated according to the clinician criteria (n = 38); and Group 4: patients with all cultures negative (n = 29). A total of 139 prosthetic components, from 89 patients, were sonicated and 59 (42%) were positive.

The concordance of the microbiological results from tissue samples and sonication is also shown in **Table 1.2**. In Group 1, there were 9 (75%) cases in which the sonicated fluid of prosthetic components was positive, and the same microorganism was identified in the tissue samples. Three cases had an additional single positive tissue culture that was discordant with the other samples (3/12 = 25%). In Group 2, concordant results were due by definition. Additionally, discordant results were observed in the sonicated fluid in two cases (2/10 = 20%) and in one tissue culture (1/10 = 10%). In contrast, discordance was established in Group 3 by definition. We identified 12 cases with a positive tissue sample (Group 3a; 12/38 = 32%) and 26 with a positive sonicated fluid sample (Group 3b). In the first subgroup, 2 patients had one positive sonicated fluid sample that was not concordant with the tissue isolation. In 7 patients from Group 3b, the two sonicated components were positive and the same bacteria were identified in 5 cases.

Table 1.1 Patients' baseline characteristics and features of the prostheses that were removed

	LCPJI (n=23)	Group 1 (n=12, 13%)	Group 2 (n=10, 11%)	Group 3 (n=38, 43%)	Group 4 (n=29, 33%)	All (group 1-4) (n=89, 100%)	
Age median (IQR)	72 (66-79)	74 (65-82)	76 (67-82)	77 (66-82)	73 (64-79)	74 (65-81)	
Sex (female)	14 (60.9%)	7 (58.3%)	4 (40%)	18 (47.4%)	19 (65.5%)	50 (56.2%)	
Underlying diseases							
Cardiovascular diseases[1]	9 (39%)	5 (41.7%)	8 (80%)	29 (76.3%)	18 (62.1%)	60 (67.4%)	
Diabetes mellitus	5 (22.7%)	1(8.3%)	1 (10%)	8 (21.1%)	4 (13.8%)	14 (15.7%)	
Cirrhosis	2 (9.1%)	1(8.3%)	0	1 (2.6%)	0	2 (2.2%)	
COPD[2]	1 (4.3%)	2 (16.7%)	2 (20%)	4 (10.5%)	3 (10.3%)	11 (12.4%)	
Other[3]	-	2 (16.7%)	1 (10%)	2 (5.2%)	4 (13.8%)	9 (10.1%)	
Localization							
Hip	8 (35%)	10 (83.3%)	7 (70%)	25 (65.8%)	18 (64.1%)	60 (67.4%)	
Knee	15 (65%)	2 (16.7%)	3 (30%)	13 (34.2%)	11 (37.9%)	29 (32.6%)	
Type of prosthesis							
Primary	14 (63.6%)	10 (83.3%)	9 (90%)	28 (73.7%)	23 (79.3%)	70 (78.7%)	
Revision	8 (36.4%)	2 (16.7%)	1 (10%)	10 (26.3%)	6 (20.7%)	19 (21.3%)	
Cemented		7 (58.3%)	4 (40%)	25 (78.1%)	18 (75%)	54 (60.7%)	
Pain for > 1 year	-	6 (50%)	4 (40%)	18 (51.4%)	11 (44%)	39 (47%)	
Prosthesis age[4] (median months, IQR)	21 (14-45)	46 (31-131)	65 (29-208)	63 (46-153)	81 (40-167)	65 (38-155)	p<0.001
Num. of components exchanged	46	18	16	59	46		
Bone lysis by component							
Minimal lysis degree (Type I, T1 and F1)	16 (35%)	11 (61%)	6 (38%)	22 (37%)	21 (47%)		

Footnote table 1.1 [1] Cardiovascular diseases include: hypertension and ischemic heart diseases. [2]**COPD**: chronic obstructive pulmonary diseases. [3]**Other**: HIV, dementia, rheumatoid arthritis, neoplasia. [4]**Prosthesis age**: time from implantation to revision arthroplasty. The median prosthesis age between groups was statistically significant (p<0.001, Kruskal-Wallis). The median prosthesis age median between (Group LCPJI + Group 1) and (Group 2-4) was statistically significant (p<0.001, U-Mann-Whitney).

Table 1.2. Microbiological findings: conventional tissue cultures and sonicated fluid cultures

PATIENTS [a]	**Group 1 (n = 12)**	**Group 2 (n = 10)**	**Group 3 (n = 38)**	**Group 4 (n = 29)**	**All (n = 89)**	
Conventional tissue samples cultures						
Positive [b]	12 (100%)	10 (100%)	12 (32%)	0	34 (38%)	
Microbiology num. cases, bacteria (num. positive samples, in group 1)	6 **CoNS** (2≥) 1 ***Corynebacterium spp*** (2≥) 1 ***P. aeruginosa*** (2≥) 1 **CoNS** (2≥)+ *B.cereus* (1) 1 **CoNS** (2≥) + *Corynebacterium spp* (1) 1 **CoNS** (2)+***S.viridans*** (2) 1 ***Corynebacterium spp*** (2≥) + *E.faecalis* (1)	8 **CoNS** 1 ***Corynebacterium spp*** 1 **CoNS +** *E.faecalis*	5 CoNS 3 *Corynebacterium spp* 1 *Anaerobic* 1 *M.luteus* 1 CoNS + *P.aeruginosa* 1 CoNS + *S.viridans*	-		
Discordant positive [c]	3 (25%)	1 (10%)	12 (32%)	0	16 (18%)	
Sonication fluid cultures						
Positive [b]	9 (75%)	10 (100%)	28 (74%)	0	47	
Discordant positive [c]	0	2 (20%)	28 (74%)	0		*p*=0.005
PROSTHETIC COMPONENTS [d]	**18**	**16**	**59**	**46**	**139**	
Sonication fluid cultures						
Positive [e]	11 (61%)	13 (81%)	35 (59%)	0	59 (42%)	
Discordant with conventional tissue samples [f]	0	2 (12%)	35 (59%)	0		
Microbiology	8 **CoNS** 1 ***Corynebacterium spp*** 2 ***P. aeruginosa***	11 **CoNS** 1 ***Corynebacterium spp*** 1 NI	26 **CoNS** 2 ***Corynebacterium spp*** 2 ***P. aeruginosa*** 2 ***Bacillus*** 1 ***M. luteus*** 1 ***A. viridans*** 1 Not identified			

Footnote table 1.2

[a] Microbiological findings are analysed by patient (n=89), detailing whether the results correspond to tissue or sonicated samples.

[b] Positive: includes patients with at least one positive culture.

[c] Discordant positive: includes patients with single positive cultures that are not-concordant with the microorganism that caused the infection (in Group 1 and Group 2) or when single positive cultures were isolated (Group 3). Differences between Group 1-Group 2 and Group 3 (p=0.005).

[d] Microbiological findings are analysed by prosthetic components (n=139).

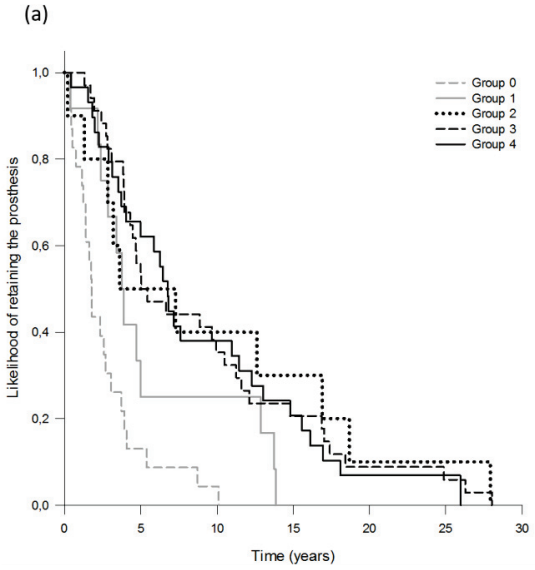
[e] Positive: includes components with positive sonicated fluid culture.

[f] Discordant with conventional tissue samples: number of components with positive cultures that are not-concordant with the correspondent tissue samples.

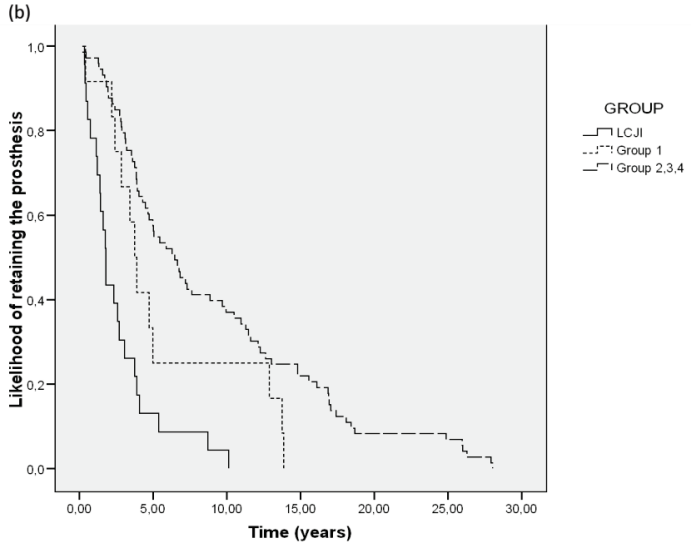
1.3 Length of prostheses according to microbiological findings

The median time from implantation to revision arthroplasty (prosthesis age) for LCPJI, and Groups 1, 2, 3 and 4 was 21, 46, 65, 63 and 81 months, respectively (P < 0.001; Table 1), whereas the percentage of patients with prolonged pain (>1-year) was similar between groups. The survival curve is shown in **Figure 1.1** We observed a different dynamic trend in prosthesis failure evolution between LCPJI, Group 1 and the last 3 groups (p < 0.001; see **Figure 1.1**).

Figure 1.1



1.1.a Group 0 (LCPJI): patients with late chronic infection by *S.epidermidis.* Time (years) = prosthesis age (time from implantation to revision arthroplasty).



1.1.b Dynamic trend in prosthesis failure between LCPJI, Group 1 and Groups (2+3+4) was statistically significant (*p*<0,001, Log Rank).

Revision arthroplasties within the first 2 years were mainly performed among the cohort of LCPJI (57%), rather than among patients with presumed AL (less than 20% in any group, and no differences between them). We found significant differences between groups in the percentage of prostheses exchanged 4 years after implantation: this intervention was performed in 83% cases within the cohort LCPJI, and in 58%, 50%, 32% and 31% in Groups 1, 2, 3 and 4, respectively (MH Test for trend $p < 0.001$).

1.4 Radiological evaluation of prosthetic loosening degree

Among all cases with presumed AL, bone lysis was notably higher in patients with older prostheses (Groups 2, 3 and 4) than in patients from Group 1, with subclinical pre-surgical infection, lower prosthesis age, and mostly a minimal degree of lysis. By contrast, patients with pre-surgical signs of prosthesis infection (LCPJI) showed higher bone lysis, even though they had the lowest prosthesis age (**Table 1**).

1.5 Follow-up after prosthetic revision

The follow-up after revision arthroplasty was recorded for cases in Group 2, due to a specific clinical interest in the evaluation of these cases with 1 single positive tissue sample and a concordant positive SF. None of these patients were treated with long-term antibiotics but only with revision surgery. After a median of 16 months (IQR 6-24) of follow-up, there was one case who presented a new prosthesis infection caused by *Staphylococcus aureus* (a different microorganism than the one isolated in the implant revision) 5 months after the implant revision, and the remaining cases were free of infection.

Several patients with suspected aseptic loosening were misdiagnosed PJI that presented with particular clinical characteristics; other patients presented a single positive intraoperative sample that is concordant with the sonication sample – which is unlikely to represent contamination- and many others patients presented a single positive sample (from sonication), suggesting the presence of microorganisms on the implant surface. These microbiological findings correlate with clinical features (e.g. prosthesis age).

A.2. Surgical management of PJI

Aim 2: to evaluate the risk of re-infection following one-stage and two-stage surgical revision with hip PJI

Article 2. *Risk of re-infection following one-stage and two-stage surgical revision of peri-prosthetic joint infection of the hip: A pooled individual participant data analysis of 44 observational cohort studies.* The global inform collaboration leaded by Sk. Kunutsor is listed at the end of the paper, and includes **A. Ribera** et al. Submitted for publication.

One-stage and two-stage revision strategies are two options for treating late chronic PJI of the hip. Although the two-stage strategy has traditionally been considered the gold standard for late chronic PJI, there is uncertainty regarding which is the best option. Therefore, these two procedures were analysed within pooled individual participant data, to compare the risk of re-infection between the two strategies.

2.1 Description of the studies included in an individual pooled data analysis to compare the risk of re-infection after one-stage or two stage surgical revision within PJI

38 articles consisting of 44 unique studies and comprising of 1,856 participants contributed to pooled analysis. Overall, there were 13 one-stage (884 patients) and 31 two-stage (972 patients) studies based in 13 countries (from North and South America, Europe, and Asia).

2.2 Description of the cases and the characteristics of the infection before the revision procedure. Management and follow-up.

Summary baseline and follow-up characteristics of the 1,856 patients with PJI of the hip treated by one-stage or two-stage revision that contributed to the analyses are shown in **Table 2.1 (a and b)**.

The one stage revision group had older patients on average (66.8 vs 63.4 y) and had a higher proportion of patients with previous PJI (39.2 vs 7.5 %) and previous hip surgery -other than the index surgery- (92.5 vs 30.7 %) compared with their two-stage counterparts. In addition, the one-stage revision group had higher median levels of blood circulating C-reactive protein and a higher proportion of patients presenting with an abscess, sinus, draining wound, or fistula before revision (31.3 vs 23.6 %). (**Table 2.1.a**)

Table 2.1.a Summary of socio-demographic features and infection characteristics prior the revision procedure

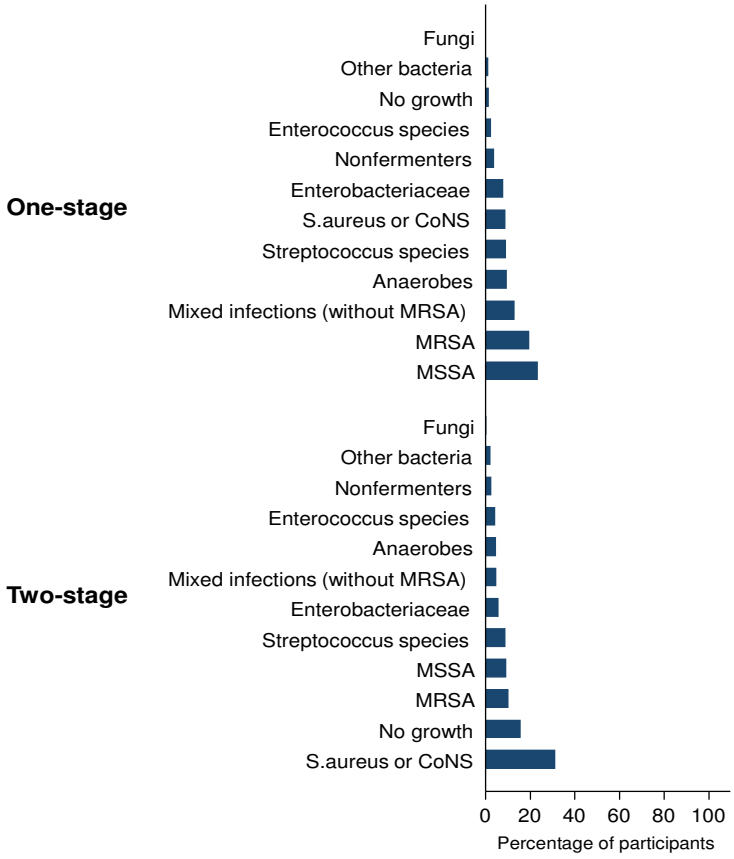
	Overall n (%)	**One-stage revision n (%)**	**Two-stage revision n (%)**
Total number of participants	1856	884	972
Socio-demographic characteristics			
Gender	N=1743	N=864	N=879
Males	926 (53.1)	458 (53.0)	468 (53.2)
Age at baseline (years), mean (SD)	65.1 (13.0)	66.8 (12.4)	63.4 (13.3)
Physical measurements			
Body mass index in kg/m^2, mean (SD)	27.6 (6.6)	27.5 (5.9)	27.8 (7.0)
Medical and surgical history			
Comorbidity Index	N=785	N=282	N=503
No previously recorded disease categories	256 (32.6)	45 (16.0)	211 (42.0)
One or two disease categories	433 (55.2)	212 (75.2)	221 (43.9)
More than two disease categories	96 (12.2)	25 (8.9)	71 (14.1)
History of previous PJI	N=321	N=120	N=201
Yes	62 (19.3)	47 (39.2)	15 (7.5)
Previous hip surgery	N=1,060	N=809	N=251
Yes	825 (77.8)	748 (92.5)	77 (30.7)
Hip involved in index implantation	N=1233	N=632	N=601
Right	676 (54.8)	348 (55.1)	328 (54.6)
Left	557 (45.2)	284 (44.9)	273 (45.4)
Baseline data before revision			
C-reactive protein (mg/l), median (IQR)	18.9 (6.1-54·0)	22.5 (9.0-56.5)	17.1 (5.8-50.5)
Neutrophils /µl, median (IQR)	4520 (2800-6000)	4800 (4100-6000)	3835 (99-5980)
Harris Hip Score, median (IQR)	55.0 (48.0-60.0)	55.5 (43.5-63.5)	55.0 (48.0-60.0)
Characteristics of infection before revision procedure			
Previous procedure performed to treat infection	N=541	N=277	N=264
Yes	137 (25.3)	70 (25.3)	67 (25.4)
Presence of abscess, sinus, draining wound, or fistula at presentation	N=588	N=278	N=310
Yes	160 (27.2)	87 (31.3)	73 (23.6)
Time from index implantation to infection (weeks), median (IQR)	102.7 (36.6-299.2)	154.3 (51.4-350.1)	102·6 (32.6-268.5)
Time from infection to revision procedure (weeks), median (IQR)	20.6 (8·4-51.4)	30·0 (10.2-94.2)	12·9 (6.4-34.3)

Footnote Table 2.1.a: N= total number of participants with this variable described.

The most common indication for the index implantation for both groups was osteoarthritis. This was followed by fractures in the one-stage group and osteonecrosis in the two-stage group.

The most common cultured microorganism responsible for a PJI after the index operation in the one-stage group was methicillin-sensitive *Staphylococcus aureus* (MSSA); whereas it was *S.aureus* or coagulase-negative staphylococci (CoNS) in the two-stage group (**Figure 2.1**).

Figure 2.1 Type of infecting microorganisms after index implantation by type of revision strategy



Footnote Figure 2.1 MRSA: methicillin-resistant *Staphylococcus aureus*, MSSA methicillin-sensitive *Staphylococcus aureus*

The median times to onset of infection from index implantation and from infection to revision surgery were longer in one-stage revision strategy patients compared with two-stage patients. The median duration of antibiotic therapy in between stages for the two-stage revision group was about two times longer than that after revision therapy in the one-stage group. Thus

patients treated with two-stage revision received a longer duration of antibiotics over the entire course of treatment (median, 18.3 weeks) compared with those treated with one-stage (median, 12.6 weeks) **(Table 2.1.b)**

The median (interquartile range) follow up time was 4.2 (2.0-8.1) years in the one-stage group and 3.3 (2.0-5.9) years in the two-stage group. During follow-up, 88 (10.0%) participants experienced a re-infection in the one stage group compared with 134 (13.8%) in the two-stage group. **(Table 2.1.b)**

Table 2.1.b Characteristics of revision procedure, management and follow-up

	Overall n (%)	**One-stage revision n (%)**	**Two-stage revision n (%)**
Total number of participants	1856	884	972
Characteristics of revision procedure and management			
Type of re-implantation	N=122	N=89	N=33
Cemented	91 (74.6)	65 (73.0)	26 (78.8)
Cementless	23 (18.9)	16 (18.0)	7 (21.2)
Hybrid	8 (6.6)	8 (9.0)	0 (0·0)
Antibiotics in cement	N=1092	N=758	N=334
Yes	750 (68.7)	584 (77.0)	166 (497)
Type of spacer	-		N=183
Unknown	-	-	1 (0.6)
Handmade	-	-	167 (91.3)
Commercial	-	-	15 (8.2)
Antibiotics in spacer	-	-	N=183
Yes	-		180 (98.4)
Systemic Antibiotic treatment			
Duration of antibiotics between stages (weeks), median (IQR)	-	-	24.0 (4.5-24.0)
Duration of antibiotic after revision (weeks), median (IQR)	12.1 (6.1-12.6)	12.6 (12.0-12.6)	1.3 (0.5-5.5)
Duration between stages (weeks), median (IQR)	-	-	14.5 (11.0-24.0)
Follow-up			
Duration of follow-up (years), median (IQR)	3·7 (2.0-6.9)	4·2 (2.0-8.1)	3.3 (2.0-5.9)
Harris Hip Score at follow up, median (IQR)	86.0 (73.0-93.0)	80.0 (52.0-90.0)	87.0 (78.0-95.0)
Number of re-infections	222	88	134

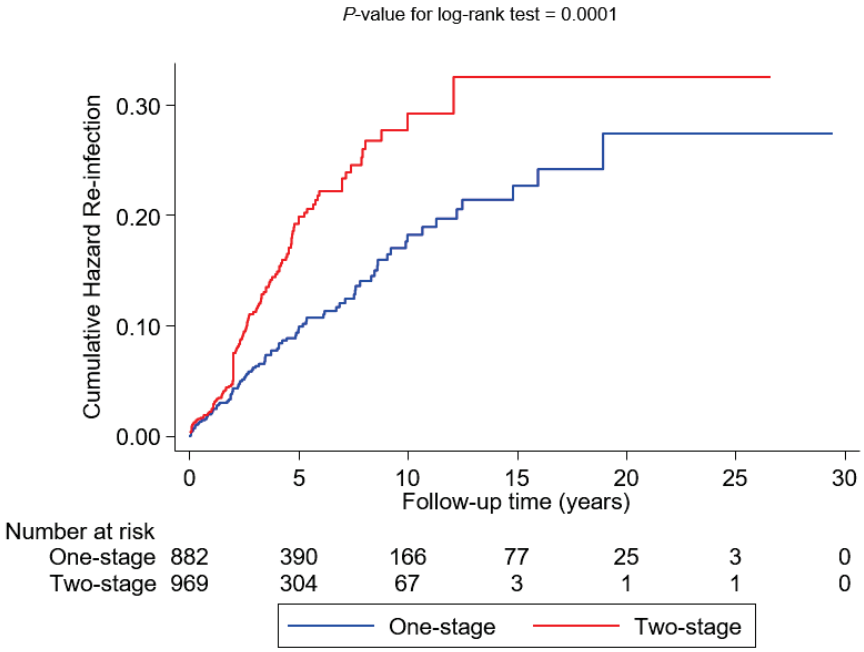
Footnote Table 1b: N= total number of participants with this variable described

2.3 Evaluation of the risk of re-infection according to the revision strategy

During a median (interquartile range) follow-up of 3.7 (2.0-6.9) years, 222 re-infections were recorded. Cumulative hazard curves demonstrated a greater risk of re-infection among two-

stage revision strategy participants compared with one-stage revision strategy participants (P = 0.0001 for log-rank test; **Figure 2.2**).

Figure 2.2 Cumulative hazard curves for re-infection by type of revision strategy



Re-infection rates per 1000 person-years of follow-up across revision strategies were 16.8 (95% CI: 13.6 to 20.7) and 32.3 (95% CI: 27.3 to 38.3) for the one-stage and two stage strategies, respectively.

Among 1,038 individuals (113 re-infections) with available survival data, comparing two- with one-stage revision, the age-adjusted Hazard ratios (HR) for re-infection was 1.69 (95% CI: 0.58 to 4.98; P=0.338). The corresponding HR remained consistent 1.70 (95% CI: 0.58 to 5.00; P=0.332) on adjusting for sex; and was attenuated to 1.33 (95% CI: 0.48 to 3.69; P=0.583) after further adjustment for previous hip surgery. The associations remained absent in analyses restricted to 439 individuals (41 re-infections) with available data on comorbidities and type of infecting organism. HRs did not vary importantly by levels or categories of pre-specified patient level characteristics (P for interaction > 0.10 for each).

The pooled available data suggest that the one-stage revision strategy may be as effective as the two-stage revision strategy when treating late chronic PJI in unselected patients.

B. ON THE ASSESSMENT OF ANTIMICROBIAL EFFICACY FOR THE TREATMENT OF ORTHOPAEDIC DEVICE-REALATED INFECTIONS

B.1 Infections by *Streptococcus spp*

Aim 3: to assess the efficacy of adding rifampicin to β-lactams for the treatment of streptococcal PJI managed with implant retention, and its impact on the prognosis

Article 3. *The Not-So-Good Prognosis of Streptococcal Periprosthetic Joint Infection Managed by Implant Retention: The Results of a Large Multicenter Study*. J. Lora-Tamayo, E. Senneville, **A. Ribera**, L. Bernard, M. Dupon, V. Zeller, HK. Li, C. Arvieux, M. Clauss, I. Uçkay, D. Vigante, T. Ferry, JA. Iribarren, TN. Peel, P. Sendi, NG. Miksić, D. Rodríguez-Pardo, MD. del Toro, M. Fernández-Sampedro, U. Dapunt, K. Huotari, JS. Davis, J. Palomino, D. Neut, BM. Clark, T. Gottlieb, R. Trebše, A. Soriano, A. Bahamonde, L. Guío, A. Rico, M. JC Salles, MJ. G Pais, N. Benito, M. Riera, L. Gómez, J. Esteban, JP. Horcajada, K. O'Connell, M. Ferrari, G. Skaliczki, R. San Juan, J. Cobo, M. Sánchez-Somolinos, A. Ramos, E. Giannitsioti, A. Jover-Sáenz, J. M Baraia-Etxaburu, JM. Barbero, P. FM Choong, N. Asseray, S. Ansart, G. Le Moal, W. Zimmerli, J. Ariza. Accepted in Clinical Infectious Diseases. doi: 10.1093/cid/cix227

Communication 3. *Streptococcal Prosthetic Joint Infection Managed with Implant Retention.* J. Lora-Tamayo, E. Senneville, **A. Ribera**, L. Bernard, V. Zeller, H. Li, P. Tattevin, M. Clauss, I. Uçkay, D. Vigante, T. Ferry, J. Ariza. 55th ICAAC. San Diego, California, 2015. (K-221)

Communication 4. *Infección estreptocócica de prótesis articular manejada con retención del implante: influencia del tratamiento con rifampicin.* J. Lora-Tamayo, **A. Ribera**, JA. Iribarren, M. Fernández, D. Rodríguez-Pardo5, MD. del Toro, J. Palomino, A. Soriano, L. Guío, A. Bahamonde, A. Rico, J. Corredoira, M. Riera, N. Benito, L. Gómez, J. Esteban, L. Sorlí, R. San-Juan, A. Ramos, A.Jover-Sáenz, JM. Baraia-Etxaburu, J.Ariza. XIX Congress SEIMC. Sevilla, Spain, 2015. (Comunicación 488)

Streptococci are a common cause of PJI, especially in hematogenous infections. Surgical management with debridement, antibiotics, and implant retention (DAIR) is thought to produce a positive prognosis in early acute PJI, but the optimal antimicrobial treatment is unknown. In terms of prognosis, some authors have suggested that streptococcal PJI may have a more favorable outcome than other etiologies, but others authors do not agree with this. As such, the clinical presentations and outcomes of a large cohort of patients with streptococcal

PJI that was managed by DAIR were analysed, focusing on the impact of adding rifampicin to β-lactams for the treatment of those infections.

3.1 Description of the selected case series with streptococcal PJI managed with DAIR

Overall, 922 cases of PJI were recorded, of which 92 (10.0%) were excluded for various reasons, leaving a cohort of 830 cases. We initially managed 462 (55.7%) by DAIR, and these cases were used as the focus of this analysis.

The median age was 72 years (IQR, 65–78 years), and 50% were men. The most frequent type of PJI was hematogenous (52%), which occurred more frequently in men, in patients with malignancy and in those with knee prostheses. Patients with hematogenous PJI more frequently presented with bacteremia and elevated temperature, along with higher leukocyte counts and C-reactive (CRP) protein levels (**Table 3.1**).

The most frequent species was *S. agalactiae* (159 cases [34.4%]) (**Table 3.2**). There were 63 (14%) polymicrobial infections which were typically postoperative (83%), presented less frequently with fever (51% vs 68%, p=0.007) and more frequently with a sinus tract (34% vs 10%, p<0.001), and had lower CRP levels (80 mg/L [IQR 41-150] vs 202 mg/L [IQR 110-291], p<0.001).

Baseline features, clinical presentation, and management were similar among the streptococcal species. Exceptions to this were the higher rate of patients with rheumatoid arthritis among episodes caused by *S. pyogenes*, and the higher rate of chronic lung disease and malignancy in PJI due to *S. pneumoniae*. Pneumococcal PJI was also more frequently hematogenous, occurred more frequently with knee prostheses, and presented with a higher leukocyte count. Penicillin minimum inhibitory concentration (MIC) was >0.125 mg/L in 24/425 cases (6%).

Table 3.1 Baseline features, clinical presentation, surgical management and outcome and comparative analysis of hematogenous and non-hematogenous cases

	All patients (n=462)	Non-hematogenous cases (n=220)	Hematogenous cases (n=242)	p
Baseline features				
Sex (men)	232 (50%)	121 (45%)	111 (54%)	0.050
Age (years)^	72 (65-78)	72 (64-78)	72 (65-78)	0.986
Diabetes	111 (24%)	50 (23%)	61 (25%)	0.533
Renal chronic disease	45 (10%)	20 (9%)	25 (10%)	0.654
Rheumatoid arthritis	37 (8%)	15 (7%)	22 (9%)	0.369
Immunosuppressive therapy	49 (11%)	22 (10%)	27 (11%)	0.687
Malignancy	29 (6%)	7 (3%)	22 (9%)	0.009
Liver cirrhosis	19 (4%)	9 (4%)	10 (4%)	0.982
Chronic lung disease	56 (12%)	27 (12%)	29 (12%)	0.924
Chronic heart disease	128 (28%)	54 (25%)	74 (31%)	0.148
Prosthesis location (knee)	273 (59%)	117 (53%)	156 (65%)	0.014
Revision prosthesis	114 (25%)	48 (22%)	66 (27%)	0.174
Clinical presentation and microbiological data				
Temperature >37^{o}C	300 (66%)	110 (51%)	190 (80%)	<0.001
Sinus tract	62 (14%)	46 (21%)	16 (7%)	<0.001
Leukocyte count (x10E9/L)^	12.0 (8.5-15.4)	11.0 (7.3-14.6)	13.0 (9.6-16.0)	0.001
C-reactive protein at diagnosis (mg/L)^	186 (85-283)	135 (55-230)	234 (130-305)	<0.001
Rx signs of infection	85 (18%)	41 (19%)	44 (18%)	0.900
Bacteremia	138 (31%)	35 (17%)	103 (45%)	<0.001
Penicillin MIC >0.125 mg/L §	24/425 (6%)	15 (8%)	9 (4%)	0.113
Polymicrobial infection	63 (14%)	52 (24%)	11 (5%)	<0.001
Surgical management				
Time to debridement (days)^$^{\phi}$	5 (2-13)	5 (2-16)	5 (2-12)	0.688
Exchange of removable components $^{\&}$	220/418 (53%)	100/200 (50%)	120/218 (55%)	0.302
Need for ≥2 debridements	42 (9%)	21 (10%)	21 (9%)	0.797
Outcome‡				
Overall failure	187/444 (42%)	92/210 (44%)	95/234 (41%)	0.494
Early failure‡	55/187 (29%)	25/92 (27%)	30/95 (32%)	0.509
Late failure‡	71/187 (38%)	34/92 (37%)	37/95 (39%)	0.779
Failure after therapy ‡	61/187 (33%)	33/92 (36%)	28/95 (30%)	0.351

Footnote Table 3.1 Data expressed as count and (percentage) except for ^continuous variables (median and interquartile range). MIC: minimal inhibitory concentration. $^{\phi}$Time from onset of symptoms to surgical debridement. $^{\&}$Data available in 418 cases. ‡444 patients evaluable for outcome, percentages given over the whole of failures.

Table 3.2 – Etiology of 462 episodes of streptococcal periprosthetic joint infection

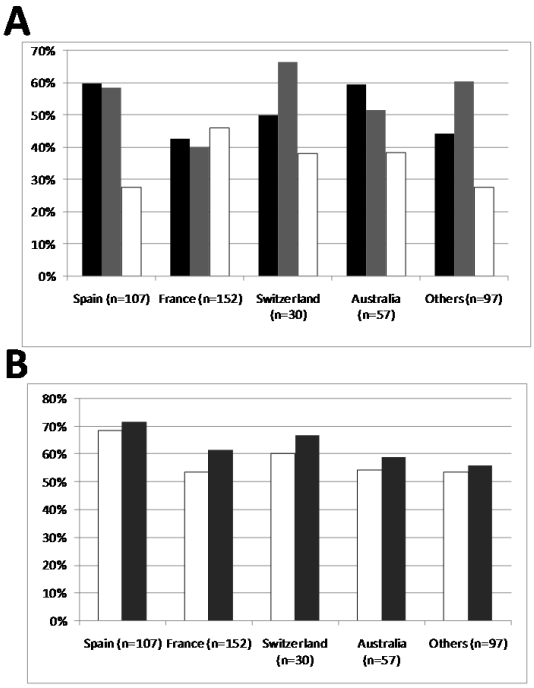
Streptococcus		
S. agalactiae		**159 (34.4%)**
S. pyogenes		**36 (7.8%)**
S. pneumoniae		**21 (4.5%)**
Other large-colony β-haemolytic streptococci		**121 (26.2%)**
S. dysagalactiae	49 (10.6%)	
Group G streptococci	40 (8.7%)	
Other β-haemolytic streptococci	28 (6.1%)	
S. equisimilis	4 (0.9%)	
S. anginosus group		**32 (6.9%)**
S. anginosus	17 (3.7%)	
S. constellatus	8 (1.7%)	
S. milleri	4 (0.9%)	
S. intermedius	3 (0.6%)	
Viridans group		**86 (18.6%)**
Unspecified viridans streptococci	25 (5.4%)	
S. mitis	25 (5.4%)	
S. oralis	17 (3.7%)	
S. sanguis	10 (2.2%)	
S. salivarius	4 (0.9%)	
S. gordonii	2 (0.4%)	
S. mutans	2 (0.4%)	
S. parasanguis	1 (0.2%)	
Other streptococci		**7 (1.5%)**
S. bovis	6 (1.3%)	
S. canis	1 (0.2%)	
Other microorganisms (polymicrobial episodes)		
Gram positive microorganisms		59
Staphylococcus aureus	29	
Coagulase-negative staphylococci^	15	
Enterococcus faecalis	7	
Corynebacterium striatum^	2	
Other Gram-positive microorganisms*	6	
Gram negative microorganisms		19
Enterobacteriaceae†	15	
Non-fermentative Gram-negative bacilli**	2	
Anaerobe Gram-negative microorganisms‡	2	

Footnote Table 3.2 *Includes *Aerococcus viridans* (n=1), *Arcanobacterium haemolyticus* (n=1), *Bacillus* spp (n=2), *Lactobacillus acidophilus* (n=1) and *Peptostreptococcus* spp (n=1); ** includes *Pseudomonas aeruginosa* (n=1), *Acinetobacter baumannii* (n=1); † includes *Escherichia coli* (n=5), *Klebsiella pneumoniae* (n=1), *Enterobacter cloacae* (n=4), *Proteus mirabilis* (n=3), *Serratia* sp (n=1), and *Citrobacter* sp (n=1); ‡includes *Veillonella* spp, and *Prevotella spp*

3.2 DAIR management and the use of rifampicin combined regimens

Although all selected patients were managed by DAIR not all were submitted to DAIR according to the IDSA criteria (Osmon et al. 2013) (**Figure 3.1**). Patients underwent debridement after a median of 5 days (IQR 2–13) from the onset of symptoms. Removable components were exchanged in 53% of cases, this being highly variable across participating centers.

Figure 3.1 Variability of success rate, surgical approach and application of IDSA criteria across participating centers.



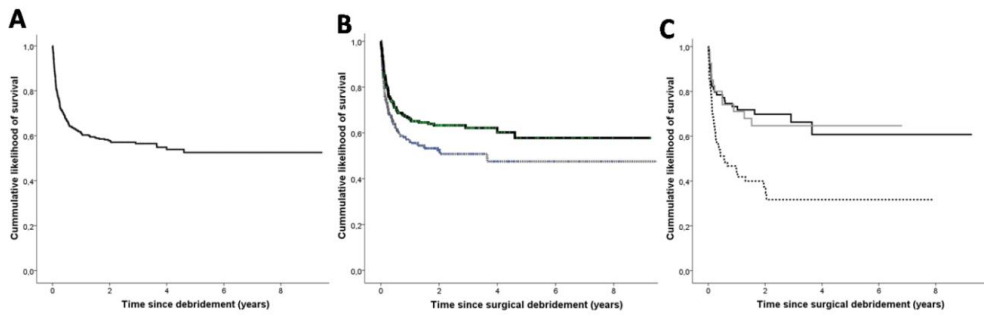
Footnote Figure 3.1
A. Variability of DAIR management. Black bars: percentage of patients submitted to DAIR according to the IDSA criteria; light grey bars: percentage of patients in whom removable components were exchanged during debridement (i.e. the polyethylene liner) (data available in 418 cases); white bars: percentage of patients that received >21 days of a rifampin-based combination (analysis performed in patients that did not fail during treatment, n=318).
B. Variability of success rates. White bars: overall rate of success; dark-grey bars: rate of success in patients who met the IDSA criteria for DAIR.
Other countries are: United Kingdom, Greece, Ireland, Slovenia, Germany, Italy, The Netherlands, Latvia, Hungary, Finland, and Brazil.

The median number of different antimicrobial classes prescribed per patient was 2 (range 1–6). Patients were usually treated with β-lactams, which were given intravenously for a mean time of 21 days ± 20 days. Rifampin-based combinations were significantly used (i.e. during >21 days) in 37% of patients, but this fraction was also highly variable across the participating hospitals (in those recruiting >10 patients, it ranged from 18–88%) (**Figure 3.1.A**, white bar). Alternative antimicrobials such as fluoroquinolones, clindamycin, or linezolid were used less often. In patients not failing while on treatment, antimicrobial therapy was continued for a median of 91 days (IQR 58–171 days).

3.3 Overall outcome

The primary endpoint was evaluable in 444 patients (96.1%). *Overall failure* occurred in 187 patients (42.1%, 95% confidence interval [95%CI]: 37.5%–46.7%) after a median of 62 days from debridement (IQR 25–160 days); by contrast, 257 patients (57.1%) did not fail and were followed up for a median of 802 days (IQR 507–1339 days) (**Figure 3.2.A**). Success rates were highly variable among the participating centers (**Figure 3.1.B**), with it ranging from 44% to 91% among hospitals recruiting >10 patients.

Figure 3.2 – Kaplan-Meier curves of patients with streptococcal periprosthetic joint infection according to the criteria for indicating debridement and implant retention.



Footnote Figure 3.2

A. Kaplan-Meier curve of all evaluable patients (n=444, 187 failures). Causes of failure were due to the streptococcal infection in 147 cases (79%). Death related to PJI was observed in 11 cases (2%).

B. Black continuous line: patients meeting IDSA criteria for DAIR (81 failures in 221 episodes of infection); grey dotted line: patients not meeting IDSA criteria for DAIR (106 failures in 223 episodes of infection); long-rank test, p = 0.017.

C. Post-surgical cases (i.e., non-hematogenous cases) (n=189, 82 failures). Black continuous line: cases with symptoms beginning within the first 30 days after the placement of the prosthesis (n=78, 25 failures); grey continuous line: cases with symptoms beginning within 31 and 90 days after the placement of the prosthesis (n=41, 13 failures); black dotted line: cases with symptoms beginning beyond 90 days after the placement of the prosthesis (n=70, 44 failures). Long-rank test, p<0.001.

Independent predictors of a poor outcome were rheumatoid arthritis (Hazard Ratio [HR] 2.36), late post-surgical infection (HR 2.20), and bacteremia (HR 1.69). The exchange of removable components was independently associated with a favorable outcome (HR 0.60). No one streptococcal species was associated with a higher likelihood of Overall Failure, although a non-significant better prognosis was observed for *S.pneumoniae* (24% failure). A high penicillin MIC (>0.125 mg/L) was also not associated with failure. Also, polymicrobial cases were not associated with a higher likelihood of failure, even when *S.aureus* was involved (data not shown). Late post-surgical infection was indeed a predictor of bad prognosis, when defined as onset of symptoms beginning >3 months after the prosthesis placement (**Figure 3.2.C**). Cases with symptoms beginning within the first and third month had a similar prognosis to that of cases with symptoms beginning within the first month after prosthesis placement. No relevant differences were observed in these two groups of patients.

The failure rate was higher in patients not fulfilling the IDSA criteria for DAIR, namely 106/223 (48%) vs 81/221 (37%) (long-rank test, p=0.017) (**Fig 3.2.B**). Indication of DAIR according to the IDSA criteria was highly variable among participating centers (**Figure 3.1.A**, black bar), ranging from 33% to 83% in those recruiting >10 patients. Independent predictors of failure among patients meeting the IDSA criteria were rheumatoid arthritis (HR 2.46 [95%CI 1.34–4.53]), bacteremia (HR 1.92 [95%CI 1.22–3.02]), and male sex (HR 1.85 [95%CI 1.18–2.91]). Interestingly, the exchange of removable components during debridement was especially beneficial in patients not meeting the IDSA criteria (37% failures vs 62%, p<0.001), in comparison with patients fulfilling them (failures 33% vs 39%, p=0.286).

3.4 Failure dynamics and antimicrobial therapy. The benefits of adding rifampicin to β-lactams on the treatment of streptococcal PJI managed with DAIR

Among the 187 patients who failed, 55 (29%) developed e*arly failure* (within the first 30 days after surgical debridement), 71 (38%) developed *late failure* (beyond the first 30 days after debridement, but still under antimicrobial therapy), and 61 developed *failure after therapy* (once patients had finished the scheduled therapy) (33%). Variables independently associated with *early failure* were age, rheumatoid arthritis, late post-surgical infection, bacteremia, and infection by *S.pyogenes* (**Table 3.3**). Characteristics associated with *late failure* were male sex, immunosuppressant therapy, revision prosthesis, debridement delay >7 days, and the need for >1 debridement to control the infection. Failure was also associated with the early use of glycopeptides during >14 days.

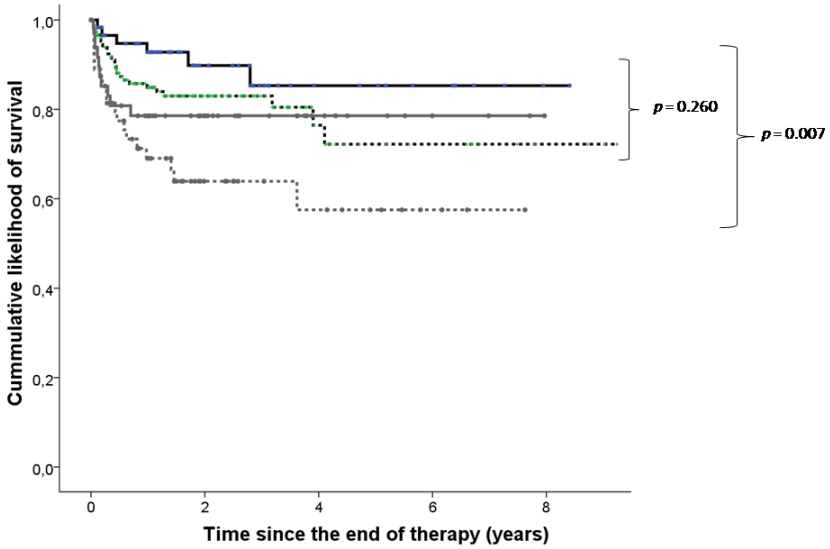
Table 3.3 Univariate and multivariate analysis of parameters predicting Early Failure, Late Failure and Failure After Therapy

	Early failure (n=444, 55 failures)				Late Failure (n=389, 71 failures)				Failure After Therapy (N=318, 61 failures)			
	OR (CI95%)	*p*	aOR (95%CI)	*p*	HR (95%CI)	*p*	aHR (CI95%)	*p*	HR (95%CI)	*p*	aHR (CI95%)	*p*
Sex (female)	1.19 (0.68-2.10	0.540			0.50 (0.31-0.81	**0.004**	0.51 (0.30-0.85	**0.009**	1.16 (0.69-1.92	0.572		
Age (per year)	1.03 (0.99-1.01	**0.076**	1.04 (1.00-1.07	**0.027**	1.00 (0.98-1.02	0.995			0.99 (0.97-1.01	0.348		
Rheumatoid arthritis	2.98 (1.35-6.56	**0.007**	3.33 (1.40-7.93	**0.007**	2.95 (1.55-5.62	**0.004**	-	-	1.19 (0.37-3.81	0.772		
Immunosuppressive therapy	1.49 (0.66-3.66	0.343			2.76 (1.56-4.89	**0.002**	2.64 (1.46-4.79	**0.001**	1.51 (0.65-3.51	0.363		
Renal chronic disease	1.67 (0.73-3.81	0.223			1.99 (1.05-3.79	**0.053**	-	-	1.17 (0.47-2.91	0.746		
Prosthesis location (knee)	1.04 (0.86-1.26	0.677			0.98 (0.83-1.14	0.753			1.18 (0.98-1.41	**0.073**	-	-
Revision prosthesis	1.53 (0.83-2.81	0.173			1.78 (1.09-2.91	**0.027**	1.77 (1.07-2.93	**0.027**	1.56 (0.90-2.70	0.129		
Chronic post-surgical inf.	1.212 (0.97-1.2	**0.091**	1.41 (1.10-1.81	**0.007**	1.12 (0.92-1.37	0.256			1.47 (1.22-1.77	**<0.00**	2.24 (1.24-4.05)	**0.008**
Sinus tract	0.75 (0.31-1.84	0.529			1.05 (0.54-2.06	0.881			1.61 (0.84-	0.175		
Bacteremia	2.17 (1.20-3.92	**0.011**	2.23 (1.80-4.20	**0.014**	1.24 (0.74-2.06	0.420			1.23 (0.70-2.19	0.478		
Rx signs of infection	1.16 (0.98-1.39	**0.091**	-	-	0.77 (0.40-1.48	0.421			2.21 (1.14-4.30	**0.025**	-	-
Infection by *S. pyogenes*	3.10 (1.41-6.85	**0.005**	3.31 (1.41-7.77	**0.006**	0.60 (0.19-1.92	0.357			1.11 (0.45-2.78	0.821		
Infection by virdidans streptococ	0.71 (0.32-1.57	0.401			1.60 (0.94-2.70	**0.094**	-	-	1.01 (0.51-1.98	0.987		
Polymicrobial infection	0.95 (0.41-2.20	0.896			1.33 (0.71-2.47	0.385			1.23 (0.61-2.49	0.579		
Time to debridement (>7 days)†	0.96 (0.54-1.72	0.899			1.60 (1.00-2.54)†	**0.050**	1.70 (1.05-2.75	**0.033**	1.33 (0.80-2.20	0.281		
Exchange of polyethylene	0.56 (0.31-1.02	**0.059**	-	-	0.75 (0.46-1.21	0.234			0.45 (0.26-0.77	**0.033**	0.44 (0.26-0.76)	**0.003**
Need for ≥ 2 debridements	1.16 (0.57-2.36	0.683			2.26 (1.63-4.36	**<0.001**	2.45 (1.45-4.15	**0.001**	0.60 (0.26-1.40	0.206		
Antimicrobial therapy‡												
B-lactams (without rifampin)	-	-			1.41 (0.88-2.27	0.155			0.62 (0.37-1.03	**0.061**	0.48 (0.28-0.84)	**0.010**
β-lactams + rifampin	-	-			0.89 (0.47-1.70	0.724			0.42 (0.18-0.98	**0.025**	0.34 (0.12-0.96)	**0.041**
Quinolones + rifampin	-	-			0.19 (0.03-1.36	**0.082**	0.21 (0.03-1.54	0.125	1.03 (0.45-2.40	0.940		
Glycopeptides without rifampi	-	-			3.97 (2.08-7.58	**<0.001**	2.82 (1.43-5.53	**0.003**	4.25 (1.32-13.7	**0.015**	-	-
Duration of therapy > 120 day	-	-			-	-			0.54 (0.29-0.90	**0.046**	-	-

Footnote Table 3.3 OR: odds ratio; aOR: adjusted odds ratio; 95%CI: 95% confidence interval; HR: hazard ratio; aHR: adjusted hazard ratio. †Time to debridement: time from onset of symptoms to the first surgical debridement. Initial models of multivariate analyses were built with variables with a *P* value <0.10 in the univariate analysis, and then selected with a stepwise backward process. ‡ Treatments included in this analysis are those received during the first 30 days after debridement, and are considered if they were administered for at least 15 days (for Late Failure) and those received during the whole period of treatment, both orally and intravenously, and are considered if they were administered for at least 22 days (for Failure After Therapy).

However, the addition of rifampin to treatment with glycopeptides neutralized this poor prognosis. The early use of rifampin plus fluoroquinolones also showed a trend toward a favorable outcome in the univariate analysis (HR 0.19, p=0.082). Late post-surgical infection was an independent predictor of *failure after therapy*, while the exchange of removable components was associated with a favorable outcome. The use of β-lactams for >21 days, both alone and combined with rifampin, were independently associated with better *outcomes* (HR 0.48 and 0.34, respectively) (**Figure 3.3**).

Figure 3.3. Prognosis after the end of therapy according to antibiotic treatment



Footnote figure 3.
Analysis performed in cases that did not fail during treatment (n=318, *failure*s = 61). Black continuous line: patients treated during >21 days with β-lactams + rifampin (n=60, failures=6); black dotted line: patients treated during > 21 days with β-lactams, but no rifampin (n=154, failures=26); grey continuous line: patients treated >21 days with a rifampin-based combination other than β-lactams plus rifampin (n=48; failures=10); grey dotted line: patients who did not receive either β-lactams or rifampin for > 21 days (n=56; failures=19). Comparisons calculated with the Long-rank test. The comparison of these 4 treatment regimes showed similar trends when the analysis was stratified for patients meeting and not meeting IDSA criteria, and for patients who did and did not undergo exchange of removable components during debridement.

The benefits of early treatment with rifampin were also observed for patients when treatment did not fail within the first 30 days after debridement (HR 0.98 per day of treatment, p=0.034).

The results of this study demonstrate a worse prognosis than previous reports, confirming the benefits of exchanging the removable components during the DAIR procedure and supporting the potential benefit of adding rifampicin to β-lactams to improve the outcome.

B.2 Infections by MDR Gram-negative bacilli

B.2.1 The use of β-lactams in continuous infusion

Aim 4: to standardize a measurement procedure based on UHPLC-MS/MS for the simultaneous measurement of multiple β-lactam antibiotic concentrations in human plasma

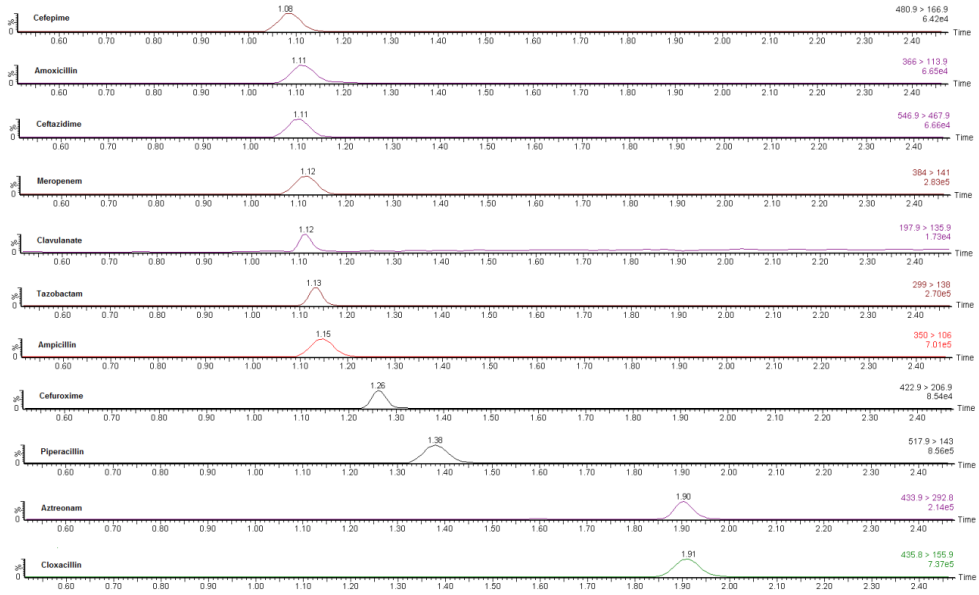
Article 4. *Development and validation of a measurement procedure based on ultra-high performance liquid chromatography-tandem mass spectrometry for simultaneous measurement of β-lactam antibiotic concentration in human plasma.* R. Rigo-Bonnin, **A. Ribera**, A. Arbiol-Roca, S. Cobo-Sacristán, A. Padullés, O. Murillo, E. Shaw, R. Granada, XL. Pérez-Fernández, F. Tubau, P. Alía. Clinica Chimica Acta 2017; 468:215-224. doi: 10.1016/j.cca.2017.03.009

Therapeutic drug monitoring of β-lactams appears to be mandatory for guiding therapy in several clinical situations (e.g. when using them in continuous or extended infusions). However, there are no commercial assays available for the routine measurement of β-lactam concentrations in patients' plasma. Thus, a UHPLC-MS/MS method was developed and validated for the simultaneous measurement of nine β-lactams to incorporate routine determinations into daily clinical practice for patients with difficult-to-treat infections that are managed with β-lactams in continuous or extended infusions.

4.1 Chromatography

Under the chromatographic conditions established for the UHPLC-MS/MS procedure, BL eluted at retention times ranging between 1.08 and 1.91 min. A typical multiple reaction monitoring chromatogram for the lowest quality control sample (3.00 mg/L) is shown in **Figure 4.1**. The UHPLC-MS/MS run time was 3.5 min, including the time needed for the solvent gradient to return to baseline conditions before the next injection.

Figure 4.1 Chromatogram



4.2 Validation data

Peak area responses were observed to test selectivity, carry-over and lower limits of quantification with proper results. Calibration curves showed a satisfactory linearity for all antibiotic plasma concertations. Data for intra-day and inter-day imprecision and relative bias data was acceptable. Imprecision values for dilution integrity, at five- and ten-fold dilution were low. Values for recovery and matrix effect were analysed and their variations were less than 15% among all concentrations. Antibiotic concentrations in plasma were stable during storage at 5±3 ºC for a period of 3 days, in the autosampler at 4 ± 1°C for 12 h, and at −75 ± 3 °C for at least 6 months. Stock solutions of antibiotics and internal standards stored at 5 ± 3 °C were stable for 3 days, and at (–75 ± 3) °C for 6 months. Data is shown in the article (attached at the end of the book).

4.3 Clinical application

Plasma antibiotic concentrations values obtained from patients treated with BL, were consistent with the clinical situations observed by the clinician. This validated method (UHPLC-MS/MS for simultaneous measurement of several β-lactams) could be applied to daily clinical laboratory practice to measure the concentration of these antibiotics in the plasma of patients with osteoarticular infections that are managed with β-lactams in continuous or extended infusion.

Aim 5: to evaluate the efficacy and safety of β-lactams in continuous infusion for difficult-to-treat osteoarticular infections caused by Gram-negative bacilli, and to validate an easy method for clinical use

Article 5. *Beta-lactams in continuous infusion for difficult-to-treat osteoarticular infections caused by Gram-negative bacilli: validation of an easy method for clinical use*. **A. Ribera**, L. Soldevila, R. Rigo, F. Tubau, A. Padullés, J. Gómez-5 Junyent, J. Ariza, O. Murillo. Submitted for publication in Antimicrobial Agents and Chemotherapy journal, a detailed revision has been sent according to editor/reviewers comments.

Communication 5. β-lactams in continuous infusion for difficult-to treat osteoarticular infections caused by Gram-negative bacilli: a preliminary validation of an easy-to-use method. **A. Ribera**, J. Gómez-Junyent, L. Soldevila, R. Rigo, F. Tubau, A. Padullés, J. Ariza, O.Murillo. 27th ECCMID. Vienna, Austria, 2017. (Abstract number 5179)

β-lactams in continuous infusion could optimize their PK/PD parameters and consequently, the outcomes of cases managed with these regimens, especially in difficult-to-treat infections caused by MDR GNB. The ideal dosage of β-lactams in continuous or extended infusion is not established and therapeutic drug monitoring is usually not available in routine clinical practice. An easy-to-use equation was therefore validated to guide this therapy. Moreover, the safety and efficacy of using β-lactams in continuous infusion to treat infections caused by GNB was evaluated.

5.1 Description of the case series

Twenty-four patients with osteoarticular infections caused by GNB were analysed: 11 osteomyelitis, 10 prosthetic joint or arthrodesis infections and three septic arthritis [median age: 66 years (IQR 54-75), 14 (58.3%) women, and 16 (66.6%) with renal impairment (CR_{CL}<90mL/min)]. The most frequent microorganism was *P. aeruginosa* (21 cases, 87.5%; 9 MDR), and there were three isolated cases caused by *Achromobacter xylosidans*, Acynetobacter baumannii and Enterobacter cloacae (**Table 5.1**).

All cases were treated with BL in CI (alone or in combination): Ceftazidime (14 cases), aztreonam (seven cases), and piperacillin-tazobactam (three). BL was combined with ciprofloxacin in quinolone susceptible strains (five cases). BL plus colistin was used in infections caused by quinolone-resistant strains (12 cases), of which nine cases were BL-resistant and

three cases were BL-susceptible. Median treatment duration was 34.5 days (IQR 20.3-42). Twenty-four patients underwent concomitant surgery such as debridement or removal of an orthopedic device.

5.2 Calculation of BL daily dose administered in CI according to the defined Equation

A theoretical daily dose of BL was calculated for each case before starting the antibiotic regimens. In several cases the theoretical dose was modify according to the clinician criteria to a Real dose. To finally analyse data we used Real doses (**Table 5.1**). After all, resistant strains required higher doses than susceptible ones: ceftazidime (median dose-grams-/24h, IQR) 6 (4-6) vs 4.5, and aztreonam 5.5 (4.3-6) vs 3, respectively.

Table 5.1 Patient characteristics, microorganisms details, antibiotic dose and plasma concentration of patients treated with ceftazidime (n=24)

Case/ ATB	Patient characteristics		Microorganisms details			Antibiotic dose (mg/24h)		Antibiotic plasma concentration (mg/L)			
	CR_{CL}	Weight (kg)	Microorganism	MIC (mg/L)	Susceptibility	TimesxMIC, Theoretical dose	Real dose	C_{pred}	C_{obs}	Δ_{Conc}	$\%\Delta_{Conc}$
1/CAZ	115.32	56	PA	8	S	4×8, 5314	6000	36.13	50.90	14.77	29
2/CAZ	148.62	72	PA	4	S	4×4, 3424	4000	18.69	23.80	5.11	21
3/CAZ	108.59	90	PA	4	S	4×4, 2502	4000	25.58	28.90	3.32	11
4/CAZ	118.08	80	PA	2	S	4×2, 1360	7000	41.17	70.40	29.23	41
5/CAZ	56.17	85	PA	8	S	4×8, 2588	9000	111.27	90.00	-21.27	-23
6/CAZ	73.21	74	PA	2	S	4×2, 843	2000	18.97	15.50	-3.47	-22
7/CAZ	88.37	70	PA	4	S	4×4, 2036	5000	39.29	65.20	25.91	40
8/CAZ	18.95	75	PA	8	S	4×8, 873	3500	128.29	94.20	-34.09	-36
9/ CAZ	77.75	80	PA	8	S	4×8, 3583	5000	44.66	25.40	-19.26	-75
10/ CAZ	87.29	75	AX	4	S	4×4, 2011	4000	31.82	46.80	14.98	32
11/ CAZ	65.39	72	PA	12	R	4×12, 4520	8000	84.96	103.00	18.04	17
12/ CAZ	22.30	80	PA	32	R	2×32, 2055	4000	124.57	104.60	-19.97	-19
13/ CAZ	71.99	68	PA	16	R	3×16, 4976	6000	57.88	82.40	24.52	30
14/ CAZ	61.12	92	PA	16	R	3×16, 4225	6000	68.17	37.20	-30.97	-83
15/ATM	238,21	64	PA	16	R	3x16, 5679	6000	50.71	62.20	11.49	18
16/ATM	74.23	75	PA	16	R	3x16, 5679	5000	42.26	42.60	0.34	1
17/ATM	69.92	50	PA	8	R	4x8, 3786	4000	33.81	82.20	48.39	59
18/ATM	204.19	110	PA	4	S	4x4, 1893	3000	25.35	77.90	52.55	67
19/ATM	155.03	65	PA	8	R	4x8, 3786	6000	50.71	115.50	64.79	56
20/ATM	58.05	73	PA	4	S	4x4, 1893	3000	25.35	39.20	13.85	35
21/ATM	109.55	75	PA	4	S	4x4, 1893	3000	25.35	49.30	23.95	48
22/TZP	86.70	100	ABAU	8	S	4x8, 7327	10000	43.68	52.40	8.72	16
23/TZP	96.04	100	ECLO	8	S	4x8, 10368	12000	37.04	45.20	8.16	18
24/TZP	34.22	40	PA	16	S	3x16, 7880	12000	73.10	33.00	-40.10	-121

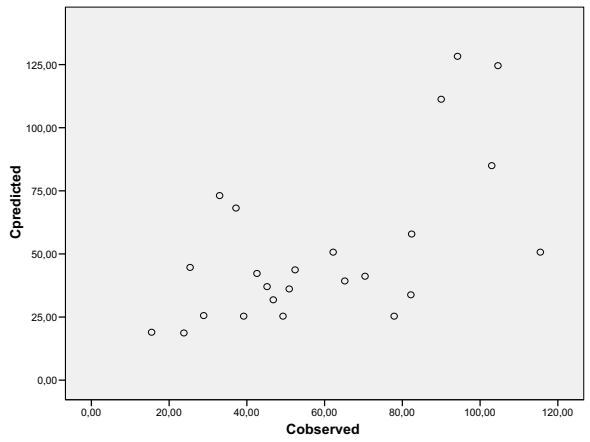
Footnote table 5.1

CAZ= ceftazidime, ATM= aztreonam, TZP= piperacillin-tazobactam, CR_{CL}= Creatinine clearence, PA= *Pseudomonas aeruginosa*, AX= *Achromobacter xylosidans*, ABAU= Acynetobacter baumannii, ECLO= Enterobacter cloacae, S= susceptible, R=resistant, mg: milligrams. TIMES×MIC= the expected number of times over the MIC, used to achieve the desired C_{ss} concentrations to calculate a daily Theoretical Dose of BL in CI (see Materials and Methods section, Equation 1). Theoretical daily dose: dose predicted by Equations 1 (see and Materials and Methods section, Equation 1). Real dose: dose finally administered to patients. C_{pred}=predicted concentration by using Equation 2 for a specific Real dose (see Materials and Methods section, Equation 2); C_{obs}= observed concentration determined by UPLC-MS/MS; $\Delta_{Conc} = C_{obs} - C_{pred}$; $\%\Delta_{Conc} = \Delta_{Conc} / C_{obs}$

5.3 Correlation between BL predicted concentration in plasma and the observed concentration levels using UHPLC-MS/MS

In total we performed 37 antibiotic plasma determinations using UHPLC-MS/MS: 24 initial determinations (Table 1) and 13 monitoring levels. The C_{obs} were higher than the C_{pred} in cases with normal renal function (difference between C_{obs}-C_{pred} –percentage-, 19% to 54%), and it was more variable with renal impairment (from -33% to +31%). *Spearman* correlation between C_{pred} and C_{obs} was: rho=0.6 (*P*=0.005), for all BL (Figure 1); and rho=0.8 (*P*<0.001), for ceftazidime exclusively. This correlation was better for patients with a lower weight (rho 0.6, <75kg) than higher (rho 0.3, ≥75kg).

Figure 5.1 Correlation between C_{obs} and C_{pred} (n= 24; Rho *Spearman*=0.6)



5.4 Efficacy and safety of BL used in CI

Finally, all patients except one (with a polymicrobial arthrodesis infection who required a supracondylar amputation), were clinically cured after a median follow-up of 18.4 months (IQR 10-32).

Overall, BL used in CI were well tolerated although some patients achieved high levels of BL plasma concentrations (around 100 mg/L) (**Table 5.1**). Only one case which was treated with ceftazidime (6 Grams/24hours–plasma levels 50.9 mg/L), presented a *Clostridium difficile* colitis that was cured with metronidazole and a reduction in ceftazidime dosage. No neurological or hematological toxicity were observed.

The proposed simple equation seems to be an easy way to estimate the BL-CI dosage and its plasma levels when TDM is not available. Moreover, the use of BL-CI appears to be a safe an effective therapeutic option for treating osteartícular infections caused by GNB.

B.2.2 The use of antibiotic combinations with colistin

Aim 6: to evaluate the benefits of the antibiotic combination of colistin and β-lactams when treating patients with MDR *Pseudomonas aeruginosa* infections

Article 6. *Osteoarticular infection caused by MDR Pseudomonas aeruginosa: the benefits of combination therapy with colistin plus beta-lactams*. **A. Ribera**, E. Benavent, J. Lora-Tamayo, F. Tubau, S. Pedrero, X. Cabo, J. Ariza, O. Murillo. Journal of Antimicrobial Chemotherapy. 2015; 70(12):3357-65. doi: 10.1093/jac/dkv281

Communication 6. *Role of Combined Therapy Including Beta-lactams on Intermittent or Continuous Infusion for the Treatment of Osteoarticular Infection (OI) by Extensively Drug-resistant Pseudomonas aeruginosa (PA.)* **A. Ribera**, O. Murillo, E. Benavent, G. Euba, J. Lora, S. Pedrero, F. Tubau, J. Cabo, J. Ariza. 54th ICAAC. Washington, USA, 2014. (L-416)

Given the emergence of MDR GNB (such as *Pseudomonas aeruginosa*), osteoarticular infections are becoming more difficult to treat. The role of β-lactams in monotherapy is questioned and older drugs need to be reconsidered and combined with β-lactams, as suggested by PK/PD and experimental models. Thus, our clinical experience with the management of these infections was evaluated; focusing on prognostic factors for failure and the impact of the combined therapy (with β-lactams plus colistin) on the final clinical outcome of osteoarticular infections caused by MDR *P.aeruginosa*.

6.1 Main characteristics of patients and initial management of a case series with osteoarticular infection (OI) caused by MDR *Pseudomonas aeruginosa*

We included 34 patients: 15 (44%) with PJI, 11 (32%) with osteoarthritis (OA) not related to an orthopaedic device and 8 (24%) with OA related to an orthopaedic device. The median age was 68.7 years (IQR 59.5–78) and 59% were men, with >70% having at least one comorbidity. Polymicrobial infection was initially present in 16 (47%) patients and 20 (59%) had a super-infection caused by MDR P. aeruginosa (**Table 6.1**).

Table 6.1 Main characteristics of patients with OI caused by MDR *P.aeruginosa*; N = 34

	Median (IQR) or n (%)
Age (median, IQR)	68.7 (59.5-78)
Sex (man)	20 (58.8%)
Comorbidities	
diabetes mellitus	6 (17.6%)
immunosuppressive therapy	8 (23.5%)
autoimmune disease	5 (14.7%)
chronic renal failure	6 (17.6%)
malignancy	4 (11.8%)
others[a]	6 (17.6%)
no comorbidity[b]	10 (29.4%)
Type of infection	
PJI	15 (44.1%)
OA (without related device)	11 (32.4%)
OA (related with an orthopaedic device)	8 (23.5%)
Polymicrobial infection	16 (47.1%)
Super-infection	20 (58.8%)
P.aeruginosa MDR/XDR	11 (32.4%) / 23 (67.6%)

Footnote Table 6.1
[a]Included patients with chronic pulmonary disease, chronic heart disease or advanced dementia.
[b]Included patients without any of the previously defined comorbidities.
Abbreviations: PJI, prosthetic joint infection; OA, osteoarthritis; MDR, multidrug-resistant; XDR, extensively drug-resistant.

Of the 34 patients, 31 (92%) initially underwent surgery. Three patients with OA (without device) were managed conservatively with antibiotics alone: two had post-surgical pubic symphysis osteomyelitis following a prostatic resection, and one had sacroiliitis because of a sacral pressure sore. Among the 23 patients with OI related to an orthopaedic device (8 OA plus 15 PJI), surgery involved debridement and device removal in 14 (60.9%; 9 PJI and 5 OA), while the device was retained in 9 (39.1%; 6 PJI and 3 OA). Monotherapy was used in 19 (56%) patients, mainly with intermitent boluses of b-lactams (14/19), but 4 patients received colistin alone. When the clinician used combination therapy (15, 44%), it was mostly with continuous infusion of a BL plus colistin (10/15). Overall, 30 patients received BL: in 12 patients, *P. aeruginosa* strains were susceptible (6 to antipseudomonal cephalosporins, 2 to piperacillin/tazobactam and 4 to carbapenems), but the other 18 were not susceptible: 2 intermediate (1 to aztreonam and 1 to carbapenem) and 16 resistant (6 to anti-pseudomonal cephalosporins, 6 to piperacillin/tazobactam, 1 to aztreonam and 3 to carbapenems). The median dose of colistin was 5 MIU/day (IQR 2.8–6), for a median of 40.5 days (IQR 26–43). Amikacin was administered only in two patients, where it was combined with intermittent boluses of b-lactams (**Table 6.2**).

Table 6.2 Initial management of patients with OI caused by MDR P.aeruginosa; N = 34

	n (%) or n
Antibiotic	
monotherapy	19 (55.9%)
colistin	4
BL-IB	14
BL-CI	1
combined therapy	15 (44.1%)
colistin + BL-IB	3
colistin + BL-CI	10
amikacin + BL-IB	2
Surgery	
no surgery	3 (8.8%)
surgery without device maintenance[a]	22 (64.7%)
debridement with device retention	9 (26.5%)

Footnote table 6.2
BL, β-lactam; IB, intermittent bolus.

Monotherapy: BL-IB, ceftazidime (4), cefepime (1), aztreonam (1), piperacillin/tazobactam (4) and carbapenem (4); and BL continuous infusion, piperacillin/tazobactam (1).

Combined therapy: colistin+BL-IB: ceftazidime (1), aztreonam (1) and carbapenem (1); colistin+BL continuous infusion: ceftazidime (5), aztreonam (2), piperacillin/tazobactam (2) and carbapenem (1); and amikacin+BL-IB: cefepime (1) and piperacillin/tazobactam (1).

[a]Includes patients with OI without a device managed by debridement and patients in which the involved devices were removed.

6.2 Prognostic factors for persistence of infection and analysis of risks of failure after the initial therapy. Benefits of using combined therapy to treat OI caused by MDR *P. aeruginosa*.

After initial therapy, the cure rate reached 50%. Among the remaining patients, 15 (44%) had persistent infection caused by MDR *P. aeruginosa* and 2 died during the initial treatment. The factors predicting treatment failure were therefore evaluated, focusing on the host, the type of infection and the therapeutic plan. XDR *P. aeruginosa* was present in 23 patients and MDR *P. aeruginosa* in 11 patients, with no differences in management (surgical or antibiotic regimen) between the groups (data not shown). Of the 11 patients with OI caused by MDR *P. aeruginosa*, just three (27%) were cured after the first therapeutic plan; but the cure rate more than doubled when the pathogen was an XDR *P. aeruginosa* strain (cure rate 14/23, 61%, P = 0.067) (**Table 6.3**).

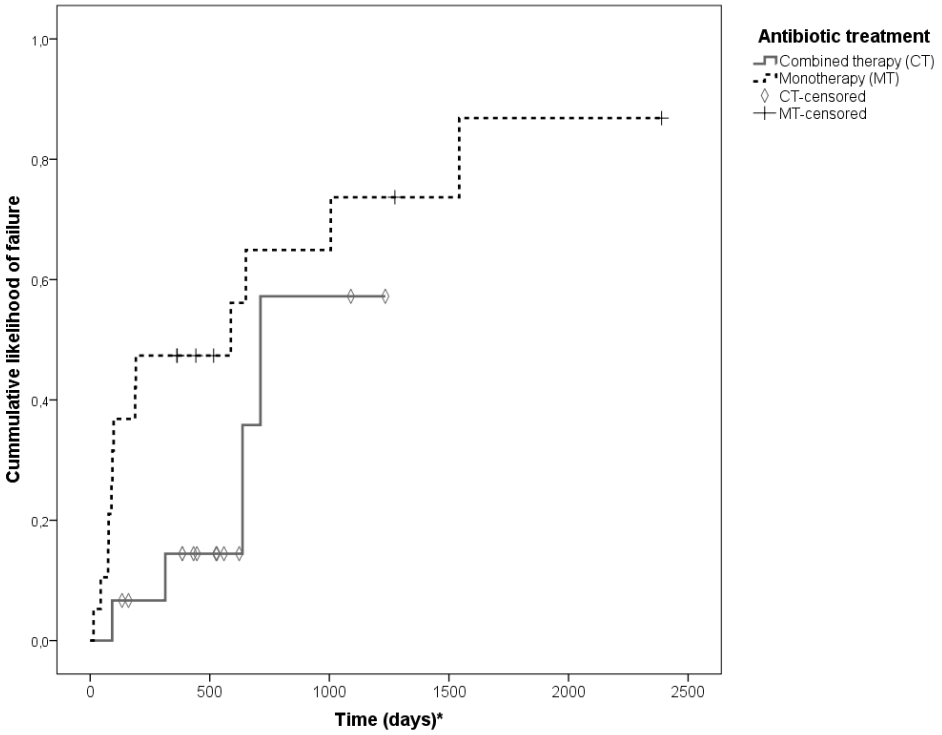
Table 6.3 Prognostic factors for persistence of infection after the initial therapy, analysis of risk failure considering main characteristics and antibiotic treatment; N =34

	Cured infection n = 17 n (%)	**Non-cured infection n = 17 n (%)**	***p***
Main characteristics			
age (years), median (IQR)	71 (59-76)	67 (51-79)	1
sex (man)	12 (70.6%)	8 (47.1%)	0.163
polymicrobial infection	6 (35.3%)	10 (58.8%)	0.169
super-infection	11 (64.7%)	9 (52.9%)	0.486
MDR PA / XDR PA	3 (17.6%) /14 (82.4%)	8 (47.1%) /9 (52.9%)	0.067
related to an orthopaedic device	10 (58.8%)	13 (76.5%)	0.271
Antibiotic			
Monotherapy	6 (35.3%)	13 (76.5%)	0.016
Combined therapy	11 (64.7%)	4 (23.5%)	
BL-IB	8 (53.3%)	11 (73.3%)	0.256
BL continuous infusion	7 (46.7%)	4 (26.7%)	

Footnote Table 6.3. PA, P.aeruginosa; BL, β-lactam; IB, intermittent bolus.

Combination therapy (mainly with colistin plus BL) was significantly more effective than monotherapy (with either b-lactams or colistin), with cure rates of 11/15 (73%) and 6/19 (32%), respectively (P = 0.016) (**Table 6.3**). **Figure 6.1** illustrates the likelihood of failure according to the antibiotic treatment and follow-up period (log-rank = 0.079). In our case series, colistin was well tolerated, and although 10 patients presented renal impairment during the treatment, creatinine was normalized after reducing the dose. The use of BL in continuous infusion was safe and seemed to offer more benefits than BL in an intermittent bolus (cure rates of 64% and 42%, respectively, P = 0.256) (**Table 6.3**).

Figure 6.1



Footnote Figure 6.1. Likelihood of failure according to the antibiotic treatment (combined therapy or monotherapy). *Time from the start of antibiòtic therapy to the end of follow-up or to failure (in cases not initially cured). Grey continuous line, combined therapy; black broken line, monotherapy. Log-rank = 0.079.

The failure rate was also analysed between the two groups by the difficulty of treatment. Patients in Group A had a higher failure rate (61.1%) compared with patients in Group B (37.5%). Focusing on those patients managed with implant retention (n = 9), three patients were cured after initial debridement (3/9, 33%), but six required further surgery for device removal (**Table 6.4**).

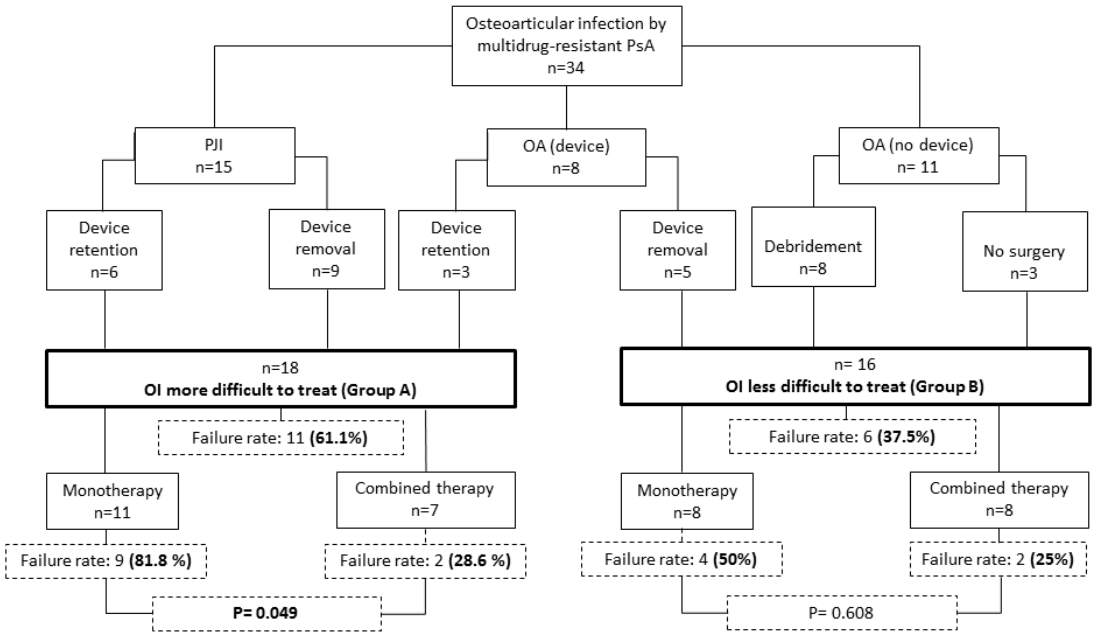
Combined antibiotic treatment (mainly with colistin plus BL) also appeared to be associated with better outcomes than monotherapy in patients with infections considered more difficult to treat (Group A), despite the added management difficulties, with cure rates of 5/7 (71%) and 2/11 (18%), respectively (P = 0.049) (**Figure 6.2**).

Table 6.4 Prognostic factors for persistence of infection after the initial therapy; analysis of risk of failure according to the difficulty of treatment; N=34.

Type of infection	Surgical management	Failure n/N (%), 17/34 (50%)		p
PJI	implant retention	4/6 (66.7%)	More difficult-to-treat OI (Group A) 11/18 (61.1%)	0.169
PJI	implant removal[a]	5/9 (55.6%)		
OA (with device)	implant retention	2/3 (66.7%)		
OA (with device)	implant removal	2/5 (40%)	Less difficult-to-treat OI (Group B) 6/16 (37.5%)	
OA (no device)	No surgery or debridement	4/11 (36.4%)		

Footnote Table 6.4
[a]Management: 3 Girdlestone (2 failures), 5 two-step revision (3 failures) and 1 arthrodesis.
Group A (more difficult to treat), prosthetic joint infections (PJIs) and osteoarthritis (OA) managed with device retention; Group B (less difficult to treat), OA managed without device retention.

Figure 6.2



Footnote Figure 6.2. Chart of OI initial management (antibiotic and surgery) according to the difficulties considered (Group A and Group B). Boxes with broken lines show percentages of failure in the various situations. PA, *P. aeruginosa*.

6.3 Rescue therapy in those patients who were not cured after the initial therapy.

Details of the treatment received by the 17 (50%) patients in whom initial therapy was not curative are summarized in Table 5. Two patients died (Table 5, cases 16 and 17). Among the patients who were not cured by initial therapy, one had a PJI that was retained with a persistent infection [Table 5, case 7, managed conservatively with careful follow-up of a persistent fistula, but without antibiotics (no oral option was possible)]. Another 14 patients required second-line treatment (7 PJIs and 7 OA), which consisted of device removal in 6 patients (always together with an antibiotic plan) or debridement in 8 patients (Table 5). In one patient, prosthetic removal consisted of an infracondylar amputation (Table 5, case 5, total knee prosthesis after resection of an osteosarcoma). The concomitant antibiotic treatment included combination therapy (7 patients), colistin monotherapy (2 patients) or b-lactam monotherapy (4 patients) (**Table 6.5**). There was no emergence of colistin-resistant strains in patients with persistent infections.

Overall, three patients died and two had infections that could not be healed, so satisfactory outcomes were achieved in up to 85% of patients (29/34). If we focus on the patients with PJI, 11 of the 15 patients (73.3%) were finally cured; of these, 4 retained a functional prosthesis (2 with the initial prosthesis and 2 with a new prosthesis), 1 with a spacer, 4 with a Girdlestone resection and 2 with an arthrodesis.

Table 6.5 Details of patients not cured after receiving initial therapy.

					Initial therapy			Rescue therapy			
					surgery[a]	antibiotic		surgery[a]	antibiotic		
Case	Age (years)	Type of infection	PA	Orthopaedic device	managed with device retention	MT/CT	BL-IB/BL continuous infusion	managed with device retention	MT/CT	BL-IB/BL continuous infusion	Final outcome
1	63	PJI	XDR	yes	device retention	MT (colistin)	—	no	MT (colistin)	—	cured
2	67	PJI	MDR	yes	device retention	MT	IB	no	CT	IB	cured
3	71	PJI	MDR	yes	no	MT	IB	no	MT	IB	cured
4	70	PJI	MDR	yes	no	MT	IB	no	CT	IB	cured
5	28	PJI	XDR	yes	device retention	CT	continuous infusion	no		—	amputation
6	78	PJI	XDR	yes	device retention	MT	IB	no	MT	IB	cured
7	79	PJI	MDR	yes	no	CT	IB		—		persistence
8	84	PJI	XDR	yes	no	MT	IB	no	CT	continuous infusion	died
9	67	OA	XDR	yes	no	MT (colistin)	—	no	CT	IB	cured
10	41	OA	XDR	yes	device retention	MT	IB	no	CT	IB	cured
11	39	OA	MDR	yes	device retention	MT	IB	no	MT	IB	cured
12	30	OA	MDR	no	no	MT	IB	no	MT (colistin)	—	cured
13	61	OA	MDR	no	no	CT	continuous infusion	no	CT	continuous infusion	cured
14	82	OA	MDR	no	—	MT	IB	no	MT	IB	cured
15	61	OA	XDR	no	no	CT	continuous infusion	no	CT	continuous infusion	cured
16	79	PJI	XDR	yes	no	MT	continuous infusion			died	
17	93	OA	XDR	yes	no	MT	IB			died	

Footnote Table 6.5 [a]'No' means no device retentions (so include cases managed without a device: because there was no previous device or it has been removed). A '—' means that there was no surgery. PA, P.aeruginosa; MT, monotherapy; CT, combined therapy; BL, β-lactam; IB, intermittent bolus.

Our results add clinical experience to the PK/PD and experimental models about the benefits of the β-lactams and colistin combination for the treatment of MDR *P. aeruginosa* infections managed with appropriate surgical treatment.

Aim 7: to study the effect of adding colistin to β-lactams against ESBL-producing *klebsiella pneumonia*e biofilm in an *in vitro* experimental model

Article 7. *Activity of colistin combined with meropenem against ESBL-producing Klebisella pneumoniae in an in vitro dynamic model for growing biofilm*. **A. Ribera**, J. Gómez-Junyent, C. El Haj, F. Tubau, S. Martí, E. Benavent, K. Jiménez, J. Ariza, O. Murillo. Under elaboration.

Comunication 7. *Eficacia comparativa de meropenem versus su combinación con colistina frente a Klebsiella pneumoniae BLEE en un modelo dinámico de biofilm in vitro*. **A. Ribera**, C. El Haj, J. Gómez-Junyent, F. Tubau, E. Benavent, K. Jiménez, J. Ariza, O. Murillo. XXI Congress SEIMC. Málaga, Spain, 2017. (Comunicación 358)

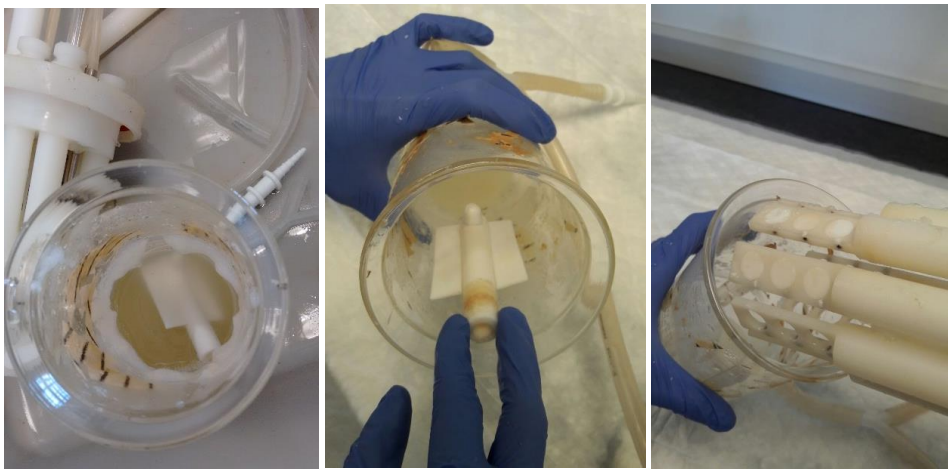
The incidence of MDR GBN infections is increasing. The efficacy of β-lactams against PJI fluoroquinolones-resistant strains has been questioned. Moreover, it has been suggested that colistin may have activity against biofilm-embedded bacteria. Thus, it is hypothesized that the combination of colistin plus carbapenems may exhibit a potential synergistic effect against biofilm-embedded ESBL-producing *Klebsiella pneumoniae*. As such, an *in vitro* dynamic model for growing *K. pneumonia*e biofilm was developed based on the CDC biofilm reactor (CBR).

According to the previously defined fixed and tested operating conditions (see Materials and Methods), different experiments were performed to evaluate the capacity of *K. pneumoniae* to form biofilm on the Teflon coupons (biofilm-embedded cells). Medium samples (considered a mixture of planktonic and stationary growing bacteria) were also enumerated. Two different strains, A and B, were studied (meropenem MICs 0.06 and 0.03 mg/L, respectively; colistin MIC 0.12 in both strains; and MBCs of meropenem 1 and 2 mg/L, respectively), as defined in Materials and Methods. Experiment 2 was performed with strain A.

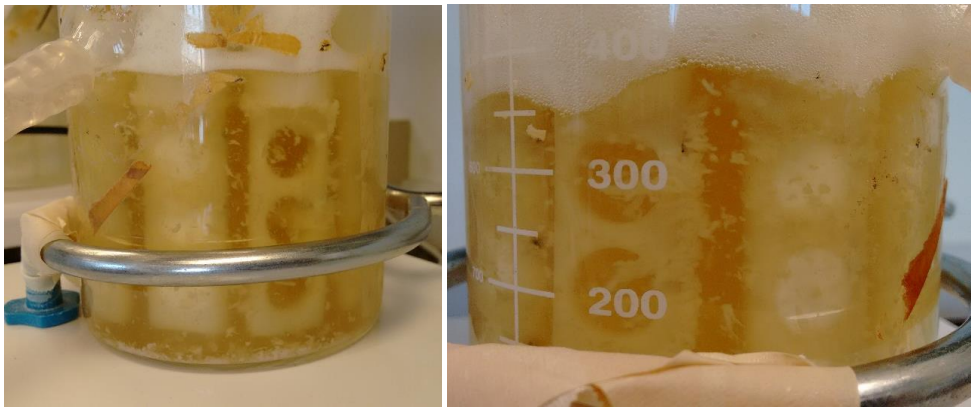
EXPERIMENT	STATIC PHASE	DYNAMIC PHASE	
	Fixed conditions	Fixed conditions	Tested conditions
1	24 h 35-37ºC 130 rpm TSB 100%	35-37ºC 130 rpm Flow rate: 13.5 mL/min	72h, TSB 20%
2			24h, TSB 100%
3			24h, TSB 20%

Figure 7.1 Pictures of the CBR at the end of the conditioning phase for growing biofilm

A



B



Footnote 7.1

A. Pictures of an open CBR at the end of the conditioning phase, the turbidity of the reactor medium is diplayed and a large amount of frothy material (containing large quantities of *K. pneumoniae*) is attached to the reactor walls, the stir bar and the rods/coupons. From Experiments 1 and 3, strain A.

B. Pictures of a CDC reactor at the end of the conditioning phase, where the turbidity of the reactor medium is displayed with many floating materials, which are also attached to the rods and coupons. From Experiment 2, strain A.

After the macroscopic results from experiment 2 demonstrated the prominence of planktonic bacteria, the decision to proceed with studying the model through experiments 1 and 3 was made.

7.1 Assessment of biofilm formation according to the different tested operating conditions

7.1.1 Enumeration of log_{10} cfu/mL determination at different conditions (for strains A and B).

- COUPONS (biofilm-embedded cells)

STRAIN A	**AFTER STATIC PHASE** (log_{10} cfu/mL, mean, SD)	**AFTER DYNAMIC PHASE** (log_{10} cfu/mL, mean, SD)
Experiment 1	5.3 (0.2)	6.5 (0.4)
Experiment 3	5.5 (0.3)	6.8 (0.3)

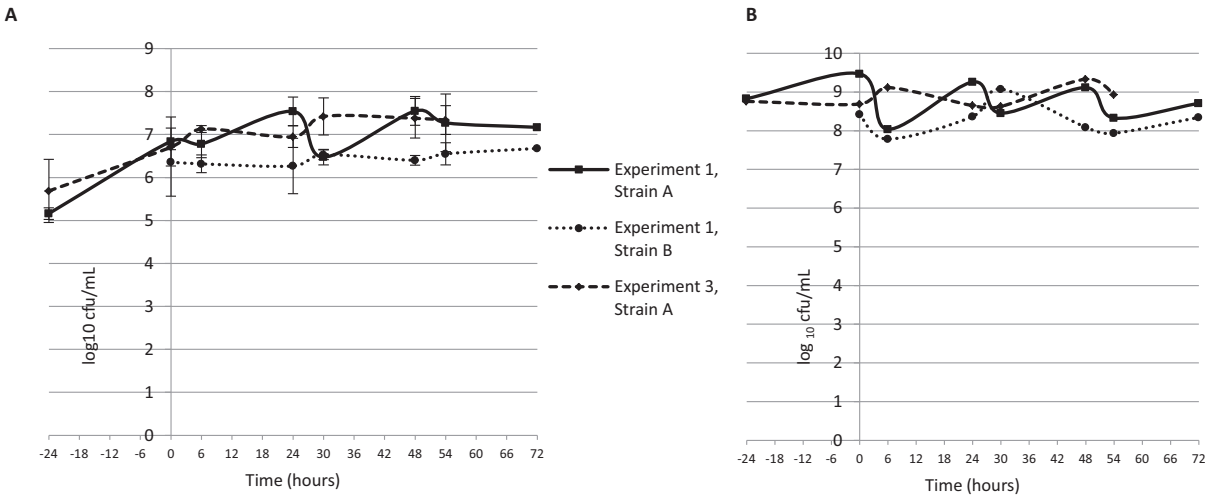
STRAIN B	**AFTER STATIC PHASE** (log_{10} cfu/mL, mean, SD)	**AFTER DYNAMIC PHASE** (log_{10} cfu/mL, mean, SD)
Experiment 1	5.7 (0.3)	6.7 (0.3)
Experiment 3	5.6 (0.08)	6.4 (0.6)

- REACTOR MEDIUM (considered a mixture of planktonic and stationary growing bacteria)

STRAIN A	**AFTER STATIC PHASE** (log_{10} cfu/mL, mean, SD)	**AFTER DYNAMIC PHASE** (log_{10} cfu/mL, mean, SD)
Experiment 1	8.7 (0.4)	8.8 (0.4)
Experiment 3	8.7 (0.3)	8.4 (0.5)

STRAIN B	**AFTER STATIC PHASE** (log_{10} cfu/mL, mean, SD)	**AFTER DYNAMIC PHASE** (log_{10} cfu/mL, mean, SD)
Experiment 1	9	8.5 (0.2)
Experiment 3	8.2	8.2

Figure 7.2 Bacterial growth over time in the absence of colistin and meropenem (growth controls) for biofilm-embedded bacteria (coupons) and reactor medium.



Footnote Figure 7.2

A. Represents bacterial growth (without antibiotics) for biofilm embedded bacteria, strains A (Experiments 1 and 3) and strain B (Experiment 1). At 72h of growth controls for Experiment 1, enumerate coupons of viable cells were 7.2 (0.4) and 6.7 (0.11) for strains A and B, respectively. In Experiment 3, enumerate coupons for viable cells at 54h were 7.3 (0.3). Enumerate cells are expressed in $\log_{10}$ cfu/mL.

B. Represents bacterial growth (without antibiotics) for reactor medium (considered a mixture of planktonic and stationary growing bacteria), strain A (Experiments 1 and 3) and strain B (Experiment 1). At 72h of growth control for Experiment 1, enumerate viable cells suspended in medium were 8.7 and 8.3 for strains A and B, respectively. In Experiment 3, enumerate viable cells suspended in medium at 54h were 8.9. Enumerate cells are expressed in $\log_{10}$ cfu/mL.

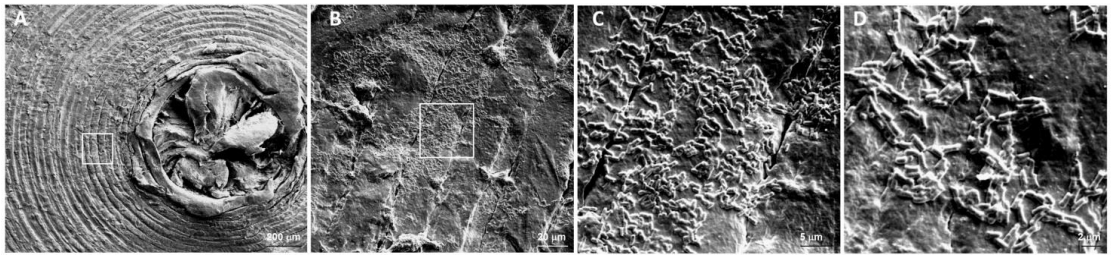
Time on the x-axis begins at -24h (immediately after the static phase), but corresponds to -72h in Experiment 1. Although the dynamic phase was 72h in experiment 1 and 24h in experiment 3, for both cases Time 0 corresponds to the moment immediately after the dynamic phase when the entire conditioning phase has occurred. The y-axis represents the $\log_{10}$ cfu/mL quantification. Data are presented as means (SD) or as means.

7.1.2 Scanning electron microscopy (SEM)

To assess biofilm formation within different operating conditions (experiments 1 and 3, strain A), SEM analysis was performed with the coupons. SEM images were also processed at the end of the therapeutic experiments to display the activity of the antibiotics. For each experiment, two coupons were analysed: one after the dynamic phase (Time 0) and one after the treatment (experiment 1, after 72 h under meropenem plus colistin treatment; experiment 3 after 54 h under treatment with meropenem).

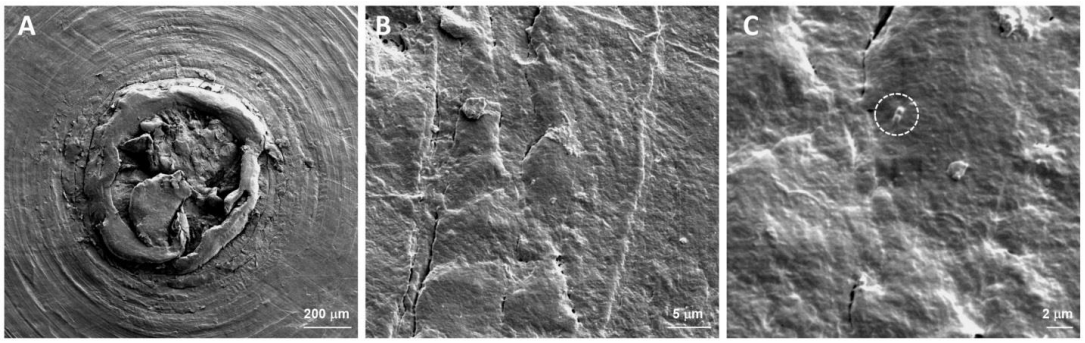
- **Experiment 1**

  - After the biofilm growing phase **(Figure 7.3)**



Footnote Figure 7.3
A. Image of the general aspect of a Teflon coupon at low magnification. **B**, **C,** and **D** are observations made at higher magnification for displaying bacilli and their organization. The bacteria distribution appreciated in images **C** and **D** suggesting that bacteria are associated between them since they are making side-to-side contact or forming a row. Scale bar A (200 µm), B (20 µm), C (5 µm), and D (2 µm). Region of square of A corresponds to image B, and square B to image C.

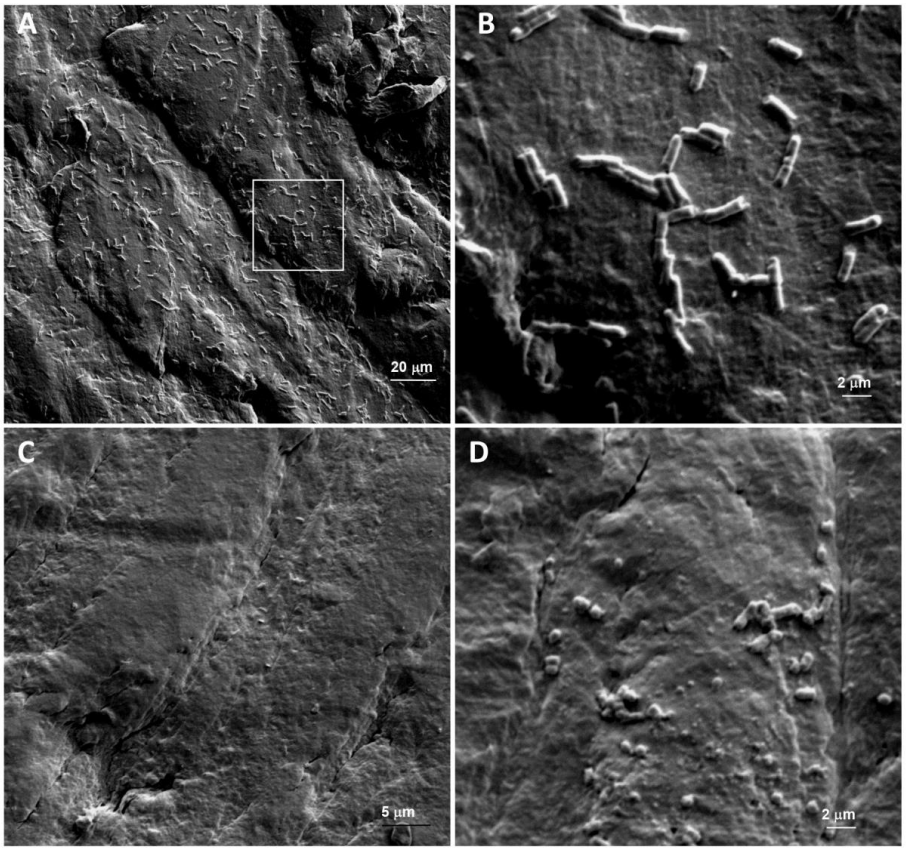
  - After treatment with meropenem plus colistin **(Figure 7.4)**



Footnote Figure 7.4
A. Image of the general aspect of a Teflon coupon at low magnification. **B** and **C** are observations made at higher magnification for displaying bacilli and their organization. In several areas, there are no bacteria (such image **B**). However, in other areas, isolated dividing bacilli are observed with the morphology shown in image **C** (marked with a round dotted line), where a single bacillus has begun to divide by bipartition and has stopped at that phase. Scale bar A (200 µm), B (5 µm), and C (2 µm).

- **Experiment 3**

  - After the biofilm growing phase and after 54 h with meropenem **(Figure 7.5)**



Footnote Figure 7.5
Images **A** and **B** correspond to 0h and **C** and **D** to the end of treatment. Compared to Experiment 3, after the biofilm growing phase there are fewer bacteria. As described in Figure 1, the bacteria distribution appreciated in images **A** and **B** suggests that bacteria are associated between them, mainly by forming a row (image **B**). After treatment (images **C** and **D**), there are several areas without bacteria, and some areas display few bacteria with a different morphology since they have begun to divide by bipartition and have stopped in that phase, as shown in image **D**. Scale bar A (200 µm), B (2 µm), C(5 µm), and D (2µm).

7.2 Pharmacokinetics analysis

Measured meropenem Cmax values were 110.3 (2.09) mg/L, mean (SD). The observed mean $t_{1/2}$ for the simulated intermittent meropenem dosage regimens was 59.6 (3.9) minutes for the targeted value of 60 minutes.

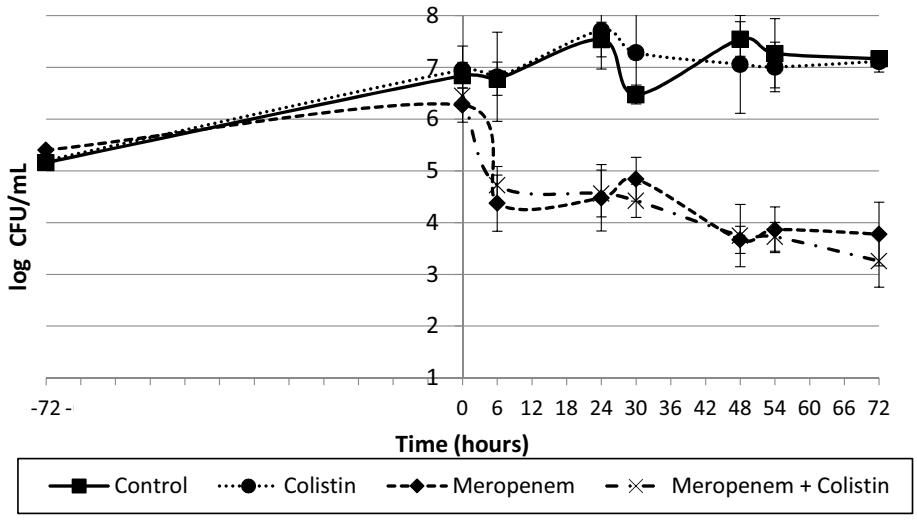
7.3 Microbiological response and emergence of antibiotic resistance

Within this model, regimens with colistin and meropenem alone or in combination were tested and followed up 54 or 72 hours. Control experiments (drug free) under the same conditions

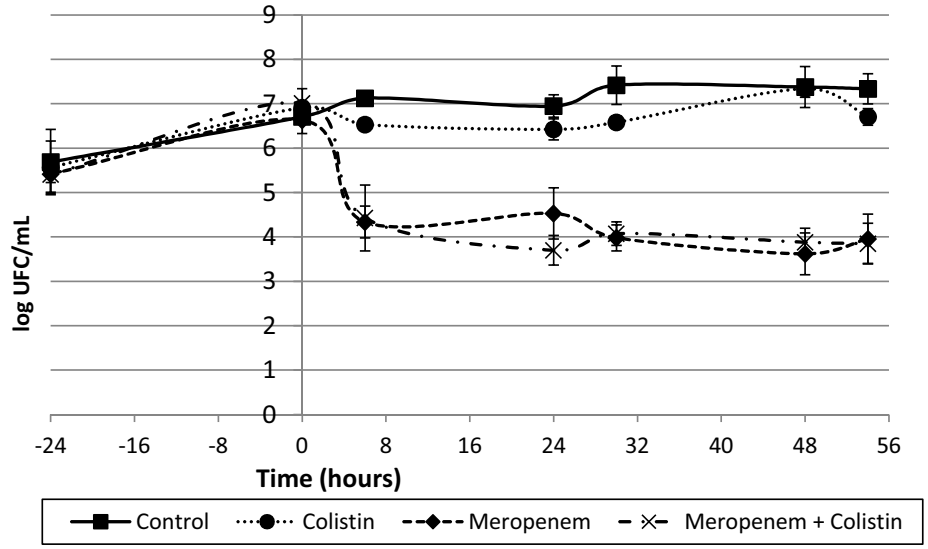
were also performed and the time-course profiles of bacterial counts of biofilm-embedded (coupon samples) and medium sample controls are shown above (**Figure 7.2**). In addition, the results of the therapeutic experiments are exposed below. Log changes in viable cells counts in the presence of colistin, meropenem, or a combination of both are outlined in **Figure 7.6** (biofilm-embedded cells)

Figure 7.6 Bacterial killing curves.

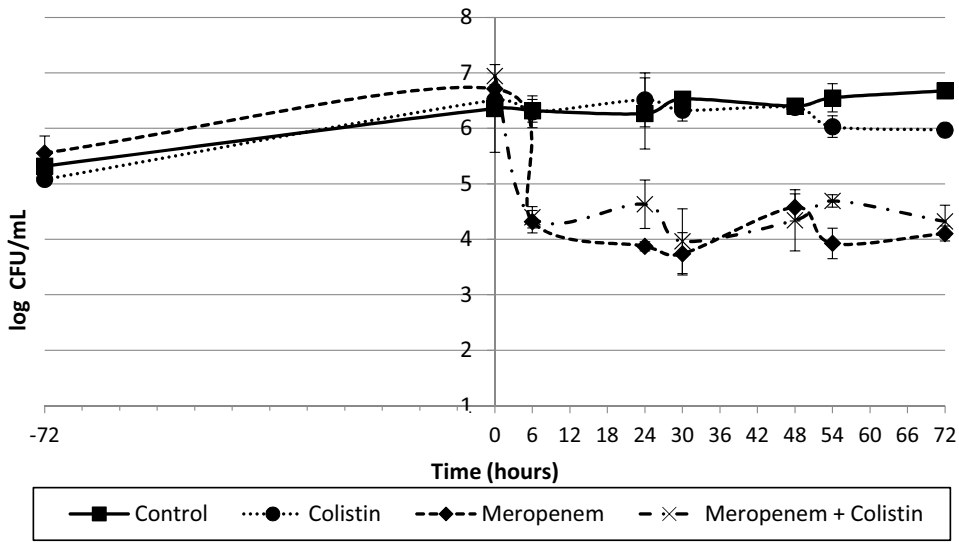
A



B



C



Footnote Figure 7.6

Bacterial killing by colistin, meropenem and a combination of both against biofilm-embedded cells of two different *K. pneumoniae* strains under two different biofilm growing conditions. The control curve is also represented. Data are presented as means (SD) or as means.

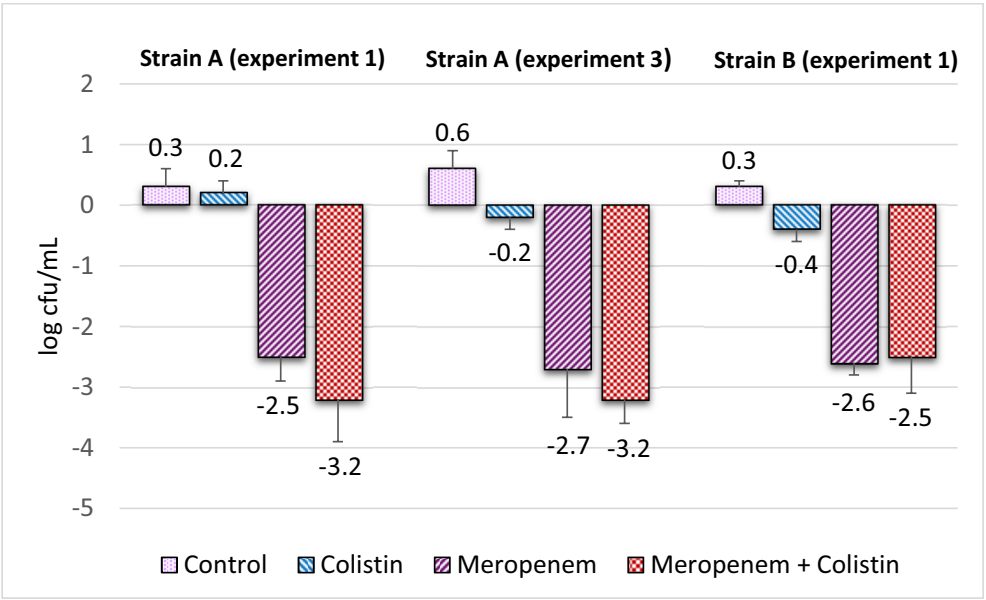
A. Represents results from strain A under biofilm growing conditions according to experiment 1
B. Represents results from strain A under biofilm growing conditions according to experiment 3
C. Represents results from strain B under biofilm growing conditions according to experiment 1

The colistin monotherapy regimen 3.5 mg/L was ineffective against both strains of *K.pneumoniae* in both situations: bacteria attached to (**Figure 7.6**) or suspended in the medium. This regimen resulted in the rapid emergence of colistin resistance in all bacterial populations within the reactor. Thus, among the biofilm-embedded bacteria, resistant strains appeared during the first 24 hours of treatment (MIC 4-6mg/L). Their presence then increased and they expressed progressively higher MIC values (MIC 12-16 mg/L at 30 h, and 32-48 mg/L at the end of treatment).

The combination of colistin and meropenem achieved rapid and sustained killing until the end of the treatment against biofilm-embedded bacteria **(Figure 7.6)**, which was also reported by SEM images **(Figure 7.4 B-C)** showing extended areas without bacteria. However, the killing rate differs between strains and biofilm growing conditions (experiments 1 and 3). Decreases in the mean $\log_{10}$ cfu/mL (SD) at the end of treatment of -3.2 (0.7) and -3.2 (0.4) were observed with strain A in experiments 1 and 3, respectively. However, this combination was not bactericidal in biofilm against strain B (under experiment 1 conditions); with a decrease of -2.5 (0.6) $\log_{10}$ cfu/mL.

Meropenem monotherapy also achieved rapid killing, which was also reported by SEM images **and Figure 7.5 C-D**, although it was not bactericidal in any of the testing strains or conditions: -2.5 (0.4), -2.7 (0.8), and -2.6 (0.2) in strain A (experiments 1 and 3) and strain B, respectively (**Figure 7.7**). The combination of colistin plus meropenem resulted in greater killing than meropenem in monotherapy within strain A, mainly in experiment 1 (-3.2 vs -2.5, *p*=0.021). This difference became less obvious in experiment 3 (-3.2 vs -2.7, *p*=NS). No emergence of colistin-resistant subpopulations was observed during the combined treatment.

Figure 7.7 Decreases in counts of biofilm-embedded bacteria from Time 0 to the end of the the treatment.



Footnote Figure 7.7.
Bacterial counts are expressed in mean log numbers of cfu/mL (± standard deviation [SD]).

Regarding the antibiotic efficacy against bacteria from the reactor medium, meropenem monotherapy produced decreases at the end of treatment, mean $\log_{10}$ cfu/mL (SD), of -2.8 (1.5) and -2.4 in experiment 1 for strains A and B strains, respectively, and a minor killing rate with a decrease of -1.2 (0.11) in experiment 3 (strain A). The killing rate was similar when using a combination of colistin and meropenem in experiment 1 (- 2.6 and -2.3 for strains A and B) without statistical significant differences when compared with the meropenem monotherapy. In experiment 3, this difference in efficacy between the two therapeutic groups was more prominent, and the combination achieved a higher killing than meropenem [-2.7 (0.03) vs -1.24 (0.11), *p*=0.026].

Overall, our preliminary results show slight benefits of adding colistin to β-lactams for biofilm-embedded ESBL-producing *K.pneumoniae.* Therefore, these results may place the combination of colistin and carbapenems in a better position than monotherapy (carbapenems) against biofilm-embedded infections caused by carbapenem-susceptible ESBL-producing *K.pneumoniae* strains. However, for these potential benefits to be generalized and clinically relevant, they should be confirmed in future studies.

DISCUSSION

Orthopaedic device-related infection is an increasingly common pathology that represents a first magnitude health-care problem, mainly in developed countries. Its prevalence is due to the extraordinary development of orthopaedic surgery with novel orthopaedic devices used for fracture fixation, and the increasing life expectancy which promotes prosthesis implantations as a consequence of degenerative bone diseases in the elderly population. Moreover, it is considered a difficult-to-treat infection mainly because of its etio-pathogenesis, which involves the participation of bacteria in a stationary phase of growth forming a biofilm structure on the device's surface. Thus, it is essential to ensure optimal management to achieve the best outcomes in terms of infection healing and in terms of the impact on patients' lives. The evaluation of antimicrobial efficacy is also fundamental to designing the best antibiotic regimes for each specific microorganism. PK/PD could help clinicians to understand the antibiotic behaviour and optimize antibiotic treatment, primarily in cases caused by multidrug-resistant strains. Finally, therapeutic drug monitoring for individualized antibiotic doses and guiding therapy in different clinical situations is necessary in the daily clinical practice.

1. ON THE MANAGEMENT OF ORTHOPAEDIC DEVICE-RELATED INFECTIONS

1.1 The importance of an accurate diagnosis for prosthetic joint loosening

Joint prosthesis loosening can be the result of either an aseptic process or infection. Thus, it is important to reach the correct diagnosis and provide appropriate treatment. Clinical characteristics are the main guide for the initial suspicion of the cause of loosening. However, surgical findings (macroscopic pus or histology) and microbiological cultures of surgical samples have proven useful for clinicians to identify some cases of infection among presumed aseptic loosening (AL) (Cobo and Del Pozo 2011; Del Pozo and Patel 2009; Tsukayama et al. 1996; Zimmerli et al. 2004). Definitive criteria of infection is considered when ≥2 positive cultures are isolated with the same microorganism, or a virulent microorganism is isolated in a single sample (Atkins et al. 1998; Osmon et al. 2013). But between having at least two from five surgical samples or having aseptic samples there are other intermediate situations that are not clearly classified, and this becomes more confusing when complementary sonication samples are performed. As such, the clinical characteristics of a case series of patients with a presumed diagnosis of AL were analysed, according to microbiological findings at the time of surgical revision, and compared with a cohort of patients with late chronic prosthetic joint infection (LCPJI).

In this study, 13% of cases with pre-surgical suspicion of AL had microbiological definitive criteria of PJI. While these cases may have belonged to a misdiagnosed group, it seems that the group has its own characteristics. When we compared them with a cohort of patients with LCPJI, we observed that this cohort had a significantly different dynamic trend in the evolution of prosthesis failure, with a shorter time from implantation to revision (prosthesis age) and notably higher bone lysis. These differences suggest a more aggressive process in cases of LCPJI, probably with a high bacterial load and obvious clinical signs of infection.

Cases with a single positive culture from intraoperative tissues were also well documented in the present study in 22 patients (29%), of which, 10 also had a concordant positive sonication culture. The classification of these cultures as infection or contamination of the surgery and laboratory processes remains challenging. The probability that some cases may represent real clinically silent PJI was previously calculated at approximately 8% (Atkins et al. 1998). Overall, the accurate interpretation of a single positive tissue culture is of great clinical concern because a diagnosis of definitive PJI or AL defines different therapeutic approaches. In recent recommendations, the sonication of the removed prosthetic components is proposed to distinguish between infection and contaminated prostheses (Piper et al. 2009; Portillo et al. 2012; Trampuz et al. 2007). Although there is no formal consensus on the sonication protocol and the number of microorganisms required for considering infection (Osmon et al. 2013), sonication samples provide new microbiological information that clinicians should interpret. In our study, we identified a group of patients with a single positive tissue sample and concordant sonication fluid culture. In this group, we could apply criteria for considering prosthesis loosening caused by infection. It is likely that centres which routinely process sonication fluid, consider these cases as definitive diagnoses of PJI and treat these patients with additional antibiotics. The sonication was concordant with conventional cultures in 75% of cases in the group with ≥2 positive tissue samples; and the low percentage of discordant results in the case series cases, supported the presence of a non-contaminant microorganism from sonicated prostheses in these situations (defined as a single positive tissue sample plus a concordant sonicated fluid sample).

Another group of patients was identified by presenting a single positive sample (tissue or sonication). Twelve patients had a single positive tissue culture that could be considered probable contamination (32%) since this proportion was similar to single discordant tissue samples also found in the group with definitive criteria of infection (25%). In contrast, 28

patients (74%) had a positive culture from the sonicated fluid and some had the same microorganism in both prosthetic components. In these cases, it is difficult to determine whether the microorganisms that were isolated in the sonication fluid are contaminants or were attached to the surface of the removed prosthesis. When comparing this percentage with that of the discordant results in cases with proven infection, significant differences were observed. These contrasting data suggest that isolated positive cultures from sonicated fluid should not always be considered contaminants. Nevertheless, the optimal therapeutic management of cases with low bacterial inoculum is not clearly defined. In the present study, patients with one positive tissue sample and a concordant sonication fluid sample were not treated with long-term antibiotics, but they did not develop persistence or relapse of initial infection. These results are in accordance with the results reported by Barrack et al. (Barrack et al. 2007), which supported that in most cases, prosthesis removal is sufficient to eradicate the low bacterial inoculum. However, considering the particularities of foreign-body infections while waiting for further clinical evidence, prudent interpretation of a single positive culture is recommended.

The evaluation of clinical findings in our cohort of cases with pre-surgical suspicion of AL showed different dynamics in the prosthesis explantation surgery between the groups that were established according to the microbiological results. There was a progressive increase in prosthesis age from patients with a clear diagnosis of PJI to cases with single positive samples. Moreover, when the results were analysed four years after implantation of the arthroplasty, revised arthroplasties were more common among patients with LCPJI (82%) and patients with presurgical suspicion of AL and microbiological findings of infection (50-58%), than among patients without findings of infection from intraoperative cultures (31-32%). These results, in accordance with previous reports, suggest that early prosthesis failure is associated with a strong likelihood of infection regardless of the presence or absence of compatible clinical signs or symptoms (Holinka et al. 2011; Ince et al. 2004; Portillo et al. 2013; Del Pozo and Patel 2009; Trampuz et al. 2007).

These findings show that high bacterial inoculum (the number of positive tissue and sonicated fluid cultures) is associated with a shorter time from primary arthroplasty to revision surgery. Thus, bacteria were real pathogens that could participate in early implant failure. No differences in the degree of bone lysis were detected among the cases in relation to the microbiological samples. However, a longer time between prosthesis implantation and revision was associated with more bone lysis. This finding supports the probable role of microorganisms in prosthesis failure but not in the degree of bone lysis, which is related to

prosthesis age. It is still not clear whether isolated low virulence organisms can survive around the implant without pathological involvement, participate in prosthetic loosening, or cause delayed low-grade infections that mimic natural aseptic failure (Nelson et al. 2005). The pathogenesis of aseptic loosening is probably a multifactorial process that is not well known.

1.2 Rate of success with one-stage or two stage surgical revision for hip PJI

This study was conducted to address the uncertainties regarding the effectiveness of one-stage and two-stage revision strategies for treating PJI of the hip, using re-infection as the outcome of interest. This large-scale study shows the differences in baseline and follow-up characteristics between one-stage and two-stage revision strategy patients. The proportion of patients with a previous hip surgery, other than the index surgery or a previous PJI, was higher in the one-stage revision strategy group than in the two-stage group. Within this one-stage revision group, patients seemed to have severe PJI at presentation compared with the two-stage group given their higher levels of circulating CRP and the higher proportion of patients presenting with an abscess, sinus, draining wound, or fistula. These findings were unexpected, as patients with severe PJI usually undergo a two-stage revision to facilitate additional antimicrobial strategies.

The one-stage revision strategy is traditionally thought to expose patients to a higher risk of reinfection by residual bacteria and should only be used in select cases, such as for patients with known organisms and sensitivities, non-immunocompromised patients, and in the absence of a sinus tract (Gulhane et al. 2012; Vanhegan et al. 2012). The results of the time to onset of infection from index implantation suggest that most PJIs in the one-stage group were late infections (more than 24 months after surgery), while those in the two-stage group were delayed infections (3 to 24 months after surgery). Given that late infections are mostly acquired by hematogenous seeding (Zimmerli et al. 2004), this might account for the severity of PJI in the one-stage revision group. *Staphylococcus* species were the most common causative organisms for PJI in both treatment groups; and these results are consistent with the literature (Hickson et al. 2015; Stefánsdóttir et al. 2009; Zimmerli et al. 2004).

Unadjusted Kaplan-Meier curves suggest a higher re-infection rate for the two-stage revision strategy compared with one-stage revision. However, given the imbalance between several baseline sociodemographic and clinical characteristics, such unadjusted results are likely confounded. In multivariate analyses, there was no evidence of a statistically significant increased risk of re-infection when the two-stage revision strategy was compared with the

one-stage revision strategy. However, there was a trend towards a higher risk of re-infection in the two-stage revision group.

For several decades, the two-stage revision strategy has been presumed to be more effective than the one-stage strategy for treating PJIs (Matthews et al. 2009; Zimmerli et al. 2004). However, the two-stage strategy has several drawbacks, such as significant pain and functional impairment, longer hospitalization periods, increased risk of mortality (Cahill et al. 2008; Matthews et al. 2009; Wolf et al. 2011), and higher healthcare costs compared to one-stage revision (Klouche et al. 2010).

The outcomes of this study suggest that one-stage revision may be as effective as the two-stage revision strategy in treating infected hip prostheses, even for patients with characteristics that were previously considered inappropriate for one-stage revision, such as those with sinus tracts at the time of presentation. This novel thought seems to concur and further extend that of recent aggregate reviews conducted on the topic (Kunutsor et al. 2015). Reinfection rates were similar between two procedures, as reported in other current studies (Beswick et al. 2012; Leonard et al. 2014) in which one-stage revision showed superior functional outcomes (Leonard et al. 2014). Therefore, the one-stage strategy might be considered a potentially effective procedure for PJI of the hip.

Despite the novelty and strengths of the current study, there are several limitations which deserve consideration. Because the revision strategy only varied between cohorts, a head-to-head comparison of the two revision strategies could not be made and appropriate inferences could only be made based on differences in re-infection rates between studies using either treatment strategy. Moreover, most studies were unable to contribute relevant clinical data, which precluded the ability to adjust for a comprehensive panel of potential confounders, thereby introducing the possibility of residual confounding. Detailed subgroup analyses were also unable to conduct by clinically relevant subgroups. Apart from the control of infection, maintenance of joint function is also considered an important factor for successful outcomes following one-stage or two-stage revision (Kendoff and Gehrke 2014; Rasul et al. 1991). Several studies focusing on outcomes after joint surgery have shown that patients are frequently more concerned with pain and joint function than clinical indices such as re-infection rates (Jeffery et al. 2011; Moore et al. 2015). However, the two revision strategies could not be compared using measures of joint function.

2. ON THE ASSESSMENT OF ANTIMICROBIAL EFFICACY FOR THE TREATMENT OF ORTHOPAEDIC DEVICE-RELATED INFECTIONS

INFECTIONS BY *STREPTOCOCCUS SPP*

2.1 The role of antibiotic combination with rifampin for streptococcal PJI and its impact on the prognosis

Within the largest series assessing the management of streptococcal PJI by DAIR, our results show an overall long-term likelihood of curing the infection and keeping the prosthesis of 57%. This represents a modest prognosis compared to several previous studies which suggested that streptococcal have a more favorable outcome than other etiologies (s*taphylococcus*, GNB), with success rates that may reach 65-100% (Betz et al. 2015; Everts et al. 2004; Meehan et al. 2003; Sendi et al. 2011; Zeller et al. 2009). However, few other studies report a poor prognosis, even with lower success rates than ours (Corvec et al. 2011; Duggan et al. 2001). It is presumably dependent on the selection criteria used.

Predictors of poor outcomes in this series were similar to those found in previous studies of PJI by staphylococci and GNB managed by DAIR. In previous reports, patients with bacteremia, who required >1 debridement or with high CRP levels had a bad prognosis (Brandt et al. 1997; Lora-Tamayo et al. 2013; Martínez-Pastor et al. 2009; Rodríguez-Pardo et al. 2014; Tornero et al. 2014; F. Vilchez et al. 2011). In this series, bacteremia and infection by *S.pyogenes* were independent predictors of *early failure*. Otherwise, the streptococcal species presented a similar pattern regarding clinical presentation and outcome, though *S.pneumoniae* presented more frequently as a hematogenous infection and was usually associated with a better prognosis (non-significant).

The percentage of hematogenous infection in this series was notably high when compared with PJI by *S.aureus* (52% vs 15%) (Lora-Tamayo et al. 2013). Although staphylococcal hematogenous PJI has been reported to carry a poor prognosis (Lora-Tamayo et al. 2013; Sendi et al. 2011; Vilchez et al. 2011), this study did not demonstrate this association. It is possible that the ability of β-lactams to clear bacteremia and planktonic infection in hematogenous PJI could be higher for streptococci than for staphylococci.

Univariate and multivariate analyses have shown that some debilitating baseline conditions are associated with a worse outcome. Together with a previous large series, rheumatoid arthritis, immunosuppressant therapy, and chronic renal insufficiency seem to be associated with a higher risk of treatment failure when attempting DAIR (Lora-Tamayo et al. 2013;

Rodríguez-Pardo et al. 2014). The exchange of removable components was associated with a favorable outcome, which has also been observed in previous studies (Choi et al. 2011; Lora-Tamayo et al. 2013). This is consistent with the physical removal of the biofilm and likely stands as a surrogate marker of an exhaustive surgical debridement

The IDSA criteria for instituting DAIR were not met by all cases in this study. Consistent with previous studies, this allowed us to confirm the usefulness of these criteria for selecting suitable candidates for DAIR (Lora-Tamayo et al. 2013; Rodríguez-Pardo et al. 2014; Sendi et al. 2011; Tschudin-Sutter et al. 2016). The definition of early postoperative PJI has varied over time in several landmark publications, ranging from one to three months (Tsukayama et al. 1996; Zimmerli et al. 1998, 2004), with the IDSA recommending that DAIR should be performed within one month after placing the prosthesis (Osmon et al. 2013). A similar prognosis was observed for patients with postoperative infection whose symptoms began within the first month after prosthesis placement and those whose symptoms started between the first and third month. A similar finding was also observed for staphylococcal PJI (Lora-Tamayo et al. 2013), which emphasizes this three-month time limit over a stricter cut-off.

Unfortunately, the possibility of performing an accurate analysis of antimicrobial efficacy was impaired by the retrospective nature of this study and the heterogeneity of the therapeutic schedules. Still, the large size of our series allows for some interesting considerations.

β-lactams have classically been the preferred therapy for streptococcal infections, including PJI, providing good activity for the initial planktonic phase of these infections (Baker et al. 1981). However, once this initial phase has passed, the antibiofilm profile of these antimicrobials is questionable because, as with any antibiotics with a mechanism of action dependent on cell wall synthesis, they will become less effective against biofilm-embedded bacteria (Costerton et al. 1999). There is now strong evidence that β-lactams have poor efficacy for staphylococcal and GNB PJI, especially when contrasted with other antibiotics that have superior antibiofilm profiles, such as rifampin against staphylococci or fluoroquinolones against GNB (Lora-Tamayo et al. 2013; Martínez-Pastor et al. 2009; Rodríguez-Pardo et al. 2014; Senneville et al. 2011; Zimmerli et al. 1998). However, these findings have not been demonstrated in streptococcal PJI, which haves been disregarded in these studies.

Our patients were mostly treated with β-lactams according to classic recommendations and routine clinical practice. The multivariate analysis of *failure after therapy* showed that this therapy was beneficial, with superiority over less effective alternatives such as glycopeptides. This beneficial effect was likely dependent on the activity of β-lactams against planktonic

bacteria in the first weeks of treatment (Sendi and Zimmerli 2012). However, other data could indicate the suboptimal antibiofilm activity of β-lactams in our series, along with evidence of a beneficial effect of rifampin. In patients who completed a long course of treatment with β-lactams, no statistical differences were observed among those receiving rifampin or not, but a tendency toward a better prognosis was found in those treated with combined therapy (10.0% failure rate vs 16.8%). In addition, initial treatment with rifampin was also identified as an independent predictor of a favorable outcome.

Our analysis has the inherent limitations of retrospective studies and the significant heterogeneity of patients included across the participating institutions, especially regarding their management. The fulfilment of the IDSA criteria, the participation of different surgical teams, and the decision about whether to use rifampin are all examples of this variability. Still, these cases form a large cohort of patients with streptococcal PJI treated by DAIR, presenting an opportunity to study their prognosis in the best and worst possible clinical scenarios. Thus, an overall perspective of the clinical problem is provided.

Within the largest case series of streptococcal PJI managed by DAIR, we showed a not-so-good prognosis than previously reported. However, the beneficial effects of exchanging the removable components during the debridement and the potential benefit of adding rifampicin could improve the overall success rate of these infections.

INFECTIONS BY MDR GRAM-NEGATIVE BACILLI

- **The use of β-lactams in continuous infusion**

2.2 The efficacy of using β-lactams in continuous infusion to treat Gram-negative bacteria through a safety position by calculating the predicted concentration of β-lactams in patients' plasma or by measuring β-lactam concentration in human plasma using UHPLC-MS/MS (if available).

The optimization of BL efficacy by administration in CI may be essential in particular scenarios of difficult-to-treat infections (Alou 2005; Cappelletty et al. 1995; Mouton and Vinks 1996, 2007), since it maintains the antibiotic concentration above the MIC for longer, particularly for bacteria with high MIC and also may recover the antimicrobial efficacy against multidrug-resistant bacteria that exhibit high MIC values (Dulhunty et al. 2013; Roberts et al. 2007). Based on PK/PD parameters, in infections caused by susceptible strains, BL-CI could achieve optimal levels with lower doses than the standard doses recommended for intermittent bolus

administration. However, the potential benefits of BL in CI administration against biofilm-related infections have not been sufficiently evaluated. During the last years we have been using these regimens in our daily clinical practice to treat patients with GNB osteoarticular infections, often caused by MDR strains, and our results have been evaluated.

The dosages of BL used in CI have not been established. Clinicians tend to prescribe the same total dose administered for IB, but this strategy may pose a risk of overdosing and toxicity (Moriyama et al. 2010). Therapeutic drug monitoring (TDM) appears to be essential for individualizing antibiotic dosages and for guiding therapy in different clinical situations (Huttner et al. 2015). However, while it is commonly used in clinical practice for some antibiotics (i.e, vancomycin, aminoglycosides), this is not the case for BL Due progressive accumulation of the drug in the organism, which is mainly observed in patients with renal failure, the use of BL in continuous infusion (or extended infusion) should be properly administered. Therefore, TDM is advisable to guide therapy and anticipate potential toxic levels (Moriyama et al. 2010), mainly during prolonged treatments.

Through an institutional program, a UHPLC-MS/MS procedure was developed and validated to simultaneously measure the concentrations of nine BL antibiotics including amoxicillin, ampicillin, cloxacillin, piperacillin, cefepime, ceftazidime, cefuroxime, aztreonam, and meropenem) and two β-lactamase inhibitors (clavulanat and tazobactam) in plasma. The specificity of tandem spectrometry permits the measurement of different quantities with minimal preparation, and the sensitivity of the detector enables the use of small sample volumes. In addition, considering the time of analysis, versatility, flexibility and the analytical performance characteristics of selectivity, capability of detection, precision, trueness, recovery, and matrix effect, the UHPLC-MS/MS procedure is well suited to routine hospital practice for TDM of antibiotics in patients. This procedure could improve dose adjustment of BL during daily clinical practice, especially in critically ill patients with unpredictable PKs and those with bone and joint infections with prolonged antibiotic therapies. Given that the procedure permits the simultaneous measurement of all established BLs, its institutional use is available since several samples from multiple patients undergoing different BL regimens are measured together in minutes.

While drug monitoring of BL concentrations should be essential for guiding this therapy in different clinical scenarios, it is currently not applied in hospital routine practice. This study demonstrates that through simple equations (described in Material and Methods) clinicians can estimate the BL-CI dosage and its plasma levels in the early hours of treatment. After using

these equations, a correlation was identified between the estimated BL concentrations (C_{pred}) in patients' plasma and the concentrations measured by UHPLC-MS/MS (C_{obs}). Nevertheless, the C_{obs} tend to be higher overall than the C_{pred}, likely because the established BL clearance values were not adequately adjusted to the population cohort. Although these equations clearly improve the individualization of clinical doses; through our experience we have learned clinicians should be cautious when using these doses for different BLs or for patients in different weight or renal function groups.

BL pharmacokinetics in humans may not be explained by conventional linear models. In this regard, several sophisticated nonlinear pharmacokinetic models can better represent the pharmacokinetics of these antibiotics (Georges et al. 2009; Roberts et al. 2014). However, these models are difficult for clinicians to apply and also lacked in particular infections (e.g. osteoarticular infections). The use of the equations that are described, as a linear pharmacokinetic model, may be considered a limitation of our study. However, after clearly stating the weakness of these equations, they seem to offer new useful information for daily clinical practice.

In our case series, all patients except for one were healed. However, a conclusion cannot be made about the efficacy of using BL in CI, mainly due to the lack of a comparative treatment and the use of concomitant antibiotics or surgery. Furthermore, in a case series of patients with osteoarticular infections by MDR *Pseudomonas aeruginosa* (also presented in this thesis), BL administered by continuous infusion showed successful outcomes that were consistent with these benefits. BL used in CI was demonstrated as a safe therapy since no serious adverse events were detected in any of the studies though high concentrations (even around 100 mg/L) were maintained for a long time.

PK/PD studies have shown that the maximum killing rate occurs at concentrations three to four times above the MIC, but remains stable after exceeding this level. In this case series, several patients achieved levels that were more than four times above the MIC, mainly when treating susceptible strains with low MIC. It is unclear whether these higher (but still safe) antibiotic levels have improved the clinical outcome of the difficult-to treat-infections presented. Moreover, BL in CI could achieve prolonged antibiotic concentrations above the MIC, making several initially resistant strains become susceptible in terms of drug PK/PD (Dulhunty et al. 2013; Van Herendael et al. 2012; Moriyama et al. 2009, 2010; Mouton and Vinks 2007; Roberts et al. 2007). All these results support the benefits of BL in CI and encourage further studies to confirm these data.

- **The use of antibiotic combinations with colistin**

2.3 The benefits of combination therapy with colistin plus β-lactam for osteoarticular infections caused by MDR *Pseudomonas aeruginosa*.

The management of osteoarticular infection caused by MDR GNB represents a new challenge for the clinician, and no specific treatment has been defined. The role of β-lactams (BL) in treatment needs to be questioned. Indeed, when treating PJI caused by ciprofloxacin resistant GNB, BL monotherapy was associated with worse outcomes than fluoroquinolone monotherapy (treatment responses of 40% and 80%, respectively) (Rodríguez-Pardo et al. 2014). This scenario is further complicated for infections caused by MDR *Pseudomonas aeruginosa*, since several strains show reduced susceptibility or resistance to BL. Thus, limited antibiotic availability has led specialists to rediscover old drugs such as colistin and apply them to new therapeutic strategies.

A case series of osteoarticular infection caused by MDR *P. aeruginosa* at our hospital is presented in this thesis. Given the few published reports on this topic (Papagelopoulos et al. 2007; Valour et al. 2013), our results provide potentially relevant information about the efficacy of BL and colistin when used in combination. In this case series of 34 patients with osteoarticular infection caused by MDR *P. aeruginosa*, the overall cure rate was 50% after first-line therapy and >85% at the final outcome after rescue therapy. This sample contained more XDR than MDR strains of *P. aeruginosa*, at rates of 68% and 32%, respectively. Despite the greater degree of resistance in the latter strain, they seemed to be easier to eradicate. This is consistent with our previous experience regarding the lower virulence and pathogenicity of XDR *P. aeruginosa* in patients with bacteremia and infections in ICUs (Peña et al. 2013), suggesting a trade-off for the acquisition of MDR.

In terms of the antibiotic treatment, combination therapy with BL plus colistin was significantly more effective than monotherapy (with either BL or colistin) overall, even against strains that are susceptible to the β-lactams used. This fact supports previous thoughts based on the specific target of each antibiotic within the biofilm structure of GNB. The benefits of combined therapy were shown in patients who were considered even more difficult to treat (PJI and osteoarthritis managed with device retention), with a failure rate of 81.8% with monotherapy and 28.6% with the combination ($P<0.05$). Although limited previous information on this topic makes it difficult to compare these results, two clinical studies were identified (Papagelopoulos et al. 2007; Valour et al. 2013). Valour et al. reported a unique case series of bone and joint infection caused by MDR GNB (16 caused by *P. aeruginosa*) (Valour et al. 2013), with a cure

rate of 41% for orthopaedic device-associated infections (despite implant removal) using colistin alone. In our results the outcome was clearly optimized by a combination of BL and colistin (cure rate 71%). These data support the potential role of colistin in synergy with BL, especially against biofilm-associated infections.

The individual contribution of each antibiotic in the combination of BL and colistin) is difficult to separate out. Our clinical results are consistent with pharmacokinetic and pharmacodynamic considerations and with the results of experimental studies on this topic (Bergen et al. 2011). Moreover, in biofilms caused by GNB, colistin has been effective against less active bacteria located in the deeper layers of the biofilm structure, which contrasts with the majority of antibiotics that operate at the upper layers only, thereby targeting different subpopulations of the biofilm (Haagensen et al. 2007; Klausen et al. 2003; Pamp et al. 2008). This observation is supported by colistin's particular bactericidal activity, which is independent of hydroxyl radical formation and consumption (Brochmann et al. 2014). In addition, BLs are known to lose activity inside biofilms (Gilbert and Brown 1998; Gilbert et al. 1990). This is because their target is on the bacterial wall during the exponential growth phase, even when strains are fully susceptible to them. In addition, little is known about the efficacy of BL (alone or in combination) when strains are resistant or not fully susceptible. Even at lower doses, the synergistic effect of BL in combination with colistin could result from colistin's properties as a cationic peptide, placing BL in a better position against resistant strains by providing better antibiotic penetration (Bergen, Tsuji, et al. 2011; Zhang et al. 2000). Therefore, if further studies confirm our results, the recommendation of combined treatment (colistin plus a BL) could be extended not only to treat osteoarticular infections caused by MDR *P. aeruginosa*, but also to treat osteoarticular infections caused by all ciprofloxacin resistant GNB.

According to a pharmacokinetic analysis, it is unlikely that intravenous administration of colistimethate sodium (colistin's prodrug) could provide the required colistin concentrations to treat planktonic (Garonzik et al. 2011; Nation and Li 2009; Plachouras et al. 2009) or biofilm-associated infections (Hengzhuang et al. 2014). Moreover, colistin heteroresistance has been described for several strains of *P. aeruginosa* (Bergen et al. 2010; Lora-Tamayo et al. 2014) as a potential problem after exposure to colistin monotherapy. Given these considerations, current recommendations for patients admitted to the ICU suggest using very high doses of colistin (4.5 MIU twice a day) after an initial loading dose of 9 MIU (Plachouras et al. 2009). Nevertheless, this should be balanced with the increased risk of renal toxicity, which is the most common dose-dependent adverse effect of colistin (Antonucci et al.). We believe that, because osteoarticular infections caused by MDR *P. aeruginosa* in biofilm-associated infections

require long-term antibiotic therapy, they represent a different scenario from acute life-threatening infection. Moreover, the difference is greater when the role of combination therapy is considered because, due to their synergistic relationship, the addition of BL should allow clinicians to use lower doses of colistin without a loading dose. In our case series, patients with normal renal function were initially given colistin at 6 MIU/day without a loading dose, which was adjusted in patients with renal failure. Tolerance of this regimen was good and, although some patients suffered renal impairment due to colistin, renal function normalized after reducing the dose in all cases. In addition, the clinical results with lower doses of colistin in combination with BL remained acceptable, without colistin resistance. Although older studies have suggested that the diffusion of colistin into bone is poor (Falagas and Kasiakou 2005), recent studies have demonstrated good outcomes using lower colistin doses without a loading dose (Valour et al. 2013).

Therefore, we have added clinical experience to the pharmacokinetic, pharmacodynamic and experimental models of colistin in combination with BL. There is growing evidence that current recommendations should consider the combination of low-dose colistin with BL as an optimized treatment for osteoarticular infections caused by MDR *P. aeruginosa*. When used as part of a comprehensive treatment plan that includes appropriate surgical treatment (which included implant removal in some situations during initial therapy and in all cases of rescue therapy), this antibiotic combination is essential for achieving good outcomes in these difficult-to-treat infections.

2.4 The effect of adding colistin to meropenem against ESBL-producing *klebsiella pneumonia* biofilm in an *in vitro* experimental model.

As mentioned before, foreign body infections by MDR Gram-negative bacteria are concerning since there are a limited number of therapeutic options. In addition, the efficacy of β-lactams in monotherapy to treat these biofilm-related infections is doubted, even against susceptible strains. Increasingly, colistin is used as a last-line therapy for the treatment of such infections (Boucher et al. 2009; Li et al. 2006; Montero et al. 2009; Papagelopoulos et al. 2007; Valour et al. 2013). However, the emergence of colistin resistance has been reported *in vitro* with colistin monotherapy (Bergen et al. 2008; Bergen et al. 2011; Lora-Tamayo et al. 2014). This observed regrowth is, in part, due to the amplification of pre-existing colistin-resistant subpopulations (Bergen et al. 2008; Bergen et al. 2011). In this study, an *in vitro* model with a CDC biofilm reactor was standardized to further explore the colistin behaviour when combined with β-lactams to treat MDR enterobacteria, such as ESBL-producing *Klebsiella pneumoniae*.

Previous studies have established the optimal conditions for growing *Staphylococcus* (McLeod and Sandvik 2010; Parra-Ruiz et al. 2010; Williams and Bloebaum 2010) and *Pseudomonas aeruginosa* within the CDC biofilm reactor (Goeres et al. 2005; Lora-Tamayo et al. 2014). However, this model has not been well defined for enterobacteria, with the exception of preliminary experiments exposed by Goeres et al (Goeres et al. 2005).

Sequential standardization was needed before testing antimicrobial regimens. Three different growing conditions were tested according to previous work with other microorganisms and the generation rate of the strains. One of the tested conditions was discarded since it demonstrated an excessive number of planktonic bacteria. Subsequently, the remaining two conditions (experiments 1 and 3) were evaluated by the enumeration of viable embedded-biofilm bacilli and the presence and structure of biofilm observed by SEM. The bacteria distribution in SEM images after the conditioning phase suggests bacteria are associated and the probable presence of a physical and/or chemical structure of exopolysaccharides to connect bacteria within a biofilm architecture (Mah and O'Toole 2001). Experiment 1, which used a longer period of biofilm growth (72 h), showed a more mature biofilm with a greater number of bacteria.

Combination therapy has been suggested as a promising approach to increasing bacterial killing against GNB and minimizing the emergences of colistin resistance (Bergen, Forrest et al. 2011; Bergen, Tsuji, et al. 2011; Garonzik et al. 2011; Herrmann et al. 2010). Among therapeutic experiments, the potential effect of adding colistin to β-lactams against two strains of ESBL-producing *K. pneumoniae* (A and B) was explored *in vitro*. Colistin was administered as a continuous infusion to simulate the flat profiles of colistin observed at steady state across sodium colistin methanesulfonate (colistin prodrug) dosages (Garonzik et al. 2011; Plachouras et al. 2009). Meropenem was administered by intermittent bolus to simulate a meropenem elimination $t_{1/2}$ of one hour in patients. As expected, colistin in monotherapy was ineffective against biofilm-embedded bacteria and it resulted in the emergence of colistin resistance within the biofilm and the suspended bacteria in the medium. Either meropenem in monotherapy or its combination with colistin achieved rapid killing rates that were maintained until the end of treatment. Meropenem monotherapy presented non-bactericidal activity against biofilm-embedded bacteria from both carbapenem-susceptible strains (A and B), and its combination with colistin showed bactericidal activity against strain A. A statistically significant high efficacy of the combined strategy vs monotherapy was observed in strain A under experiment 1 conditions (with a larger biofilm), but this difference was not as prominent in experiment 3.

Overall, slightly better results were observed when adding colistin to β-lactams against biofilm embedded bacteria in our *in vitro* CBR model, even for carbapenem-susceptible strains. This hypothetic effect was mainly observed in strain A (meropenem MIC 0.06 mg/L) under conditions that produce a greater biofilm. The combined regimen avoided the emergence of resistant subpopulations of biofilm-embedded bacteria. Since these are preliminary results, further confirmatory experiments are needed to investigate the *in vitro* effects of adding colistin to β-lactams against ESBL-producing *K. pneumoniae* and to determine their clinical relevance.

CONCLUSIONS

A. On the management of orthopaedic device-related infections

A.1. Diagnostic aspects of PJI

- **Aim 1:** to analyse the microbiological and clinical findings in patients with suspected prosthetic joint aseptic loosening, and to compare to patients with chronic PJI.

  1.1 Even after following appropriate current guidelines, several patients with suspected prosthetic aseptic loosening have misdiagnosed PJI or some microorganisms in their samples.

  1.2 Sonication samples provide additional microbiological information that should help clinicians with the diagnosis of delayed low-grade infections that mimic natural aseptic failure but have one positive intraoperative tissue sample.

  1.3 Clinical parameters that determine the final prosthesis removal are correlated with the number of positive peri-prosthetic samples, supporting the probable role of microorganisms in the prosthesis failure rate.

A.2. Surgical management of PJI

- **Aim 2**: to evaluate the risk of re-infection following one-stage and two-stage surgical revision within hip PJI.

  2.1 The one-stage revision strategy may be as effective as the two-stage revision strategy, with similar re-infections rates between the two procedures.

B. On the assessment of antimicrobial efficacy for the treatment of orthopaedic device-related infections

B.1. Infections by *Streptococcus spp*

- **Aim 3**: to assess the efficacy of adding rifampicin to β-lactams for the treatment of streptococcal PJI managed with implant retention, and its impact on the prognosis.

  3.1 For the largest case series of stretopcoccal PJI managed with DAIR, this pathology showed a not-so-good prognosis as expected.

  3.2 The classical treatment with β-lactams seems ideal for fighting the planktonic component of streptococcal PJI; the addition of rifampin some days or weeks after

debridement could have a role in the antibiofilm profile to improve the current modest outcomes of this disease.

3.3 A concomitant and optimal surgical procedure is advised, following IDSA criteria and ensuring the exchange of removal components during the debridement. Similar prognosis results were observed when the IDSA criteria for DAIR were cutoff at the third month of revision rather than the first month.

B.2. Infections by MDR Gram-negative bacilli

The use of β-lactams in continuous infusion

- **Aim 4**: to standardize a measurement procedure based on UHPLC-MS/MS for the simultaneous determination of multiple β-lactam concentrations in human plasma.

  4.1 The development of a single UHPLC-MS/MS method for the simultaneous measurement of multiple β-lactam concentrations in human plasma enable the applicability of this method to routine clinical practice and the validation of an easy-to use equation for clinical use.

- **Aim 5**: to evaluate the efficacy and safety of β-lactams in continuous infusion for difficult-to-treat osteoarticular infections caused by Gram-negative bacilli, and to validate an easy method for clinical use.

  5.1 The use of β-lactams in continuous infusion is safe and effective, and may recover previously resistant strains that became susceptible in terms of their pharmacodynamic parameters. Lower doses could be used by BL-CI for susceptible strains.

  5.2 A simple equation could help clinicians to estimate the β-lactams continuous infusion dosage and its plasma levels in the early hours of treatment when UHPLC-MS/MS is not available.

The use of antibiotic combinations with colistin

- **Aim 6:** to evaluate the benefits of the combination of colistin and β-lactams to treat patients with MDR *Pseudomonas aeruginosa* infections.

  6.1 There is growing evidence to support that current recommendations should consider the combination of low-dose colistin with β-lactams as an optimized treatment for

osteoarticular infections caused by MDR *P. aeruginosa*. Further studies are needed to consider this therapy for ciprofloxacin-resistant GNB.

6.2 When used as part of a comprehensive treatment plan that includes appropriate surgical treatment, this antibiotic combination is essential for achieving positive outcomes for these difficult-to-treat infections.

- **Aim 7:** to study the effect of adding colistin to β-lactams against ESBL-producing *klebsiella pneumonia*e biofilm in an *in vitro* experimental model.

7.1 As expected, colistin in monotherapy was ineffective against biofilm-embedded bacteria and resulted in the emergence of colistin resistant strains.

7.2 Meropenem in monotherapy and its combination with colistin achieved rapid killing rates that were maintained until the end of treatment. However, only the combination showed bactericidal activity in one of the tested strains of ESBL-producing *Klebsiella pneumoniae* and its effect was more pronounced under conditions that produced a greater biofilm. The combined therapy avoided the emergence of colistin-resistant strains.

7.3 Our preliminary results may indicate a slight overall superiority *in vitro* of adding colistin to β-lactams against carbapenem-susceptible ESBL-producing *klebsiella pneumoniae*. Furthermore, studies are planned to explore this field and determine their clinical relevance.

REFERENCES

Ahsman MJ, **Wildschut ED**, **Tibboel D**, **Mathot RA**. 2009. Microanalysis of beta-lactam antibiotics and vancomycin in plasma for pharmacokinetic studies in neonates. Antimicrob Agents Chemother **53**:75–80.

Alou L. 2005. Is there a pharmacodynamic need for the use of continuous versus intermittent infusion with ceftazidime against Pseudomonas aeruginosa? An in vitro pharmacodynamic model. J Antimicrob Chemother **55**:209–213.

Antonucci E, **Taccone FS**, **Regolisti G**, **Cabassi A**, **Morabito S**, **Pistolesi V**, **Di Motta T**, **Fiaccadori E**. 2014. [Colistin: a review]. G Ital Nefrol **31**.

Ariza J, **Euba G**, **Murillo O**. 2008. [Orthopedic device-related infections]. Enferm Infecc Microbiol Clin **26**:380–390.

Atkins BL, **Athanasou N**, **Deeks JJ**, **Crook DW**, **Simpson H**, **Peto TE**, **McLardy-Smith P**, **Berendt AR**. 1998. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. The OSIRIS Collaborative Study Group. J Clin Microbiol

Baker CN, **Thornsberry C**, **Facklam RR**. 1981. Synergism, killing kinetics, and antimicrobial susceptibility of group A and B streptococci. Antimicrob Agents Chemother **19**:716–25.

Banit DM, **Kaufer H**, **Hartford JM**. 2002. Intraoperative frozen section analysis in revision total joint arthroplasty. Clin Orthop Relat Res 230–8.

Barrack RL, **Aggarwal A**, **Burnett RSJ**, **Clohisy JC**, **Ghanem E**, **Sharkey P**, **Parvizi J**. 2007. The fate of the unexpected positive intraoperative cultures after revision total knee arthroplasty. J Arthroplasty **22**:94–9.

Benito N, **Franco M**, **Ribera A**, **Soriano A**, **Rodriguez-Pardo D**, **Sorlí L**, **Fresco G**, **Fernández-Sampedro M**, **Dolores del Toro M**, **Guío L**, **Sánchez-Rivas E**, **Bahamonde A**, **Riera M**, **Esteban J**, **Baraia-Etxaburu JM**, **Martínez-Alvarez J**, **Jover-Sáenz A**, **Dueñas C**, **Ramos A**, **Sobrino B**, **Euba G**, **Morata L**, **Pigrau C**, **Coll P**, **Mur I**, **Ariza J**, **Barcenilla F**, **Pérez-Villar F**, **Prats-Gispert L**, **Cisterna R**, **Ibarra S**, **López Í**, **Santamaría JM**, **Cabo J**, **García D**, **Lora-Tamayo J**, **Murillo O**, **Pedrero S**, **Álvarez-Parrondo S**, **Muedra-Font R**, **Raya-Fernández C**, **Rodríguez-Alonso C**, **Moreno A**, **Blanco-Martínez-de-Morentin MA**, **Cabo-Magadan R**, **Combalia A**, **García S**, **Martínez-Pastor JC**, **Tornero E**, **Merino-Pérez J**, **Montejo JM**, **Alier A**, **Horcajada JP**, **Plasencia V**, **Puig L**, **Auñon Á**, **Blanco A**, **García-Cañete J**, **Sandoval E**, **Fakkas-Fernández M**, **Garcés-**

Zarzalejo C, **Fariñas-Alvarez C**, **Fariñas MC**, **Martinez-Martinez L**, **Salas-Venero C**, **Cobo J**, **Ruiz-Carbajosa P**, **Jordán M**, **Crusi X**, **Marinescu C**, **Montaner F**, **Ramírez A**, **Corona PS**, **Lung M**, **Muniain-Ezcurra MÁ**, **Peñas-Espinar C**, **Suárez AI**, **Álvarez R**, **Cordero J-A**, **López-Pliego M**, **Palomino J**, **Puente A**. 2016. Time trends in the aetiology of prosthetic joint infections: a multicentre cohort study. Clin Microbiol Infect **22**:732.e1-732.e8.

Bergen PJ, **Bulitta JB**, **Forrest A**, **Tsuji BT**, **Li J**, **Nation RL**. 2010. Pharmacokinetic/ Pharmacodynamic Investigation of Colistin against Pseudomonas aeruginosa Using an In Vitro Model. Antimicrob Agents Chemother **54**:3783–3789.

Bergen PJ, **Forrest A**, **Bulitta JB**, **Tsuji BT**, **Sidjabat HE**, **Paterson DL**, **Li J**, **Nation RL**. 2011. Clinically relevant plasma concentrations of colistin in combination with imipenem enhance pharmacodynamic activity against multidrug-resistant Pseudomonas aeruginosa at multiple inocula. Antimicrob Agents Chemother **55**:5134–42.

Bergen PJ, **Li J**, **Nation RL**, **Turnidge JD**, **Coulthard K**, **Milne RW**. 2008. Comparison of once-, twice- and thrice-daily dosing of colistin on antibacterial effect and emergence of resistance: studies with Pseudomonas aeruginosa in an in vitro pharmacodynamic model. J Antimicrob Chemother **61**:636–42.

Bergen PJ, **Tsuji BT**, **Bulitta JB**, **Forrest A**, **Jacob J**, **Sidjabat HE**, **Paterson DL**, **Nation RL**, **Li J**. 2011. Synergistic killing of multidrug-resistant Pseudomonas aeruginosa at multiple inocula by colistin combined with doripenem in an in vitro pharmacokinetic/pharmacodynamic model. Antimicrob Agents Chemother **55**:5685–95.

Beswick AD, **Elvers KT**, **Smith AJ**, **Gooberman-Hill R**, **Lovering A**, **Blom AW**. 2012. What is the evidence base to guide surgical treatment of infected hip prostheses? systematic review of longitudinal studies in unselected patients. BMC Med **10**:18.

Betz M, **Abrassart S**, **Vaudaux P**, **Gjika E**, **Schindler M**, **Billières J**, **Zenelaj B**, **Suvà D**, **Peter R**, **Uçkay I**. 2015. Increased risk of joint failure in hip prostheses infected with Staphylococcus aureus treated with debridement, antibiotics and implant retention compared to Streptococcus. Int Orthop **39**:397–401.

Boselli E, **Allaouchiche B**. 1999. [Diffusion in bone tissue of antibiotics]. Presse Med **28**:2265–76.

Boucher HW, **Talbot GH**, **Bradley JS**, **Edwards JE**, **Gilbert D**, **Rice LB**, **Scheld M**, **Spellberg B**, **Bartlett J**. 2009. Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases

Society of America. Clin Infect Dis **48**:1–12.

Brandt CM, **Sistrunk WW**, **Duffy MC**, **Hanssen AD**, **Steckelberg JM**, **Ilstrup DM**, **Osmon DR**. 1997. Staphylococcus aureus prosthetic joint infection treated with debridement and prosthesis retention. Clin Infect Dis **24**:914–9.

Brochmann RP, **Toft A**, **Ciofu O**, **Briales A**, **Kolpen M**, **Hempel C**, **Bjarnsholt T**, **Høiby N**, **Jensen PØ**. 2014. Bactericidal effect of colistin on planktonic Pseudomonas aeruginosa is independent of hydroxyl radical formation. Int J Antimicrob Agents **43**:140–7.

Buckingham-Meyer K, **Goeres DM**, **Hamilton MA**. 2007. Comparative evaluation of biofilm disinfectant efficacy tests. J Microbiol Methods **70**:236–44.

Byren I, **Bejon P**, **Atkins BL**, **Angus B**, **Masters S**, **McLardy-Smith P**, **Gundle R**, **Berendt A**. 2009. One hundred and twelve infected arthroplasties treated with “DAIR” (debridement, antibiotics and implant retention): antibiotic duration and outcome. J Antimicrob Chemother **63**:1264–71.

Cabo J, **Euba G**, **Saborido A**, **González-Panisello M**, **Domínguez MA**, **Agulló JL**, **Murillo O**, **Verdaguer R**, **Ariza J**. 2011. Clinical outcome and microbiological findings using antibiotic-loaded spacers in two-stage revision of prosthetic joint infections. J Infect **63**:23–31.

Cahill JL, **Shadbolt B**, **Scarvell JM**, **Smith PN**. 2008. Quality of life after infection in total joint replacement. J Orthop Surg (Hong Kong) **16**:58–65.

Callaghan JJ, **Katz RP**, **Johnston RC**. 1999. One-stage revision surgery of the infected hip. A minimum 10-year followup study. Clin Orthop Relat Res 139–43.

Cappelletty DM, **Kang SL**, **Palmer SM**, **Rybak MJ**. 1995. Pharmacodynamics of ceftazidime administered as continuous infusion or intermittent bolus alone and in combination with single daily-dose amikacin against Pseudomonas aeruginosa in an in vitro infection model. Antimicrob Agents Chemother **39**:1797–801.

Carlier M, **Stove V**, **De Waele JJ**, **Verstraete AG**. 2015. Ultrafast quantification of β-lactam antibiotics in human plasma using UPLC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci **978–979**:89–94.

Carlier M, **Stove V**, **Roberts JA**, **Van de Velde E**, **De Waele JJ**, **Verstraete AG**. 2012. Quantification of seven β-lactam antibiotics and two β-lactamase inhibitors in human plasma using a validated UPLC-MS/MS method. Int J Antimicrob Agents **40**:416–22.

Cazorla-Reyes R, **Romero-González R**, **Frenich AG**, **Rodríguez Maresca MA**, **Martínez Vidal JL**. 2014. Simultaneous analysis of antibiotics in biological samples by ultra high performance liquid chromatography–tandem mass spectrometry. J Pharm Biomed Anal **89**:203–212.

Ceri H, **Olson ME**, **Stremick C**, **Read RR**, **Morck D**, **Buret A**. 1999. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol **37**:1771–6.

Chambers JR, **Sauer K**. 2013. The MerR-like regulator BrlR impairs Pseudomonas aeruginosa biofilm tolerance to colistin by repressing PhoPQ. J Bacteriol **195**:4678–88.

Chiang W-C, **Pamp SJ**, **Nilsson M**, **Givskov M**, **Tolker-Nielsen T**. 2012. The metabolically active subpopulation in Pseudomonas aeruginosa biofilms survives exposure to membrane-targeting antimicrobials via distinct molecular mechanisms. FEMS Immunol Med Microbiol **65**:245–56.

Choi H-R, **von Knoch F**, **Zurakowski D**, **Nelson SB**, **Malchau H**. 2011. Can implant retention be recommended for treatment of infected TKA? Clin Orthop Relat Res **469**:961–9.

Churchwell MI, **Twaddle NC**, **Meeker LR**, **Doerge DR**. 2005. Improving LC-MS sensitivity through increases in chromatographic performance: comparisons of UPLC-ES/MS/MS to HPLC-ES/MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci **825**:134–43.

Clinical and Laboratory Standard Institute. 2016. Performance standards for antimicrobial susceptibility testing: twenty-sixth edition. Wayne, Pennsylvania.

Cobo J, **Del Pozo JL**. 2011. Prosthetic joint infection: diagnosis and management. Expert Rev Anti Infect Ther **9**:787–802.

Cockcroft DW, **Gault MH**. 1976. Prediction of creatinine clearance from serum creatinine. Nephron **16**:31–41.

Cohen-Wolkowiez M, **White NR**, **Bridges A**, **Benjamin DK**, **Kashuba ADM**. 2011. Development of a liquid chromatography-tandem mass spectrometry assay of six antimicrobials in plasma for pharmacokinetic studies in premature infants. J Chromatogr B Analyt Technol Biomed Life Sci **879**:3497–506.

Colin P, **De Bock L**, **T'jollyn H**, **Boussery K**, **Van Bocxlaer J**. 2013. Development and validation of a fast and uniform approach to quantify β-lactam antibiotics in human plasma by solid phase extraction-liquid chromatography-electrospray-tandem mass spectrometry. Talanta **103**:285–93.

Corvec S, **Illiaquer M**, **Touchais S**, **Boutoille D**, **van der Mee-Marquet N**, **Quentin R**, **Reynaud A**, **Lepelletier D**, **Bemer P**. 2011. Clinical Features of Group B Streptococcus Prosthetic Joint Infections and Molecular Characterization of Isolates. J Clin Microbiol **49**:380–382.

Costerton JW, **Stewart PS**, **Greenberg EP**. 1999. Bacterial biofilms: a common cause of persistent infections. Science **284**:1318–22.

Couet W, **Grégoire N**, **Gobin P**, **Saulnier PJ**, **Frasca D**, **Marchand S**, **Mimoz O**. 2011. Pharmacokinetics of Colistin and Colistimethate Sodium After a Single 80-mg Intravenous Dose of CMS in Young Healthy Volunteers. Clin Pharmacol Ther **89**:875–879.

Craig WA. 1998. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis **26**:1-10–2.

Darouiche RO. 2004. Treatment of infections associated with surgical implants. N Engl J Med **350**:1422–9.

Del Pozo JL, **Patel R**. 2009. Clinical practice. Infection associated with prosthetic joints. N Engl J Med **361**:787–94.

Del Pozo JL, **Rouse MS**, **Patel R**. 2008. Bioelectric effect and bacterial biofilms. A systematic review. Int J Artif Organs **31**:786–95.

del Prado G, **Ruiz V**, **Naves P**, **Rodríguez-Cerrato V**, **Soriano F**, **del Carmen Ponte M**. 2010. Biofilm formation by Streptococcus pneumoniae strains and effects of human serum albumin, ibuprofen, N-acetyl-l-cysteine, amoxicillin, erythromycin, and levofloxacin. Diagn Microbiol Infect Dis **67**:311–8.

Della Valle CJ, **Sporer SM**, **Jacobs JJ**, **Berger RA**, **Rosenberg AG**, **Paprosky WG**. 2007. Preoperative testing for sepsis before revision total knee arthroplasty. J Arthroplasty **22**:90–3.

Denooz R, **Charlier C**. 2008. Simultaneous determination of five beta-lactam antibiotics (cefepim, ceftazidim, cefuroxim, meropenem and piperacillin) in human plasma by high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Analyt Technol Biomed Life Sci **864**:161–7.

Dobbins JJ, **Seligson D**, **Raff MJ**. 1988. Bacterial colonization of orthopedic fixation devices in the absence of clinical infection. J Infect Dis **158**:203–5.

Donlan RM, **Costerton JW**. 2002. Biofilms: survival mechanisms of clinically relevant

microorganisms. Clin Microbiol Rev **15**:167–93.

Donlan RM, **Piede JA**, **Heyes CD**, **Sanii L**, **Murga R**, **Edmonds P**, **El-Sayed I**, **El-Sayed MA**. 2004. Model system for growing and quantifying Streptococcus pneumoniae biofilms in situ and in real time. Appl Environ Microbiol **70**:4980–8.

Drusano GL. 2004. Antimicrobial pharmacodynamics: critical interactions of "bug and drug". Nat Rev Microbiol **2**:289–300.

Duggan JM, **Georgiadis G**, **VanGorp C**, **Kleshinski J**. 2001. Group B streptococcal prosthetic joint infections. J South Orthop Assoc **10**:209–14.

Dulhunty JM, **Roberts JA**, **Davis JS**, **Webb SAR**, **Bellomo R**, **Gomersall C**, **Shirwadkar C**, **Eastwood GM**, **Myburgh J**, **Paterson DL**, **Lipman J**. 2013. Continuous infusion of beta-lactam antibiotics in severe sepsis: a multicenter double-blind, randomized controlled trial. Clin Infect Dis **56**:236–44.

Eagle H, **Fleischman R**, **Musselman AD**. 1950. Effect of schedule of administration on the therapeutic efficacy of penicillin; importance of the aggregate time penicillin remains at effectively bactericidal levels. Am J Med **9**:280–99.

El Espera I, **Blondet C**, **Moullart V**, **Saïdi L**, **Havet E**, **Mertl P**, **Canarelli B**, **Schmit J-L**, **Meyer M-E**. 2004. The usefulness of 99mTc sulfur colloid bone marrow scintigraphy combined with 111In leucocyte scintigraphy in prosthetic joint infection. Nucl Med Commun **25**:171–5.

El Haj C, **Murillo O**, **Ribera A**, **Vivas M**, **Garcia-Somoza D**, **Tubau F**, **Cabo J**, **Ariza J**. 2014. Comparative Efficacies of Cloxacillin-Daptomycin and the Standard Cloxacillin-Rifampin Therapies against an Experimental Foreign-Body Infection by Methicillin-Susceptible Staphylococcus aureus. Antimicrob Agents Chemother **58**:5576–5580.

El Haj C, **Murillo O**, **Ribera A**, **Vivas M**, **Garcia-Somoza D**, **Tubau F**, **Cabellos C**, **Cabo J**, **Ariza J**. 2015. Daptomycin combinations as alternative therapies in experimental foreign-body infection caused by meticillin-susceptible Staphylococcus aureus. Int J Antimicrob Agents **46**:189–195.

Engh GA, **Ammeen DJ**. 1998. Classification and preoperative radiographic evaluation: knee. Orthop Clin North Am **29**:205–17.

European Medicines Agency. 2011. Guideline on bioanalytical method validation (EMA/CHMP/EWP/192217/2009). EMA, London, United Kingdom.

Everts RJ, **Chambers ST**, **Murdoch DR**, **Rothwell AG**, **McKie J**. 2004. Successful antimicrobial therapy and implant retention for streptococcal infection of prosthetic joints. ANZ J Surg **74**:210–4.

Falagas ME, **Kasiakou SK**. 2005. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis **40**:1333–41.

García-Castillo M, **Morosini MI**, **Valverde A**, **Almaraz F**, **Baquero F**, **Cantón R**, **del Campo R**. 2007. Differences in biofilm development and antibiotic susceptibility among Streptococcus pneumoniae isolates from cystic fibrosis samples and blood cultures. J Antimicrob Chemother **59**:301–4.

Garonzik SM, **Li J**, **Thamlikitkul V**, **Paterson DL**, **Shoham S**, **Jacob J**, **Silveira FP**, **Forrest A**, **Nation RL**. 2011. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. Antimicrob Agents Chemother **55**:3284–94.

Garrigos C, **Murillo O**, **Euba G**, **Verdaguer R**, **Tubau F**, **Cabellos C**, **Cabo J**, **Ariza J**. 2010. Efficacy of Usual and High Doses of Daptomycin in Combination with Rifampin versus Alternative Therapies in Experimental Foreign-Body Infection by Methicillin-Resistant Staphylococcus aureus. Antimicrob Agents Chemother **54**:5251–5256.

Garrigos C, **Murillo O**, **Lora-Tamayo J**, **Verdaguer R**, **Tubau F**, **Cabellos C**, **Cabo J**, **Ariza J**. 2013. Fosfomycin-daptomycin and other fosfomycin combinations as alternative therapies in experimental foreign-body infection by methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother **57**:606–10.

Georges B, **Conil J-M**, **Seguin T**, **Ruiz S**, **Minville V**, **Cougot P**, **Decun J-F**, **Gonzalez H**, **Houin G**, **Fourcade O**, **Saivin S**. 2009. Population pharmacokinetics of ceftazidime in intensive care unit patients: influence of glomerular filtration rate, mechanical ventilation, and reason for admission. Antimicrob Agents Chemother **53**:4483–9.

Gilbert P, **Brown MR**. 1998. Biofilms and beta-lactam activity. J Antimicrob Chemother **41**:571–2.

Gilbert P, **Collier PJ**, **Brown MR**. 1990. Influence of growth rate on susceptibility to antimicrobial agents: biofilms, cell cycle, dormancy, and stringent response. Antimicrob Agents Chemother **34**:1865–8.

Goeres DM, **Loetterle LR**, **Hamilton MA**, **Murga R**, **Kirby DW**, **Donlan RM**. 2005. Statistical assessment of a laboratory method for growing biofilms. Microbiology **151**:757–62.

Granchi D, **Verri E**, **Ciapetti G**, **Stea S**, **Savarino L**, **Sudanese A**, **Mieti M**, **Rotini R**, **Dallari D**, **Zinghi G**, **Montanaro L**. 1998. Bone-resorbing cytokines in serum of patients with aseptic loosening of hip prostheses. J Bone Joint Surg Br **80**:912–7.

Gristina AG, **Costerton JW**. 1985. Bacterial adherence to biomaterials and tissue. The significance of its role in clinical sepsis. J Bone Joint Surg Am **67**:264–73.

Gulhane S, **Vanhegan IS**, **Haddad FS**. 2012. Single stage revision: regaining momentum. J Bone Jt Surg - Br Vol **94**–**B**:120–122.

Gumustas M, **Kurbanoglu S**, **Uslu B**, **Ozkan SA**. 2013. UPLC versus HPLC on Drug Analysis: Advantageous, Applications and Their Validation Parameters. Chromatographia **76**:1365–1427.

Haagensen JAJ, **Klausen M**, **Ernst RK**, **Miller SI**, **Folkesson A**, **Tolker-Nielsen T**, **Molin S**. 2007. Differentiation and distribution of colistin- and sodium dodecyl sulfate-tolerant cells in Pseudomonas aeruginosa biofilms. J Bacteriol **189**:28–37.

Hancock RE, **Wong PG**. 1984. Compounds which increase the permeability of the Pseudomonas aeruginosa outer membrane. Antimicrob Agents Chemother **26**:48–52.

Hancock RE. 1997. Peptide antibiotics. Lancet **349**:418–422.

Hayashi Y, **Roberts JA**, **Paterson DL**, **Lipman J**. 2010. Pharmacokinetic evaluation of piperacillin-tazobactam. Expert Opin Drug Metab Toxicol **6**:1017–31.

Hengzhuang W, **Høiby N**, **Ciofu O**. 2014. Pharmacokinetics and pharmacodynamics of antibiotics in biofilm infections of Pseudomonas aeruginosa in vitro and in vivo. Methods Mol Biol **1147**:239–54.

Hengzhuang W, **Wu H**, **Ciofu O**, **Song Z**, **Høiby N**. 2012. In vivo pharmacokinetics/ pharmacodynamics of colistin and imipenem in Pseudomonas aeruginosa biofilm infection. Antimicrob Agents Chemother **56**:2683–90.

Herrmann G, **Yang L**, **Wu H**, **Song Z**, **Wang H**, **Høiby N**, **Ulrich M**, **Molin S**, **Riethmüller J**, **Döring G**. 2010. Colistin-tobramycin combinations are superior to monotherapy concerning the killing of biofilm Pseudomonas aeruginosa. J Infect Dis **202**:1585–92.

Hickson CJ, **Metcalfe D**, **Elgohari S**, **Oswald T**, **Masters JP**, **Rymaszewska M**, **Reed MR**,

Sprowson AP. 2015. Prophylactic antibiotics in elective hip and knee arthroplasty: an analysis of organisms reported to cause infections and National survey of clinical practice. Bone Joint Res **4**:181–9.

Hoenders CSM, **Harmsen MC**, **van Luyn MJA**. 2008. The local inflammatory environment and microorganisms in "aseptic" loosening of hip prostheses. J Biomed Mater Res B Appl Biomater **86**:291–301.

Høiby N, **Bjarnsholt T**, **Givskov M**, **Molin S**, **Ciofu O**. 2010. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents **35**:322–332.

Holinka J, **Bauer L**, **Hirschl AM**, **Graninger W**, **Windhager R**, **Presterl E**. 2011. Sonication cultures of explanted components as an add-on test to routinely conducted microbiological diagnostics improve pathogen detection. J Orthop Res **29**:617–22.

Hsieh P-H, **Lee MS**, **Hsu K-Y**, **Chang Y-H**, **Shih H-N**, **Ueng SW**. 2009. Gram-negative prosthetic joint infections: risk factors and outcome of treatment. Clin Infect Dis **49**:1036–43.

Huttner A, **Harbarth S**, **Hope WW**, **Lipman J**, **Roberts JA**. 2015. Therapeutic drug monitoring of the β-lactam antibiotics: what is the evidence and which patients should we be using it for?: Figure 1. J Antimicrob Chemother dkv201.

Ince A, **Rupp J**, **Frommelt L**, **Katzer A**, **Gille J**, **Löhr JF**. 2004. Is "aseptic" loosening of the prosthetic cup after total hip replacement due to nonculturable bacterial pathogens in patients with low-grade infection? Clin Infect Dis **39**:1599–603.

Jeffery AE, **Wylde V**, **Blom AW**, **Horwood JP**. 2011. "It's there and I'm stuck with it": patients' experiences of chronic pain following total knee replacement surgery. Arthritis Care Res (Hoboken) **63**:286–92.

Kadurugamuwa JL, **Sin L V**, **Yu J**, **Francis KP**, **Purchio TF**, **Contag PR**. 2004. Noninvasive optical imaging method to evaluate postantibiotic effects on biofilm infection in vivo. Antimicrob Agents Chemother **48**:2283–7.

Karaiskos I, **Friberg LE**, **Pontikis K**, **Ioannidis K**, **Tsagkari V**, **Galani L**, **Kostakou E**, **Baziaka F**, **Paskalis C**, **Koutsoukou A**, **Giamarellou H**. 2015. Colistin Population Pharmacokinetics after Application of a Loading Dose of 9 MU Colistin Methanesulfonate in Critically Ill Patients. Antimicrob Agents Chemother **59**:7240–8.

Kendoff D, **Gehrke T**. 2014. Surgical Management of Periprosthetic Joint Infection: One-Stage

Exchange. J Knee Surg **27**:273–278.

Klausen M, **Aaes-Jørgensen A**, **Molin S**, **Tolker-Nielsen T**. 2003. Involvement of bacterial migration in the development of complex multicellular structures in Pseudomonas aeruginosa biofilms. Mol Microbiol **50**:61–8.

Klouche S, **Sariali E**, **Mamoudy P**. 2010. Total hip arthroplasty revision due to infection: a cost analysis approach. Orthop Traumatol Surg Res **96**:124–32.

Kunutsor SK, **Whitehouse MR**, **Blom AW**, **Beswick AD**, **INFORM Team**. 2015. Re-Infection Outcomes following One- and Two-Stage Surgical Revision of Infected Hip Prosthesis: A Systematic Review and Meta-Analysis. PLoS One **10**:e0139166.

Kunutsor SK, **Whitehouse MR**, **Lenguerrand E**, **Blom AW**, **Beswick AD**, **INFORM Team**. 2016. Re-Infection Outcomes Following One- And Two-Stage Surgical Revision of Infected Knee Prosthesis: A Systematic Review and Meta-Analysis. PLoS One **11**:e0151537.

Kurtz S, **Ong K**, **Lau E**, **Mowat F**, **Halpern M**. 2007. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am **89**:780–5.

Kurtz SM, **Lau E**, **Watson H**, **Schmier JK**, **Parvizi J**. 2012. Economic Burden of Periprosthetic Joint Infection in the United States. J Arthroplasty **27**:61–65.e1.

Landersdorfer CB, **Bulitta JB**, **Kinzig M**, **Holzgrabe U**, **Sörgel F**. 2009. Penetration of antibacterials into bone: pharmacokinetic, pharmacodynamic and bioanalytical considerations. Clin Pharmacokinet **48**:89–124.

Legrand T, **Vodovar D**, **Tournier N**, **Khoudour N**, **Hulin A**. 2016. Simultaneous Determination of Eight β-Lactam Antibiotics, Amoxicillin, Cefazolin, Cefepime, Cefotaxime, Ceftazidime, Cloxacillin, Oxacillin, and Piperacillin, in Human Plasma by Using Ultra-High-Performance Liquid Chromatography with Ultraviolet Detection. Antimicrob Agents Chemother **60**:4734–4742.

Leonard HAC, **Liddle AD**, **Burke O**, **Murray DW**, **Pandit H**. 2014. Single- or two-stage revision for infected total hip arthroplasty? A systematic review of the literature. Clin Orthop Relat Res **472**:1036–42.

Lew DP, **Waldvogel FA**. 2004. Osteomyelitis. Lancet **364**:369–79.

Li J, **Nation RL**, **Turnidge JD**, **Milne RW**, **Coulthard K**, **Rayner CR**, **Paterson DL**. 2006. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet

Infect Dis **6**:589–601.

Lora-Tamayo J, **Murillo O**, **Bergen PJ**, **Nation RL**, **Poudyal A**, **Luo X**, **Yu HY**, **Ariza J**, **Li J**. 2014. Activity of colistin combined with doripenem at clinically relevant concentrations against multidrug-resistant Pseudomonas aeruginosa in an in vitro dynamic biofilm model. J Antimicrob Chemother **69**:2434–42.

Lora-Tamayo J, **Murillo O**, **Iribarren JA**, **Soriano A**, **Sánchez-Somolinos M**, **Baraia-Etxaburu JM**, **Rico A**, **Palomino J**, **Rodríguez-Pardo D**, **Horcajada JP**, **Benito N**, **Bahamonde A**, **Granados A**, **del Toro MD**, **Cobo J**, **Riera M**, **Ramos A**, **Jover-Sáenz A**, **Ariza J**, **REIPI Group for the Study of Prosthetic Infection G**, **Cabo X**, **Pedrero S**, **Goenaga MA**, **Elola M**, **Moreno E**, **Garcia-Ramiro S**, **Martinez-Pastor JC**, **Tornero E**, **Garcia-Lechuz JM**, **Marin M**, **Villanueva M**, **Lopez I**, **Cisterna R**, **Santamaria JM**, **Gomez M-J**, **Puente A**, **Cano P**, **Pigrau C**, **Sorde R**, **Flores X**, **Sorli L**, **Gonzalez-Miguez P**, **Puig L**, **Franco M**, **Jordan M**, **Coll P**, **Amador-Mellado J**, **Fuster-Foz C**, **Garcia-Paino L**, **Nieto I**, **Muniain MA**, **Suarez A-I**, **Maseguer MA**, **Garagorri E**, **Pintado V**, **Marinescu C**, **Ramirez A**, **Munez E**, **Alvarez T**, **Garcia R**, **Barcenilla F**, **Prat L**, **Perez F**. 2013. A large multicenter study of methicillin-susceptible and methicillin-resistant Staphylococcus aureus prosthetic joint infections managed with implant retention. Clin Infect Dis **56**:182–94.

Lorian V. 1971. The mode of action of antibiotics on gram-negative bacilli. Arch Intern Med **128**:623–32.

Mackowiak PA, **Jones SR**, **Smith JW**. 1978. Diagnostic value of sinus-tract cultures in chronic osteomyelitis. JAMA **239**:2772–5.

Magiorakos A-P, **Srinivasan A**, **Carey RB**, **Carmeli Y**, **Falagas ME**, **Giske CG**, **Harbarth S**, **Hindler JF**, **Kahlmeter G**, **Olsson-Liljequist B**, **Paterson DL**, **Rice LB**, **Stelling J**, **Struelens MJ**, **Vatopoulos A**, **Weber JT**, **Monnet DL**. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis Soc Clin Microbiol Infect Dis **18**:268–81.

Mah TF, **O'Toole GA**. 2001. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol **9**:34–9.

Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 8th Edition (2015). Authors: Bennett JE, Dolin R, Blaser MJ. Elsevier Saunders.

Marculescu CE, **Berbari EF**, **Hanssen AD**, **Steckelberg JM**, **Harmsen SW**, **Mandrekar JN**, **Osmon**

DR. 2006. Outcome of prosthetic joint infections treated with debridement and retention of components. Clin Infect Dis **42**:471–8.

Martínez-Pastor JC, **Muñoz-Mahamud E**, **Vilchez F**, **García-Ramiro S**, **Bori G**, **Sierra J**, **Martínez JA**, **Font L**, **Mensa J**, **Soriano A**. 2009. Outcome of acute prosthetic joint infections due to gram-negative bacilli treated with open debridement and retention of the prosthesis. Antimicrob Agents Chemother **53**:4772–7.

Matthews PC, **Berendt AR**, **McNally MA**, **Byren I**. 2009. Diagnosis and management of prosthetic joint infection. BMJ **338**:b1773–b1773.

McDonald DJ, **Fitzgerald RH**, **Ilstrup DM**. 1989. Two-stage reconstruction of a total hip arthroplasty because of infection. J Bone Joint Surg Am **71**:828–34.

McKinnon PS, **Paladino JA**, **Schentag JJ**. 2008. Evaluation of area under the inhibitory curve (AUIC) and time above the minimum inhibitory concentration (T>MIC) as predictors of outcome for cefepime and ceftazidime in serious bacterial infections. Int J Antimicrob Agents **31**:345–351.

McLeod BR, **Sandvik EL**. 2010. A biofilm growth protocol and the design of a magnetic field exposure setup to be used in the study of magnetic fields as a means of controlling bacterial biofilms. Bioelectromagnetics **31**:56–63.

McWhinney BC, **Wallis SC**, **Hillister T**, **Roberts JA**, **Lipman J**, **Ungerer JPJ**. 2010. Analysis of 12 beta-lactam antibiotics in human plasma by HPLC with ultraviolet detection. J Chromatogr B Analyt Technol Biomed Life Sci **878**:2039–43.

Meehan AM, **Osmon DR**, **Duffy MCT**, **Hanssen AD**, **Keating MR**. 2003. Outcome of penicillin-susceptible streptococcal prosthetic joint infection treated with debridement and retention of the prosthesis. Clin Infect Dis **36**:845–9.

Mirra JM, **Marder RA**, **Amstutz HC**. 1982. The pathology of failed total joint arthroplasty. Clin Orthop Relat Res 175–83.

Mohd Hafiz A-A, **Staatz CE**, **Kirkpatrick CMJ**, **Lipman J**, **Roberts JA**. 2012. Continuous infusion vs. bolus dosing: implications for beta-lactam antibiotics. Minerva Anestesiol **78**:94–104.

Montero M, **Horcajada JP**, **Sorlí L**, **Alvarez-Lerma F**, **Grau S**, **Riu M**, **Sala M**, **Knobel H**. 2009. Effectiveness and safety of colistin for the treatment of multidrug-resistant Pseudomonas aeruginosa infections. Infection **37**:461–5.

Moore AJ, **Blom AW**, **Whitehouse MR**, **Gooberman-Hill R**. 2015. Deep prosthetic joint infection: a qualitative study of the impact on patients and their experiences of revision surgery. BMJ Open **5**:e009495.

Moriyama B, **Henning SA**, **Childs R**, **Holland SM**, **Anderson VL**, **Morris JC**, **Wilson WH**, **Drusano GL**, **Walsh TJ**. 2010. High-dose continuous infusion beta-lactam antibiotics for the treatment of resistant Pseudomonas aeruginosa infections in immunocompromised patients. Ann Pharmacother **44**:929–35.

Moriyama B, **Henning SA**, **Neuhauser MM**, **Danner RL**, **Walsh TJ**. 2009. Continuous-infusion beta-lactam antibiotics during continuous venovenous hemofiltration for the treatment of resistant gram-negative bacteria. Ann Pharmacother **43**:1324–37.

Mouton JW, **Vinks AA**. 1996. Is continuous infusion of beta-lactam antibiotics worthwhile?--efficacy and pharmacokinetic considerations. J Antimicrob Chemother **38**:5–15.

Mouton JW, **Vinks AA**. 2007. Continuous infusion of beta-lactams. Curr Opin Crit Care **13**:598–606.

Murillo O, **Doménech A**, **Garcia A**, **Tubau F**, **Cabellos C**, **Gudiol F**, **Ariza J**. 2006. Efficacy of high doses of levofloxacin in experimental foreign-body infection by methicillin-susceptible Staphylococcus aureus. Antimicrob Agents Chemother **50**:4011–7.

Murillo O, **Garrigos C**, **Pachon ME**, **Euba G**, **Verdaguer R**, **Cabellos C**, **Cabo J**, **Gudiol F**, **Ariza J**. 2009. Efficacy of High Doses of Daptomycin versus Alternative Therapies against Experimental Foreign-Body Infection by Methicillin-Resistant Staphylococcus aureus. Antimicrob Agents Chemother **53**:4252–4257.

Murillo O, **Grau I**, **Lora-Tamayo J**, **Gomez-Junyent J**, **Ribera A**, **Tubau F**, **Ariza J**, **Pallares R**. 2015. The changing epidemiology of bacteraemic osteoarticular infections in the early 21st century. Clin Microbiol Infect **21**:254.e1-8.

Murillo O, **Pachon ME**, **Euba G**, **Verdaguer R**, **Tubau F**, **Cabellos C**, **Cabo J**, **Gudiol F**, **Ariza J**. 2008. Antagonistic Effect of Rifampin on the Efficacy of High-Dose Levofloxacin in Staphylococcal Experimental Foreign-Body Infection. Antimicrob Agents Chemother **52**:3681–3686.

Nation RL, **Li J**. 2009. Colistin in the 21st century. Curr Opin Infect Dis **22**:535–43.

Nelson CL, **McLaren AC**, **McLaren SG**, **Johnson JW**, **Smeltzer MS**. 2005. Is aseptic loosening

truly aseptic? Clin Orthop Relat Res 25–30.

Nemutlu E, **Kir S**, **Katlan D**, **Beksaç MS**. 2009. Simultaneous multiresponse optimization of an HPLC method to separate seven cephalosporins in plasma and amniotic fluid: application to validation and quantification of cefepime, cefixime and cefoperazone. Talanta **80**:117–26.

Nguyen LL, **Nelson CL**, **Saccente M**, **Smeltzer MS**, **Wassell DL**, **McLaren SG**. 2002. Detecting bacterial colonization of implanted orthopaedic devices by ultrasonication. Clin Orthop Relat Res 29–37.

Nivbrant B, **Karlsson K**, **Kärrholm J**. 1999. Cytokine levels in synovial fluid from hips with well-functioning or loose prostheses. J Bone Joint Surg Br **81**:163–6.

Nováková L, **Matysová L**, **Solich P**. 2006. Advantages of application of UPLC in pharmaceutical analysis. Talanta **68**:908–18.

Ohmori T, **Suzuki A**, **Niwa T**, **Ushikoshi H**, **Shirai K**, **Yoshida S**, **Ogura S**, **Itoh Y**. 2011. Simultaneous determination of eight β-lactam antibiotics in human serum by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci **879**:1038–42.

Olson ME, **Ceri H**, **Morck DW**, **Buret AG**, **Read RR**. 2002. Biofilm bacteria: formation and comparative susceptibility to antibiotics. Can J Vet Res **66**:86–92.

Osmon DR, **Berbari EF**, **Berendt AR**, **Lew D**, **Zimmerli W**, **Steckelberg JM**, **Rao N**, **Hanssen A**, **Wilson WR**. 2013. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis **56**:e1–e25.

Osmon DR, **Berbari EF**. 2002. Outpatient intravenous antimicrobial therapy for the practicing orthopaedic surgeon. Clin Orthop Relat Res 80–6.

Pamp SJ, **Gjermansen M**, **Johansen HK**, **Tolker-Nielsen T**. 2008. Tolerance to the antimicrobial peptide colistin in Pseudomonas aeruginosa biofilms is linked to metabolically active cells, and depends on the pmr and mexAB-oprM genes. Mol Microbiol **68**:223–40.

Papagelopoulos PJ, **Mavrogenis AF**, **Giannitsioti E**, **Kikilas A**, **Kanellakopoulou K**, **Soucacos PN**. 2007. Management of a multidrug-resistant Pseudomonas aeruginosa infected total knee arthroplasty using colistin. A case report and review of the literature. J Arthroplasty **22**:457–63.

Paprosky WG, **Perona PG**, **Lawrence JM**. 1994. Acetabular defect classification and surgical

reconstruction in revision arthroplasty. A 6-year follow-up evaluation. J Arthroplasty **9**:33–44.

Parra-Ruiz J, **Vidaillac C**, **Rose WE**, **Rybak MJ**. 2010. Activities of high-dose daptomycin, vancomycin, and moxifloxacin alone or in combination with clarithromycin or rifampin in a novel in vitro model of Staphylococcus aureus biofilm. Antimicrob Agents Chemother **54**:4329–34.

Pasmore M, **Costerton JW**. 2003. Biofilms, bacterial signaling, and their ties to marine biology. J Ind Microbiol Biotechnol **30**:407–413.

Patel R. 2005. Biofilms and antimicrobial resistance. Clin Orthop Relat Res 41–7.

Peel TN, **Cheng AC**, **Buising KL**, **Choong PFM**. 2012. Microbiological aetiology, epidemiology, and clinical profile of prosthetic joint infections: are current antibiotic prophylaxis guidelines effective? Antimicrob Agents Chemother **56**:2386–91.

Peel TN, **Dowsey MM**, **Daffy JR**, **Stanley PA**, **Choong PFM**, **Buising KL**. 2011. Risk factors for prosthetic hip and knee infections according to arthroplasty site. J Hosp Infect **79**:129–133.

Peña C, **Gómez-Zorrilla S**, **Oriol I**, **Tubau F**, **Dominguez MA**, **Pujol M**, **Ariza J**. 2013. Impact of multidrug resistance on Pseudomonas aeruginosa ventilator-associated pneumonia outcome: predictors of early and crude mortality. Eur J Clin Microbiol Infect Dis **32**:413–20.

Piper KE, **Jacobson MJ**, **Cofield RH**, **Sperling JW**, **Sanchez-Sotelo J**, **Osmon DR**, **McDowell A**, **Patrick S**, **Steckelberg JM**, **Mandrekar JN**, **Fernandez Sampedro M**, **Patel R**. 2009. Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication. J Clin Microbiol **47**:1878–84.

Plachouras D, **Karvanen M**, **Friberg LE**, **Papadomichelakis E**, **Antoniadou A**, **Tsangaris I**, **Karaiskos I**, **Poulakou G**, **Kontopidou F**, **Armaganidis A**, **Cars O**, **Giamarellou H**. 2009. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by gram-negative bacteria. Antimicrob Agents Chemother **53**:3430–6.

Portillo ME, **Salvadó M**, **Alier A**, **Sorli L**, **Martínez S**, **Horcajada JP**, **Puig L**. 2013. Prosthesis Failure Within 2 Years of Implantation Is Highly Predictive of Infection. Clin Orthop Relat Res **471**:3672–3678.

Portillo ME, **Salvadó M**, **Sorli L**, **Alier A**, **Martínez S**, **Trampuz A**, **Gómez J**, **Puig L**, **Horcajada JP**. 2012. Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint

infection and aseptic failure. J Infect **65**:541–8.

Poudyal A, **Howden BP**, **Bell JM**, **Gao W**, **Owen RJ**, **Turnidge JD**, **Nation RL**, **Li J**. 2008. In vitro pharmacodynamics of colistin against multidrug-resistant Klebsiella pneumoniae. J Antimicrob Chemother **62**:1311–1318.

Pulido L, **Ghanem E**, **Joshi A**, **Purtill JJ**, **Parvizi J**. 2008. Periprosthetic Joint Infection: The Incidence, Timing, and Predisposing Factors. Clin Orthop Relat Res **466**:1710–1715.

Rasul AT, **Tsukayama D**, **Gustilo RB**. 1991. Effect of time of onset and depth of infection on the outcome of total knee arthroplasty infections. Clin Orthop Relat Res 98–104.

Raut V V, **Siney PD**, **Wroblewski BM**. 1994. One-stage revision of infected total hip replacements with discharging sinuses. J Bone Joint Surg Br **76**:721–4.

Roberts JA, **Abdul-Aziz M-H**, **Davis JS**, **Dulhunty JM**, **Cotta MO**, **Myburgh J**, **Bellomo R**, **Lipman J**. 2016. Continuous versus Intermittent β-Lactam Infusion in Severe Sepsis. A Meta-analysis of Individual Patient Data from Randomized Trials. Am J Respir Crit Care Med **194**:681–691.

Roberts JA, **Abdul-Aziz MH**, **Lipman J**, **Mouton JW**, **Vinks AA**, **Felton TW**, **Hope WW**, **Farkas A**, **Neely MN**, **Schentag JJ**, **Drusano G**, **Frey OR**, **Theuretzbacher U**, **Kuti JL**, **International Society of Anti-Infective Pharmacology and the Pharmacokinetics and Pharmacodynamics Study Group of the European Society of Clinical Microbiology and Infectious Diseases**. 2014. Individualised antibiotic dosing for patients who are critically ill: challenges and potential solutions. Lancet Infect Dis **14**:498–509.

Roberts JA, **Paratz J**, **Paratz E**, **Krueger WA**, **Lipman J**. 2007. Continuous infusion of beta-lactam antibiotics in severe infections: a review of its role. Int J Antimicrob Agents **30**:11–8.

Roberts JA, **Webb S**, **Paterson D**, **Ho KM**, **Lipman J**. 2009. A systematic review on clinical benefits of continuous administration of β-lactam antibiotics*. Crit Care Med **37**:2071–2078.

Rodríguez-Pardo D, **Pigrau C**, **Lora-Tamayo J**, **Soriano A**, **del Toro MD**, **Cobo J**, **Palomino J**, **Euba G**, **Riera M**, **Sánchez-Somolinos M**, **Benito N**, **Fernández-Sampedro M**, **Sorli L**, **Guio L**, **Iribarren JA**, **Baraia-Etxaburu JM**, **Ramos A**, **Bahamonde A**, **Flores-Sánchez X**, **Corona PS**, **Ariza J**. 2014. Gram-negative prosthetic joint infection: outcome of a debridement, antibiotics and implant retention approach. A large multicentre study. Clin Microbiol Infect **20**:O911-9.

Saleh-Mghir A, **Muller-Serieys C**, **Dinh A**, **Massias L**, **Crémieux A-C**. 2011. Adjunctive rifampin is crucial to optimizing daptomycin efficacy against rabbit prosthetic joint infection due to

methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother **55**:4589–93.

Schäfer P, **Fink B**, **Sandow D**, **Margull A**, **Berger I**, **Frommelt L**. 2008. Prolonged Bacterial Culture to Identify Late Periprosthetic Joint Infection: A Promising Strategy. Clin Infect Dis **47**:1403–1409.

Sendi P, **Banderet F**, **Graber P**, **Zimmerli W**. 2011. Clinical comparison between exogenous and haematogenous periprosthetic joint infections caused by Staphylococcus aureus. Clin Microbiol Infect **17**:1098–100.

Sendi P, **Christensson B**, **Uçkay I**, **Trampuz A**, **Achermann Y**, **Boggian K**, **Svensson D**, **Widerström M**, **Zimmerli W**, **GBS PJI study group**. 2011. Group B streptococcus in prosthetic hip and knee joint-associated infections. J Hosp Infect **79**:64–9.

Sendi P, **Zimmerli W**. 2012. Antimicrobial treatment concepts for orthopaedic device-related infection. Clin Microbiol Infect **18**:1176–1184.

Senneville E, **Joulie D**, **Legout L**, **Valette M**, **Dezèque H**, **Beltrand E**, **Roselé B**, **d'Escrivan T**, **Loïez C**, **Caillaux M**, **Yazdanpanah Y**, **Maynou C**, **Migaud H**. 2011. Outcome and predictors of treatment failure in total hip/knee prosthetic joint infections due to Staphylococcus aureus. Clin Infect Dis **53**:334–40.

Sime FB, **Roberts MS**, **Roberts JA**, **Robertson TA**. 2014. Simultaneous determination of seven β-lactam antibiotics in human plasma for therapeutic drug monitoring and pharmacokinetic studies. J Chromatogr B Analyt Technol Biomed Life Sci **960**:134–44.

Spangehl MJ, **Masri BA**, **O'Connell JX**, **Duncan CP**. 1999. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. J Bone Joint Surg Am **81**:672–83.

Spellberg B, **Lipsky BA**. 2012. Systemic antibiotic therapy for chronic osteomyelitis in adults. Clin Infect Dis **54**:393–407.

Sporer SM, **Paprosky WG**. 2003. Revision total hip arthroplasty: the limits of fully coated stems. Clin Orthop Relat Res 203–9.

Stefánsdóttir A, **Johansson D**, **Knutson K**, **Lidgren L**, **Robertsson O**. 2009. Microbiology of the infected knee arthroplasty: Report from the Swedish Knee Arthroplasty Register on 426 surgically revised cases. Scand J Infect Dis **41**:831–840.

Stewart PS, **Costerton JW**. 2001. Antibiotic resistance of bacteria in biofilms. Lancet (London, England) **358**:135–8.

Strange S, **Whitehouse MR**, **Beswick AD**, **Board T**, **Burston A**, **Burston B**, **Carroll FE**, **Dieppe P**, **Garfield K**, **Gooberman-Hill R**, **Jones S**, **Kunutsor S**, **Lane A**, **Lenguerrand E**, **MacGowan A**, **Moore A**, **Noble S**, **Simon J**, **Stockley I**, **Taylor AH**, **Toms A**, **Webb J**, **Whittaker J-P**, **Wilson M**, **Wylde V**, **Blom AW**. 2016. One-stage or two-stage revision surgery for prosthetic hip joint infection – the INFORM trial: a study protocol for a randomised controlled trial. Trials **17**:90.

Suarez C, **Peña C**, **Arch O**, **Dominguez MA**, **Tubau F**, **Juan C**, **Gavaldá L**, **Sora M**, **Oliver A**, **Pujol M**, **Ariza J**. 2011. A large sustained endemic outbreak of multiresistant Pseudomonas aeruginosa: a new epidemiological scenario for nosocomial acquisition. BMC Infect Dis **11**:272.

Tande AJ, **Patel R**. 2014. Prosthetic Joint Infection. Clin Microbiol Rev **27**:302–345.

Tattevin P, **Crémieux AC**, **Pottier P**, **Huten D**, **Carbon C**. 1999. Prosthetic joint infection: when can prosthesis salvage be considered? Clin Infect Dis **29**:292–5.

The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0, 2015. http://www.eucast.org.

Tigges S, **Stiles RG**, **Roberson JR**. 1994. Appearance of septic hip prostheses on plain radiographs. AJR Am J Roentgenol **163**:377–80.

Tornero E, **Martínez-Pastor JC**, **Bori G**, **García-Ramiro S**, **Morata L**, **Bosch J**, **Mensa J**, **Soriano A**. 2014. Risk factors for failure in early prosthetic joint infection treated with debridement. Influence of etiology and antibiotic treatment. J Appl Biomater Funct Mater **12**:129–134.

Trampuz A, **Hanssen AD**, **Osmon DR**, **Mandrekar J**, **Steckelberg JM**, **Patel R**. 2004. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. Am J Med **117**:556–62.

Trampuz A, **Piper KE**, **Jacobson MJ**, **Hanssen AD**, **Unni KK**, **Osmon DR**, **Mandrekar JN**, **Cockerill FR**, **Steckelberg JM**, **Greenleaf JF**, **Patel R**. 2007. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med **357**:654–63.

Trampuz A, **Zimmerli W**. 2005. Prosthetic joint infections: update in diagnosis and treatment. Swiss Med Wkly **135**:243–51.

Trampuz A, **Zimmerli W**. 2006. Diagnosis and treatment of infections associated with fracture-

fixation devices. Injury **37 Suppl 2**:S59-66.

Trampuz A, **Zimmerli W**. 2008. Diagnosis and treatment of implant-associated septic arthritis and osteomyelitis. Curr Infect Dis Rep **10**:394–403.

Trebse R, **Pisot V**, **Trampuz A**. 2005. Treatment of infected retained implants. J Bone Joint Surg Br **87**:249–56.

Tschudin-Sutter S, **Frei R**, **Dangel M**, **Jakob M**, **Balmelli C**, **Schaefer DJ**, **Weisser M**, **Elzi L**, **Battegay M**, **Widmer AF**. 2016. Validation of a treatment algorithm for orthopaedic implant-related infections with device-retention—results from a prospective observational cohort study. Clin Microbiol Infect **22**:457.e1-457.e9.

Tsukayama DT, **Estrada R**, **Gustilo RB**. 1996. Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. J Bone Joint Surg Am **78**:512–23.

Tunney MM, **Patrick S**, **Curran MD**, **Ramage G**, **Hanna D**, **Nixon JR**, **Gorman SP**, **Davis RI**, **Anderson N**. 1999. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J Clin Microbiol **37**:3281–90.

Tunney MM, **Patrick S**, **Gorman SP**, **Nixon JR**, **Anderson N**, **Davis RI**, **Hanna D**, **Ramage G**. 1998. Improved detection of infection in hip replacements. A currently underestimated problem. J Bone Joint Surg Br **80**:568–72.

Valour F, **Dutronc H**, **Dinh A**, **Cazorla C**, **Pavèse P**, **Lesens O**, **Uçkay I**, **Chidiac C**, **Ferry T**. 2013. Difficult-to-treat Gram-negative bone and joint infections: efficacy and safety of prolonged intravenous colistin. Int J Antimicrob Agents **41**:197–9.

Van Herendael B, **Jeurissen A**, **Tulkens PM**, **Vlieghe E**, **Verbrugghe W**, **Jorens PG**, **Ieven M**. 2012. Continuous infusion of antibiotics in the critically ill: The new holy grail for beta-lactams and vancomycin? Ann Intensive Care **2**:22.

Vanhegan IS, **Morgan-Jones R**, **Barrett DS**, **Haddad FS**. 2012. Developing a strategy to treat established infection in total knee replacement: A review of the latest evidence and clinical practice. Bone Joint J **94**–**B**:875–881.

Verdier M-C, **Tribut O**, **Tattevin P**, **Le Tulzo Y**, **Michelet C**, **Bentué-Ferrer D**. 2011. Simultaneous determination of 12 beta-lactam antibiotics in human plasma by high-performance liquid chromatography with UV detection: application to therapeutic drug

monitoring. Antimicrob Agents Chemother **55**:4873–9.

Vergidis P, **Greenwood-Quaintance KE**, **Sanchez-Sotelo J**, **Morrey BF**, **Steinmann SP**, **Karau MJ**, **Osmon DR**, **Mandrekar JN**, **Steckelberg JM**, **Patel R**. 2011. Implant sonication for the diagnosis of prosthetic elbow infection. J shoulder Elb Surg **20**:1275–81.

Vergidis P, **Rouse MS**, **Euba G**, **Karau MJ**, **Schmidt SM**, **Mandrekar JN**, **Steckelberg JM**, **Patel R**. 2011. Treatment with linezolid or vancomycin in combination with rifampin is effective in an animal model of methicillin-resistant Staphylococcus aureus foreign body osteomyelitis. Antimicrob Agents Chemother **55**:1182–6.

Vilchez F, **Martínez-Pastor JC**, **García-Ramiro S**, **Bori G**, **Maculé F**, **Sierra J**, **Font L**, **Mensa J**, **Soriano A**. 2011. Outcome and predictors of treatment failure in early post-surgical prosthetic joint infections due to Staphylococcus aureus treated with debridement. Clin Microbiol Infect **17**:439–444.

Vilchez F, **Martínez-Pastor JC**, **García-Ramiro S**, **Bori G**, **Tornero E**, **García E**, **Mensa J**, **Soriano A**. 2011. Efficacy of debridement in hematogenous and early post-surgical prosthetic joint infections. Int J Artif Organs **34**:863–869.

Vogelman B, **Gudmundsson S**, **Leggett J**, **Turnidge J**, **Ebert S**, **Craig WA**. 1988. Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. J Infect Dis **158**:831–47.

Widmer AF, **Frei R**, **Rajacic Z**, **Zimmerli W**. 1990. Correlation between in vivo and in vitro efficacy of antimicrobial agents against foreign body infections. J Infect Dis **162**:96–102.

Widmer AF, **Wiestner A**, **Frei R**, **Zimmerli W**. 1991. Killing of nongrowing and adherent Escherichia coli determines drug efficacy in device-related infections. Antimicrob Agents Chemother **35**:741–6.

Widmer AF. 2001. New developments in diagnosis and treatment of infection in orthopedic implants. Clin Infect Dis **33 Suppl 2**:S94-106.

Williams DL, **Bloebaum RD**. 2010. Observing the Biofilm Matrix of Staphylococcus epidermidis ATCC 35984 Grown Using the CDC Biofilm Reactor. Microsc Microanal **16**:143–152.

Windsor RE, **Insall JN**, **Urs WK**, **Miller D V**, **Brause BD**. 1990. Two-stage reimplantation for the salvage of total knee arthroplasty complicated by infection. Further follow-up and refinement of indications. J Bone Joint Surg Am **72**:272–8.

Wolf CF, **Gu NY**, **Doctor JN**, **Manner PA**, **Leopold SS**. 2011. Comparison of one and two-stage revision of total hip arthroplasty complicated by infection: a Markov expected-utility decision analysis. J Bone Joint Surg Am **93**:631–9.

Wolff F, **Deprez G**, **Seyler L**, **Taccone F**, **Hites M**, **Gulbis B**, **Vincent J-L**, **Jacobs F**, **Cotton F**. 2013. Rapid quantification of six β-lactams to optimize dosage regimens in severely septic patients. Talanta **103**:153–60.

Wolska KI, **Grudniak AM**, **Rudnicka Z**, **Markowska K**. 2016. Genetic control of bacterial biofilms. J Appl Genet **57**:225–238.

Xu H, **Zhou W**, **Zhou D**, **Li J**, **Al-Huniti N**. 2017. Evaluation of Aztreonam Dosing Regimens in Patients With Normal and Impaired Renal Function: A Population Pharmacokinetic Modeling and Monte Carlo Simulation Analysis. J Clin Pharmacol **57**:336–344.

Zeller V, **Lavigne M**, **Biau D**, **Leclerc P**, **Ziza JM**, **Mamoudy P**, **Desplaces N**. 2009. Outcome of group B streptococcal prosthetic hip infections compared to that of other bacterial infections. Joint Bone Spine **76**:491–6.

Zhang L, **Dhillon P**, **Yan H**, **Farmer S**, **Hancock RE**. 2000. Interactions of bacterial cationic peptide antibiotics with outer and cytoplasmic membranes of Pseudomonas aeruginosa. Antimicrob Agents Chemother **44**:3317–21.

Zimmerli W, **Ochsner PE**. 2003. Management of infection associated with prosthetic joints. Infection **31**:99–108.

Zimmerli W, **Trampuz A**, **Ochsner PE**. 2004. Prosthetic-joint infections. N Engl J Med **351**:1645–54.

Zimmerli W, **Widmer AF**, **Blatter M**, **Frei R**, **Ochsner PE**. 1998. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. JAMA **279**:1537–41.

Zürcher-Pfund L, **Uçkay I**, **Legout L**, **Gamulin A**, **Vaudaux P**, **Peter R**. 2013. Pathogen-driven decision for implant retention in the management of infected total knee prostheses. Int Orthop **37**:1471–5.

ANNEXES

ANNEXE 1

Standardized protocol for collecting data of PJI in the Osteoarticular Infection Unit (Hospital Universitari de Bellvitge)

Episodi

RESUM PACIENT

Nom i cognoms

NHC

Pròtesi

Tipus infecció

Ingrés (mes/any)

Micro

Infecció

Recidiva (s/n)

Superinfecció / Reinfecció

IQ

ATB

DATOS DEL PACIENTE

Nombre | Apellidos

Iniciales | Sexo

NHC

F. Nacimiento: | Teléfono:

Hospital:

E. Base 1: | E. Base 2:

E. Base 3:

Clasificación:

aguda postq | hematógena | postquir tardía | cultivo IO +

Tipo de prótesis:

PTC | PTR | HAC | Osteosínt | Codo | Hombro

Fecha de colocación prótesis:

CIRUGIA

Prótesis:

primaria | secundaria | terciaria | cementada con ATB | cementada sin ATB

híbrida

Material prótesis:

cromocobalto | ac. inox | titanio | cerámica | polietileno

hidroxiapatita | otros

Cirugía:

profilaxis ATB | ASA | Duración (min):

DIAGNOSTICO

Fecha Diagnóstico:

Duración clínica (días): (fecha inicio de síntomas)
(días desde diagnóstico hasta el día del tratamiento-quirúrgico o ATB)

Duración ingreso (días):
(suma total de días de todos los ingresos relacionados con el episodio)

Evento previo (en 1 año):

Artroscopia	Administración intracavitaria de fármaco
Bursitis prerotuliana	Infección superficial
Infección previa de articulación	Bacteriemia mismo gèrmen
Maniobras bacteriémicas	Endocarditis
Infección respiratoria	Infección GI
Infección urinaria	Otras infecciones

Clínica:

Dolor	S. Inflamatorios	Supuración
Fístula	Fiebre	
Merle A	Knee society	Leucocitos

Rx simple:

Osteolisi geodas	Lisis periprótesis lineal
Reacción perióstica	Aflojamiento protésico

AP:

Pus macroscópico		Bx peroperatoria >10 leucos/c
Bx sinovial:	PMN	Reacc cuerpo extraño MS
Bx ósea:	PMN	Reacc cuerpo extraño MS

Líquido articular

Gluc (mg/dl)	Proteínas (g/l)	Nº céls	Tipo céls

PCR

Fecha	Valor

VSG

Fecha	Valor

Exploracion de imagen

Fecha	Infección (S/N)

MICROORGANISMO

Nombre:

Papel: Infección / Recidiva/Persistencia / Superinfección

Fecha muestra quirúrgica:

ATB previo (s/n):

	Realizado (nº)	Positivo (nº)
Frotis 1		
Frotis 2		
M. sinovial		
Cemento		
Prótesis		
BH cótilo		
BH fémur		
BH tibia		
M. periprót		
L. articular		

Muestra no quirúrgica 1: Fecha 1

Muestra no quirúrgica 2: Fecha 2

Muestra no quirúrgica 3: Fecha 3

Sensibilidad ATB

Penicilina	Oxacilina	Gentamicina	Cotrimoxazol
Clindamicina	Rifampicina	Ciprofloxacino	Vancomicina
Teicoplanina	Linezolid	Amoxi-clavul	Cefotaxima
Ampicilina	Imipenem	Eritromicina	Aztreonam
Piperacilina	Ceftazidima		

TRATAMIENTO

TRAT QUIRÚRGICO:

Fecha	Tipo

Fecha	Tipo

Opciones: Desbridamiento, Rec 1T, Rec 2T 1º, Rec 2T 2º, Girldstone, Artrodesis, Fij ext,
Retirada material + fij ext, Amputación, Cir plástical, Rec 1T cótilo, Rec 1T vástago
Desbridamiento + rosario genta, Desbridamiento + retirada material

Espaciador (s/n): ATB espaciador:

Cemento (s/n)): ATB cemento:

Hueso de banco (s/n):

Cultivo en el 2º tiempo (s/n):

Microorganismo en 2º tiempo:

Material protésico

Tipo de tto quirúrgico: Desbridam Recambio 1T

Recambio 2T Artrodesis

Retirada + implante misma prótesis

TRAT ANTIBIÓTICO:

ATB (solo/combinación)	Inicio	Final

Efectos secundarios:

Tipo Ef. secundario	Leve/Grave	ATB	Fecha

EVOLUCION

(Desde la finalización del tratamiento ATB)

6 meses:

Curación | Recidiva | Reinfección

Supresión con ATB | Desconocida

Merle

Knee

1 año:

Curación | Recidiva | Reinfección

Supresión con ATB | Desconocida

Merle

Knee

1,5 años:

Curación | Recidiva | Reinfección

Supresión con ATB | Desconocida

Merle

Knee

2 años:

Curación | Recidiva | Reinfección

Supresión con ATB | Desconocida

Merle

Knee

Exitus

Causa: Relacionada / No relacionada

NOTAS

ANNEXE 2

Articles

Journal of Infection (2014) 69, 235–243

ELSEVIER

BIAM British Infection Association

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Clinical and microbiological findings in prosthetic joint replacement due to aseptic loosening

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Accepted 8 May 2014
Available online 23 May 2014

KEYWORDS
Prosthetic joint;
Aseptic loosening;
Microorganisms

Summary *Objectives:* A role for microorganisms in aseptic prosthetic loosening (AL) is postulated. We analyse the microbiological and clinical findings of patients with suspected AL, and compare them with patients with chronic prosthetic joint infection (PJI).
Methods: Prospective study (2011–2012) of patients with presumed AL. Evaluation of tissue samples (≥5; TS) at the time of surgery and sonication fluid (SF) of prosthesis.
Results: According to positive culture in TS/SF, 89 patients were divided into: Group 1: (≥2 positive-TS; $n = 12$); Group 2: single positive-TS and concordant SF ($n = 10$); Group 3: one positive or non-concordant TS or SF ($n = 38$); and Group 4: cultures negative ($n = 29$). Positive-SF was always concordant with TS in Group 1 (75%); it was positive in 74% in Group 3. Median months (prosthesis-age: implantation to revision arthroplasty) for PJI and Group 1–4 was 21, 46, 65, 63 and 81, respectively ($P < 0.001$); they also had a different dynamic trend in prosthesis failure ($P < 0.001$).
Conclusions: Several patients with suspected AL are misdiagnosed PJI. Results from SF correlated well with TS in Group 1, led us to consider single positive-TS as significant (Group 2) and to suggest that microorganisms were on the prosthesis (Group 3). We observed a correlation between microbiology and prosthesis-age, which supports that early loosening is more often caused by hidden PJI than late loosening.

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0163-4453/$36
http://dx.doi.org/10.1016/j.jinf.2014.05.003

Introduction

The number of primary arthroplasties has been increasing in recent years. Consequently, it is estimated that total hip and knee prosthetic replacements will have doubled in number by the year 2015 (knee) and 2026 (hip).[1,2]

The most common cause of implant failure is aseptic loosening (AL), followed by prosthetic joint infection (PJI). The pathogenesis of aseptic loosening is not well known, but involves a local inflammatory process in which several cells and cytokines activate osteoclasts involved in bone resorption.[3–5] However, prosthesis loosening can also be the consequence of low-grade infection, usually produced by low-virulence microorganisms that can survive in biofilm populations on the implant surface.[6–8] Currently, an initial suspicion of implant failure etiology based on clinical and biochemical aspects, histopathology and mainly microbiological findings, helps physicians to diagnose prosthesis loosening.[7,9]

In recent years, new and sophisticated technologies (mainly based on recovering bacteria attached to the prosthesis) have been applied in the setting of implant failure revisions. Thus, Tunney et al. used prosthesis sonication and microscopy techniques (scanning electron and immunofluorescence microscopy) to show the presence of microorganism aggregates in sonicated fluid from the explanted prosthesis. They, as well as other authors, have postulated that PJI is underdiagnosed among cases of prosthesis loosening.[10–15] In contrast, other studies identified the presence of microorganisms such as coagulase-negative staphylococci (CoNS), which the authors interpreted as contaminants.[16]

Since most of the new technologies, except sonication, are difficult to incorporate in clinical practice, recent efforts have been made to validate the results obtained by this methodology by comparing them with results of histopathology or periprosthetic tissue cultures.[17–20] Nowadays, controversy still exists regarding the universal use of sonication in clinical practice,[9] but some personal opinions recommend the inclusion of the sonication technique in evaluations of prosthesis failure, to improve the etiologic diagnosis of infection.[21]

The presence of a single positive culture (either from tissue or from prosthesis sonication) still remains a matter of concern due to the difficulties in distinguishing infection (active or subclinical) from contamination.[22,23] To our knowledge, only a few studies have attempted to establish the relationship between microbiological cultures and clinical findings in cases of suspected prosthesis loosening.[16,24,25] In fact, the evaluation of AL remains a challenge, in part due to the absence of a reliable gold-standard for PJI diagnosis.

The aim of the present article was to analyse the microbiological and clinical findings of a cohort of patients with suspected AL at the time of revision arthroplasty to determine the incidence of PJI among this cohort, and to compare their clinical and microbiological characteristics with a cohort of patients with late chronic PJI.

Material and methods

Setting

The study was performed in the Osteoarticular Infection Unit of two Spanish tertiary-care teaching hospitals in Barcelona. The research groups involved have wide experience and have published several papers on clinical aspects of this field.

Study design

From January 2011 to December 2012 all patients who underwent a revision of hip or knee arthroplasty due to presumed AL were prospectively included in this observational study.

Management protocol

A diagnosis of presumed AL was made when patients had joint pain and radiological signs of prosthesis loosening in the absence of signs or symptoms of infection (local inflammatory signs, sinus tract, or systemic symptoms of infection), and the C-reactive protein (CRP) or the erythrosedimentation rate (ESR) were considered not clinically relevant (values lower than 15 mg/L and 40 mm/h, respectively). Diagnosis of PJI was established according to the last recommendations[9]; it was considered on the basis of: i/the presence of signs and symptoms of infections (defined above) or purulence around the prosthesis during surgery, ii/the histopathologic findings (at least five neutrophils per high-power field -x400- found in at least five separate microscopic fields: Feldman's criterion), or iii/the microbiological results obtained from preoperative and intraoperative cultures (two or more cultures that yielded the same organism, or the growth of a virulent microorganism in a single sample).

All patients included in the study underwent one-step revision arthroplasty, in which one or two prosthetic components were removed according to radiological signs and/or surgical findings of loosening. During the surgery, intraoperative samples from periprosthetic tissues, including bone and synovial membranes (hereafter named as tissue samples), were obtained for cultures in aerobic and anaerobic conditions, and the prosthetic components were removed. Both were then transported to the microbiology laboratory for processing (see below). Patients received standard peri-operative antibiotic prophylaxis (Cefazolin or Teicoplanin plus ceftazidime) one or two dose depending on the duration of the operation (less or more than 6 h), immediately after surgical samples were collected.

After surgery, patients were classified as having a diagnosis of: i/definitive PJI when visible purulence was observed around the prosthesis, and/or presented histopathology and microbiologic findings as defined above; and ii/definitive AL, in cases that did not meet the criteria for PJI.[26] Among the latter group, further analyses were made on the basis of the presence of only 1 positive culture from tissue samples and the existence of a positive or negative culture from the sonication fluid of prosthesis.

A control group of patients with late chronic prosthetic joint infection (LCPJI) due to CoNS were recorded from patients admitted and treated in one of the hospitals. All these patients were diagnosed with LCPJI on the basis of the presence of clinical criteria for PJI (as defined above) developed more than 1 month after total arthroplasty, and the results of histopathology and/or microbiological cultures of material obtained from joint aspiration before surgery and/or from first-stage surgery.

Patient baseline and clinical characteristics, radiological and microbiological findings were prospectively gathered in a database.

Microbiology processes

In all cases, $\geq$5 periprosthetic tissue samples were sent and processed in the Microbiology Laboratory. These specimens were cultured in 5% horse blood, chocolate, MacConkey agar plates and in thioglycolate medium with prolonged incubation (10 days) at 30—35 °C. All microorganisms were identified by standard biochemical reactions and susceptibility was studied by the disk diffusion method or by a microdilution system (Phoenix System, Becton Dickinson, Franklin Lakes, NJ, USA). Criteria of susceptibility or resistance to the various antibiotics were established according to CLSI recommendations.[27]

At the time of revision surgery, the prosthetic components were removed and introduced separately into sterile air-tight containers in the operating theatre as follows: acetabular component plus polyethylene, femoral component plus femoral head, femoral component or tibial component plus polyethylene. This process allowed us to analyse the relationship between the bone loosening of each component and the microbiological culture. Once in the Microbiology Laboratory, 150 mL of Luria—Bertani (LB) medium was added to the sterile container to cover the prosthetic material. Then, the container was introduced into an ultrasound bath (Branson 3510, Bransonic Danbury, USA) for 5 min at 40 Hz. After that, 100 μl of the sonicated fluid was inoculated in a blood-agar plate for a first colony count, and aliquots of 1 mL of this fluid were kept frozen at −80 °C for further microbiological analyses. The container with the removed component and the remaining fluid was incubated overnight at 37 °C. The next day, a new blood agar plate and a thioglycollate medium were processed and cultured for 48 h. Finally, fluid from sonication was considered negative if there were no macroscopic bacterial growth. Microorganisms were identified as indicated above.

All the microbiological processes were performed in a laminar flow cabinet to assure that manipulation was not a cause of contamination.

Radiological evaluation

Radiological bone loosening was blinded evaluated by a senior orthopedic surgeon. Results of acetabular and femoral bone lysis were interpreted according to the Paprosky classification (in hip arthroplasties), and femoral and tibiae lysis according to the Engh classification (in knee arthroplasties).[28—30]

Acetabular defects are typed from 1 to 3 by the Paprosky classification. Type I defects have bone lysis around cement anchor sites; type IIA and B defects display progressive bone loss superiorly; type IIC has medial wall defects; and type IIIA and B defects have progressive amounts of superior rim deficiencies.

Femoral bone loss is typed from 1 to 4 by the Paprosky classification. Type I is defined as minimal metaphyseal bone loss; type II defects have extensive metaphyseal bone loss with an intact diaphysis, type IIIA and B also have extensive metaphyseal bone loss, but have different degrees of cortical bone defects in the diaphysis; type IV has extensive metaphyseal bone loss and a non-supportive diaphysis.

Engh classified bone lysis into three types for the tibia (T1, T2, T3) and femur (F1, F2, F3). Type 1 is defined as no cortical defects and minimum bone loss not compromising component stability; type 2 implies unilateral or bilateral metaphyseal bone damage with prosthesis migration, type 3 is defined by significant bone loss compromising a major portion of the plateau, which may involve detachment of the patellar tendon.

Type I acetabular and femoral defects (Paprosky classification), and Type I tibia and femur bone lysis (Engh classification) were considered the minimal lysis for further analysis.

Statistical analysis

Continuous variables were expressed as the median and interquartile range (IQR), and were compared by means of the Mann—Whitney *U* test or the Kruskall—Wallis test, as appropriate. Categorical variables were expressed as count and valid percentage, and were compared with the Chi-square test or Fisher's exact test, as appropriate. Changing trends in categorical parameters were evaluated with the Mantel—Haenszel X^2 test for trends. A comparison of the age of the prosthesis was made with Kaplan—Meier curves and the long-rank test. Statistical significance was defined as a two-tailed *P* value <0.05. Data were analysed using the SPSS program (version 20.0, Chicago, IL).

Results

A total of 89 patients with presumed AL were included in the study: 60 (67%) had undergone hip replacement, and 29 (33%) knee replacement. The median age was 74 years (IQR: 65—81) and 50 (56%) patients were female. The main comorbidities were cardiovascular diseases in 60 patients (67.4%), diabetes mellitus in 14 (16%) and chronic pulmonary disease in 11 (12%). The removed prostheses were primary in 70 cases (79%) and revision prostheses in 19 (21%); 61% of the prostheses were cemented. The general characteristics of the presumed AL group and the control LCPJI group were similar, except in terms of the prosthesis location (Table 1).

The microbiology results of all cases included in the study are shown in Tables 2 and 3. According to standard and sonication cultures, AL were divided into Group 1 ("Definitive PJI"): those with $\geq$2 concordant positive tissue samples, disregarding the results in the sonication culture, which were treated with long-term antibiotics ($n = 12$); Group 2: cases with a single positive intraoperative tissue culture plus a concordant positive sonication culture with the same microorganism (defined as same species name and susceptibility pattern), which were treated with long-

Table 1 Patients' baseline characteristics and features of the prostheses that were removed.

	LCPJI (n = 23)	Group 1 (n = 12, 13%)	Group 2 (n = 10, 11%)	Group 3 (n = 38, 43%)	Group 4 (n = 29, 33%)	All (group 1–4) (n = 89, 100%)	
Age median (IQR)	72 (66–79)	74 (65–82)	76 (67–82)	77 (66–82)	73 (64–79)	74 (65–81)	
Sex (female)	14 (60.9%)	7 (58.3%)	4 (40%)	18 (47.4%)	19 (65.5%)	50 (56.2%)	
Underlying diseases							
Cardiovascular diseases[a]	9 (39%)	5 (41.7%)	8 (80%)	29 (76.3%)	18 (62.1%)	60 (67.4%)	
Diabetes mellitus	5 (22.7%)	1 (8.3%)	1 (10%)	8 (21.1%)	4 (13.8%)	14 (15.7%)	
Cirrhosis	2 (9.1%)	1 (8.3%)	0	1 (2.6%)	0	2 (2.2%)	
COPD[b]	1 (4.3%)	2 (16.7%)	2 (20%)	4 (10.5%)	3 (10.3%)	11 (12.4%)	
Other[c]	–	2 (16.7%)	1 (10%)	2 (5.2%)	4 (13.8%)	9 (10.1%)	
Localization							
Hip	8 (35%)	10 (83.3%)	7 (70%)	25 (65.8%)	18 (64.1%)	60 (67.4%)	
Knee	15 (65%)	2 (16.7%)	3 (30%)	13 (34.2%)	11 (37.9%)	29 (32.6%)	
Type of prosthesis							
Primary	14 (63.6%)	10 (83.3%)	9 (90%)	28 (73.7%)	23 (79.3%)	70 (78.7%)	
Revision	8 (36.4%)	2 (16.7%)	1 (10%)	10 (26.3%)	6 (20.7%)	19 (21.3%)	
Cemented		7 (58.3%)	4 (40%)	25 (78.1%)	18 (75%)	54 (60.7%)	
Pain for > 1-year	–	6 (50%)	4 (40%)	18 (51.4%)	11 (44%)	39 (47%)	
Prosthesis age[d] (median months, IQR)	21 (14–45)	46 (31–131)	65 (29–208)	63 (46–153)	81 (40–167)	65 (38–155)	$P < 0.001$
Number of components exchanged	46	18	16	59	46		
Bone lysis by component							
Minimal lysis degree (Type I, T1 and F1)	16 (35%)	11 (61%)	6 (38%)	22 (37%)	21 (47%)		

Footnote: Group 1: those patients with ≥2 positive intraoperative tissue samples, disregarding the results in the sonication culture. Group 2: cases with a single positive intraoperative tissue culture plus a concordant positive sonication culture with the same microorganism (defined as same species name and susceptibility pattern); Group 3: cases with one positive culture (standard or sonication) or a non-concordant microorganism either from the tissue sample or the sonication fluid; and Group 4: patients with all cultures negative. LCPJI: diagnosis of PJI (according to standard criteria) developed more than 1 month after total arthroplasty.

[a] Cardiovascular diseases include: hypertension and ischaemic heart diseases.

[b] COPD: chronic obstructive pulmonary diseases.

[c] Other: HIV, dementia, rheumatoid arthritis, neoplasia.

[d] Prosthesis age: time from implantation to revision arthroplasty. Heart diseases. The median prosthesis age between groups was statistically significant ($p < 0.001$, Kruskal–Wallis). The median prosthesis age median between (Group LCPJI + Group 1) and (Group 2–4) was statistically significant ($p < 0.001$, U-Mann–Whitney).

term antibiotics or were left untreated according to the clinician criteria (n = 10); Group 3: cases with one positive culture (standard or sonication) or a non-concordant microorganism either from the tissue sample or the sonication fluid, which were treated with antibiotics or were left untreated according to the clinician criteria (n = 38); and Group 4: patients with all cultures negative (n = 29). A total of 139 prosthetic components, from 89 patients, were sonicated and 59 (42%) were positive.

The concordance of the microbiological results from tissue samples and sonication is also shown in Tables 2 and 3. In Group 1, there were 9 (75%) cases in which the sonicated fluid of prosthetic components was positive, and the same microorganism was identified in the tissue samples. Three cases had an additional single positive tissue culture that was discordant with the other samples (3/12 = 25%). In Group 2, concordant results were due by definition. Additionally, discordant results were observed in the sonicated fluid in two cases (2/10 = 20%) and in one tissue culture (1/10 = 10%). In contrast, discordance was established in Group 3 by definition. We identified 12 cases with a positive tissue sample (3a; 12/38 = 32%) and 26 with a positive sonicated fluid sample (3b). In the first subgroup, 2 patients had one positive sonicated fluid sample that was not concordant with the tissue isolation. In 7 patients from Group 3b, the two sonicated components were positive and the same bacteria were identified in 5 cases.

The median time from implantation to revision arthroplasty (prosthesis age) for LCPJI, and Groups 1, 2, 3 and 4 was 21, 46, 65, 63 and 81 months, respectively ($P < 0.001$; Table 1), whereas the percentage of patients with prolonged pain (>1-year) was similar between groups. The survival curve is shown in Fig. 1. We observed a different dynamic trend in prosthesis failure evolution between LCPJI, Group 1 and the last 3 groups ($p < 0.001$; see Fig. 1). Revision arthroplasties within the first 2 years were mainly performed among the cohort of LCPJI (57%), rather than among patients with presumed AL (less than 20% in any group, and no differences between them). We found significant differences between groups in the percentage of prostheses exchanged 4 years after

Table 2 Microbiological findings: conventional tissue cultures and sonicated fluid cultures.

Patients[a]	Group 1 12	Group 2 10	Group 3 38	Group 4 29	All 89	
Conventional tissue samples cultures						
Positive[b]	12 (100%)	10 (100%)	12 (32%)	0	34 (38%)	
Microbiology num. cases, bacteria (num. positive samples, in group 1)	6 CoNS (≥2) 1 *Corynebacterium spp* (≥2) 1 *P. aeruginosa* (≥2) 1 CoNS (≥2) + *B. cereus* (1) 1 CoNS (≥2) + *Corynebacterium spp* (1) 1 CoNS (2) + *S. viridans* (2) 1 *Corynebacterium spp.* (≥2) + *E. faecalis* (1)	8 CoNS 1 *Corynebacterium spp.* 1 CoNS + *E. faecalis*	5 CoNS 3 *Corynebacterium spp* 1 *Anaerobic* 1 *M. luteus* 1 CoNS + *P. aeruginosa* 1 CoNS + *S. viridans*	–		
Discordant positive[c]	3 (25%)	1 (10%)	12 (32%)	0	16 (18%)	
Sonication fluid cultures						
Positive[b]	9 (75%)	10 (100%)	28 (74%)	0	47	
Discordant positive[c]	0	2 (20%)	28 (74%)	0		$p = 0.005$
Prosthetic components[d]	18	16	59	46	139	
Sonication fluid cultures						
Positive[e]	11 (61%)	13 (81%)	35 (59%)	0	59 (42%)	
Discordant with conventional tissue samples[f]	0	2 (12%)	35 (59%)	0		
Microbiology	8 CoNS 1 *Corynebacterium spp* 2 *P. aeruginosa*	11 CoNS 1 *Corynebacterium spp.* 1 NI	26 CoNS 2 *Corynebacterium spp* 2 *P. aeruginosa* 2 *Bacillus* 1 *M. luteus* 1 *A. viridans* 1 Not identified			

[a] Microbiological findings are analysed by patient ($n = 89$), detailing whether the results correspond to tissue or sonicated samples.
[b] Positive: includes patients with at least one positive culture.
[c] Discordant positive: includes patients with single positive cultures that are not-concordant with the microorganism that caused the infection (in Group 1 and Group 2) or when single positive cultures were isolated (Group 3). Differences between Group 1—Group 2 and Group 3 ($p = 0.005$).
[d] Microbiological findings are analysed by prosthetic components ($n = 139$).
[e] Positive: includes components with positive sonicated fluid culture.
[f] Discordant with conventional tissue samples: number of components with positive cultures that are not-concordant with the correspondent tissue samples.

implantation: this intervention was performed in 83% cases within the cohort LCPJI, and in 58%, 50%, 32% and 31% in Groups 1, 2, 3 and 4, respectively (MH Test for trend $p < 0.001$).

Among all cases with presumed AL, bone lysis was notably higher in patients with older prostheses (Groups 2, 3 and 4) than in patients from Group 1, with subclinical pre-surgical infection, lower prosthesis age, and mostly a minimal degree of lysis. By contrast, patients with pre-surgical signs of prosthesis infection (LCPJI) showed higher bone lysis, even though they had the lowest prosthesis age (Table 1).

The follow-up after revision arthroplasty was recorded for cases in Group 2, due to a specific clinical interest in the evaluation of these cases with 1 single positive TS and a concordant positive SF. None of these patients were treated with long-term antibiotics but only with revision surgery. After a median of 16 months (IQR 6—24) of follow-up, there was one case who presented a new prosthesis infection caused by *Staphylococcus aureus* (a different

Table 3 Microbiological findings from patients of Group 3 ($n = 38$).

	Group 3a Single positive tissue sample	Group 3b Single positive sonicated sample	All
Patients (n)	12	26	38
[a]PC positive/total PC	2[b]/19	33/40	35/59
Patients with 2 positive PC	0	7[c]	–
Concordant	–	5	–
Discordant		2	

Footnote: Microbiological findings in Group 3 were analysed in two subgroups depending on the provenance of the positive cultures (tissue sample or sonicated sample).

[a] PC: prosthesis components. PC samples were analysed in both subgroups.

[b] 2 PC in Group 3a also had positive PC cultures that were discordant with the tissue samples.

[c] 7 patients in Group 3b had 2 positive PC, in 5 of which were concordant microorganisms (CoNS).

microorganism than the one isolated in the implant revision) 5 months after the implant revision, and the remaining cases were free of infection.

Discussion

In the present study, we analyse the clinical characteristics of patients with a presumed diagnosis of AL, according to microbiological findings at the time of surgical revision, and compare them with a cohort of patients with LCPJI.

Joint prosthesis loosening can be the result of either an aseptic process or infection, thus it is important to reach the correct diagnosis to provide the appropriate treatment. Clinical characteristics are the main guide in the initial suspicion of the cause of loosening. Thus, the absence of local inflammatory signs or sinus tract supports the diagnosis of an aseptic process and in addition, normal levels of C-reactive protein can also be used with limited specificity.[31] However, surgical findings (macroscopic pus or histology) and microbiological cultures of surgical samples have proved useful for clinicians to identify some cases of infection among presumed AL.[6,7,21,32] This situation was previously well defined by Tsukayama et al. as a particular type of infection ("Intraoperative positive cultures"), or more recently by other authors as subclinical PJI. Currently, the recommendation is to obtain at least 5 tissue samples at the time of revision arthroplasty. Definitive criteria of infection are considered when $\geq$2 positive cultures are isolated with the same microorganism or a virulent microorganism is isolated in a single sample.[9,22]

In our study, 12 cases of pre-surgical suspicion of AL had microbiological definitive criteria of PJI (Group 1). While this fact might lead us to question whether these cases belong to a misdiagnosed group, it seems that the group has its own characteristics. When we compared patients in Group 1 with a cohort of patients with LCPJI, we observed that the latter had a significantly different dynamic trend in the evolution of prosthesis failure, with a shorter time from implantation to revision (prosthesis age) and notably higher bone lysis. These differences suggest a more aggressive process in cases of LCPJI, probably with a high bacterial load, and obvious clinical signs of infection. Moreover, is interesting to note that the percentage of hip prosthesis were clearly higher in patients with pre-surgical diagnosis of AL than among cases with LCPJI. These findings support the less obvious signs of infections in patients with hip prosthesis as compared to those with knee prosthesis, and

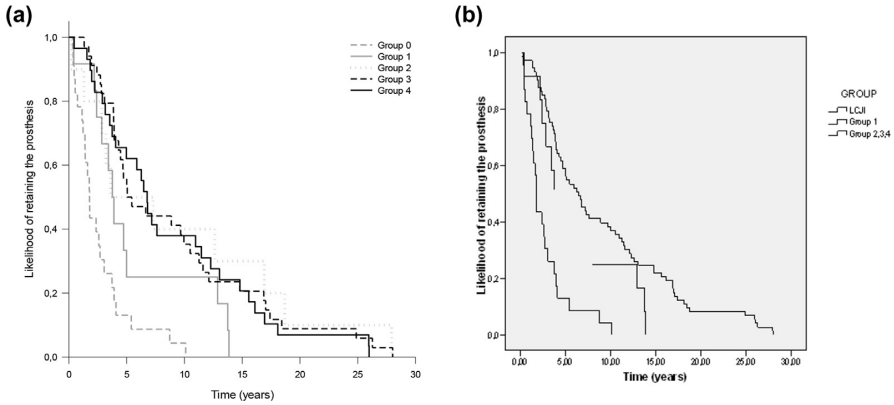

Figure 1 Analysis of free-survival of prosthesis in the different groups, according to microbiological findings at the time of revision. **1a**. Group 0 (LCPJI): patients with late chronic infection by CoNS. Time (years) = prosthesis age (time from implantation to revision arthroplasty). **1b**. Dynamic trend in prosthesis failure between LCPJI, Group 1 and Groups (2 + 3 + 4) was statistically significant ($p < 0.001$, Log Rank).

this could be related to the different soft tissue conditions at the two locations. Overall, in accordance with previous reports, our results also underline the importance of a systematic search for infection when loosening appears in the first years after implantation.[17,21,33]

Cases with a single positive culture from intraoperative tissues are also well documented in the present study in 22 patients (29%): 10 from Group 2 and 12 from Group 3. The classification of these cultures as infection or as contamination of the surgery and laboratory processes still remains a challenge. The probability that some cases may represent real "clinically silent" PJI was previously calculated to be around 8%.[22] Overall, the accurate interpretation of a single positive tissue culture is of great clinical concern, because a diagnosis of definitive PJI or AL defines different therapeutic approaches.

In recent years, new technologies applied to the diagnosis of PJI have focused on recovering bacteria adhered to the prosthesis in a biofilm population. In the first consistent studies in this setting,[10,12] the authors used scraping and sonication of the implant surface and observed bacteria within a confluent biofilm, either by electronic microscopy or immunofluorescence techniques. Thus, they considered it unlikely that these bacteria represented contamination. Unfortunately, most of these technologies, except prosthesis sonication, are difficult to incorporate into clinical practice and thus efforts have been focused on validating results with this methodology.[17–19] In recent recommendations, the vortexing and quantification of the number of microorganisms in the sonicated fluid (using a breakpoint of 50 colony forming units/ml) has been proposed to distinguish between infected and contaminant prostheses.[17,19,20] Although there is no formal consensus on the sonication protocol and the number of microorganisms required to consider infection,[9] sonication samples provide new microbiological information that clinicians should interpret.

In the present study, we analysed the value of cultures from prosthesis sonication among cases of presumed AL. On the basis of these results, we identified a group of patients with a single positive tissue sample and concordant sonication fluid culture (Group 2). In this Group, we could apply criteria for considering prosthesis loosening caused by infection; in all likelihood, some centres that processed routinely the sonication fluid, consider these cases as definitive diagnosis of PJI and treat these patients with additional antibiotics. Second, the sonication was concordant with conventional cultures in 75% of cases in Group 1, and the low percentage of discordant results in Groups 1 and 2 (0% and 2/16, 12%), supported the presence of a "non-contaminant" microorganism from sonicated prostheses in these two groups, which were comprised of a total of 22 out of 89 patients (25%) from the overall series. The evaluation of the microbiological findings in Group 3 is difficult and deserves particular attention. Twelve patients had a single positive tissue culture that could be considered probable contamination (12/38 = 32%); this proportion was similar to that of Group 1 (25%). In contrast, 28 patients (74%) had a positive culture from the sonicated fluid, and some had the same microorganism in both prosthetic components. In these cases it is difficult to determine whether the microorganisms isolated in the sonication fluid are contaminants or were really attached to the surface of the removed prosthesis. When we compared this percentage with that of the discordant results in Groups 1 and 2 (cases with "proven infection", 0 and 20%, respectively), we observed significant differences (chi-square $p = 0.005$). In our opinion, these contrasting data suggest that it is unlikely that isolated positive cultures from sonicated fluid should always be considered contaminant. Nevertheless, the optimal therapeutic management of those cases with low bacterial inoculum is still not clearly defined. In the present work, all patients of Group 2 were not treated additionally with long-term antibiotics but they did not develop persistence or relapse of initial infection. These results are in accordance with that reported for Barrack et al.,[16] which supported that in most of cases prosthesis removal could be enough to eradicate the low bacterial inoculum. However, taking into account the particularities of foreign-body infections and while waiting for further clinical evidence, prudent interpretation of a single positive culture is recommended.

The evaluation of clinical findings in our cohort of cases with pre-surgical suspicion of AL showed different dynamics in the prosthesis explantation surgery between the groups, established according to the microbiological results. We observed a progressive increase in prosthesis age among patients from Group 1 to Group 4, and this difference was statistically significant when cases with a clear diagnosis of PJI (LCPJI and Group 1) were compared with the remaining groups. Considering the implantation date of the analysed prosthesis, the number of revised arthroplasties performed during the first two years after prosthesis implantation was very low among any group of presumed AL, whereas it was almost 60% among the cohort of LCPJI. Of note, when we analysed the results four years after implantation of the arthroplasty, we observed that revised arthroplasties were more common among patients with LCPJI (82%) and patients with presurgical suspicion of AL, but with microbiological findings of infection (Group 1 – 58%, and 2 – 50%), than among patients without findings of infection from intraoperative cultures (Groups 3 – 32%, and 4 – 31%). These results suggest that early prosthesis failure is associated with a strong likelihood of infection, disregarding the presence or absence of compatible clinical signs or symptoms.[33–35] In addition, our data show that the higher the bacterial inoculum (the number of positive tissue and sonicated fluid cultures), the shorter the time from primary arthroplasty to revision surgery. These microbiological results suggest that bacteria were real pathogens that could participate in the earlier implant failure.

We did not detect differences in the degree of bone lysis among the cases in Groups 2, 3 and 4. Therefore, we could not confirm the results observed in a previous study,[36] in which an association was found between bone lysis and microbiological results obtained from explanted prosthesis sonication. However, we did find that a longer time between prosthesis implantation and revision was associated with a higher bone lysis degree. It is reasonable to think that patients in Group 1, which were early submitted to revision surgery with low degree of bone lysis, had additional clinical characteristics that unfortunately we did not collect but justified this early surgery.

Overall, we observed a correlation between microbiological findings and clinical parameters (mainly prosthesis age) in our case series. This supports the probable role of microorganisms in prosthesis failure but not in the degree of bone lysis, which is related to prosthesis age. Even today, it is not clear whether isolated low-virulence organisms can survive around the implant without pathological involvement, participate in prosthetic loosening, or cause delayed low-grade infections that mimic natural aseptic failure.[15] The pathogenesis of aseptic loosening is probably a multifactorial process that is not well known. The role of microorganisms in this setting has been postulated and related to the production of an inflammatory response,[3–5] but it should be further investigated.

Our study has some limitations that should be stated. Several difficulties in microbiological interpretation were inherent to the sonication technique: we did not incorporate vortexing and quantitative counts in our protocol, despite this is recommended by some authors, that would allow us to better interpret discordant results; and secondly, bacterial molecular identification was not performed. Both considerations could have contributed to differentiating contaminated from non-contaminated microorganisms. In contrast, we showed a homogenous AL case series, evaluated from a careful clinical perspective and taking into account both conventional peri-prosthetic tissue and fluid sonication samples, with the aim of finally developing a detailed discussion.

We conclude that, even after following appropriate current guidelines, several patients with suspected AL are really misdiagnosed PJI or have some microorganisms present in their samples. Results from prosthesis sonication among patients with presumed AL showed good correlation in cases of PJI diagnosed by conventional tissues (Group 1). This led us to consider that several cases with a single positive tissue sample were significant (Group 2), and to suggest that microorganisms were present on the implant surface in many other cases with negative tissue cultures (Group 3). We observed a correlation between the microbiological findings and prosthesis age (time from implantation to revision arthroplasty), which supports the probable role of microorganisms in the prosthesis failure rate. It remains a challenge to differentiate between contaminant and non-contaminant microorganisms isolated at the time of implant removal. However, universal consensus on the sonication process and on the interpretation of results is essential to offer an appropriate therapeutic approach.

Acknowledgements

This work was supported by a research grant from Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (FIS 10/01573), and supported by Ministerio de Economía y Competitividad, Instituto de Salud Carlos III — co-financed by European Development Regional Fund "A way to achieve Europe" ERDF, Spanish Network for the Research in Infectious Diseases (REIPI RD12/0015).

A.R. is the recipient of a research grant from the Bellvitge Biomedical Research Institute (IDIBELL).

Presented in part: 23rd European Congress of Clinical Microbiology and Infectious Diseases, Berlin 27—30 April 2013.

References

1. Kurtz S, Mowat F, Ong K, Chan N, Lau E, Halpern M. Prevalence of primary and revision total hip and knee arthroplasty in the United States from 1990 through 2002. *J Bone Jt Surg Am* 2005;**87**:1487—97.
2. Kurtz S, Ong k, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. *J Bone Jt Surg Am* 2007;**89**:780—5.
3. Hoenders CS, Harmesen MC, van Luyn MJ. The local inflammatory environment and microorganisms in "aseptic" loosening of hip prostheses. *J Biomed Mater Res B Appl Biomater* 2008;**86**:291—301.
4. Granchi D, Verri E, Ciapetti G, Stea S, Savarino L, Sudanese A, et al. Bone-resorbing cytokines in serum of patients with aseptic loosening of hip prostheses. *J Bone Jt SurgBr* 1998;**80**:912—7.
5. Nivbrant B, Karlsson K, Kärrholm J. Cytokine levels in synovial fluid from hips with well-functioning or loose prostheses. *J Bone Jt Surg Br* 1999;**81**:163—6.
6. Tsukayama DT, Estrada R, Gustilo RB. Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. *J Bone Jt Surg Am* 1996;**78**:512—23.
7. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med* 2004;**351**:1645—54.
8. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999;**284**:1318—22.
9. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2013;**56**:1—25.
10. Gristina AG, Costernon JW. Bacterial adherence to biomaterials and tissue. The significance of its role in clinical sepsis. *J Bone Jt Surg Am* 1985;**67**:264—73.
11. Dobbins JJ, Seligson D, Raff MJ. Bacterial colonization of orthopedic fixation devices in the absence of clinical infection. *J Infect Dis* 1988;**158**:203—5.
12. Tunney MM, Patrick S, Gorman SP, Nixon JR, Anderson N, Davis RI, et al. Improved detection of infection in hip replacements. *J Bone Jt Surg Br* 1998;**80**:568—72.
13. Tunney MM, Patrick S, Curran MD, Ramage G, Hanna D, Nixon JR, et al. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S RNA gene. *J Clin Microbiol* 1999;**37**:3281—90.
14. Nguyen LL, Nelson CL, Saccente M, Smeltzer MS, Wassell DL, McLaren SG. Detecting bacterial colonization of implanted orthopaedic devices by ultrasonication. *Clin Orthop Relat Res* 2002;**403**:29—37.
15. Nelson CL, McLaren AC, McLaren SG, Johnson JW, Smeltzer MS. Is aseptic loosening truly aseptic? *Clin Orthop Relat Res* 2005;**437**:25—30.
16. Barrack RL, Aggarwal A, Burnett SJ, Clohisy JC, Ghanem, Sharkey P, et al. The fate of the unexpected positive intraoperative cultures after revision total knee arthroplasty. *J Arthroplasty* 2007;**22**:94—9.
17. Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. *N Engl J Med* 2007;**357**:654—63.
18. Vergidis P, Greenwood-Quaintance KE, Sánchez-Sotelo J, Morrey BF, Steinmann SP, Karau MJ, et al. Implant sonication

for the diagnosis of the prosthetic elbow infection. *J Shoulder Elb Surg* 2011;**20**:1275—81.
19. Piper KE, Jacobson MJ, Cofield RH, Sperling JW, Sanchez-Sotelp J, Osmon DR, et al. Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication. *J Clin Microbiol* 2009;**47**:1878—84.
20. Portillo ME, Salvadó M, Sorli LL, Alier A, Martínez S, Trampuz A, et al. Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure. *J Infect* 2012;**65**:541—8. http://dx.doi.org/10.1016/j.jinf.2012.08.018.
21. Del Pozo JL, Patel R. Clinical practice. Infection associated with prosthetic joints. *N Engl J Med* 2009;**361**:787—94.
22. Atkins BL, Athanasou N, Deeks J, Crook DWM, Simpson H, Peto TA, et al. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. *J Clin Microbiol* 1998;**36**:2932—9.
23. Mirra JM, Marder RA, Amstutz HC. The pathology of failed total joint arthroplasty. *Clin Orthop Relat Res* 1982;**170**:175—83.
24. Spangehl MJ, Masri BA, O'Connell JX, Duncan CP. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. *J Bone Jt Surg Am* 1999;**81**:672—83.
25. Lonner JH, Desai P, Dicesare PE, Steiner G, Zuckerman JD. The reliability of analysis of intraoperative frozen sections for identifying active infection during revision hip or knee arthroplasty. *J Bone Jt Surg Am* 1996;**78**:1553—8.
26. Feldman DS, Lonner JH, Desai P, Zuckerman JD. The role of intraoperative frozen sections in revision total joint arthroplasty. *J Bone Joint Surg Am* 1995;**77**:1807—13.
27. Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard*. 8th ed. Wayne, PA: CLSI; 2009M7—A8.
28. Paprosky WG, Perona PG, Lawrence JM. Acetabular defect classification and surgical reconstruction in revision arthroplasty. A 6-year follow-up evaluation. *J Arthroplasty* 1994;**9**:33—44.
29. Sporer SM, Paprosky WG. Revision total hip arthroplasty: the limits of fully coated stems. *Clin Orthop Relat Res* 2003;**417**:203—9.
30. Engh GA, Ammeen DJ. Classification and preoperative radiographic evaluation: knee. *Orthop Clin North Am* 1998;**29**:205—17.
31. Bori G, Soriano A, García S, Gallart X, Casanova L, Mallofre C, et al. Low sensitivity of histology to predict the presence of microorganisms in suspected aseptic loosening of a joint prosthesis. *Mod Pathol* 2006;**19**:874—7.
32. Cobo J, Del Pozo JL. Prosthetic joint infection: diagnosis and management. *Expert Rev Anti Infect Ther* 2011;**9**:787—802.
33. Portillo ME, Salvadó M, Alier A, Sorli LL, Martínez S, Horcajada JP, et al. Prosthesis failure within 2 years of implantation is highly predictive of infection. *Clin Orthop Relat Res* 2013;**471**:3672—8.
34. Holinka J, Bauer L, Hirschl AM, Graninger W, Windhager R, Presterl E. Sonication cultures of explanted components as an add-on test to routinely conducted microbiological diagnostics improve pathogen detection. *J Orthop Res* 2011;**29**:617—22.
35. Ince A, Rupp J, Frommelt L, Katzer A, Gille J, Löhr JF. Is "aseptic" loosening of the prosthetic cup after total hip replacement due to nonculturable bacterial pathogens in patients with low-grade infection? *Clin Infect Dis* 2004;**39**:1599—603.
36. Sierra JM, García S, Martínez-Pastor JC, Tomás X, Gallart X, Vila J, et al. Relationship between the degree of osteolysis and cultures obtained by sonication of the prostheses in patients with aseptic loosening of a hip or knee arthroplasty. *Arch Orthop Trauma Surg* 2011;**131**:1357—61.

Risk of re-infection following one- and two-stage surgical revision of peri-prosthetic joint infection of the hip: A pooled individual participant data analysis of 44 observational cohort studies

The Global Infection Orthopaedic Management Collaboration*

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Abstract

Background One-stage and two-stage revision strategies are the two main options for treating established peri-prosthetic joint infection (PJI) of the hip; however, there is uncertainty regarding which is the best treatment option. Comparisons between the two revision strategies are confounded by several limitations of aggregate published data and the absence of clinical trial data. We aimed to examine re-infection rates among patients with PJI of the hip who have undergone one- or two stage revision and compare the risk of re-infection between the two revision strategies using pooled individual participant data (IPD).

Methods Observational cohort studies with PJI of the hip treated exclusively by one- or two-stage revision and reporting re-infection outcomes were retrieved by searching MEDLINE, EMBASE, Web of Science, Cochrane Library, and the WHO International Clinical Trials Registry Platform; as well as manual search of bibliographies and email contact with investigators. No clinical trials were identified. Investigators were invited to contribute individual level data. We analysed IPD of 1,856 participants with PJI of the hip from 44 cohorts across four continents. The primary outcome was re-infection (recurrence of infection by the same organism(s) and/or re-infection with a new organism(s)). Hazard ratios (HRs) for re-infection were calculated using Cox proportional frailty hazards models.

Results After a median follow-up of 3.7 years, 222 re-infections were recorded. Re-infection rates per 1000 person-years of follow-up were 16.8 (95% CI: 13.6-20.7) and 32.3 (95% CI: 27.3-38.3) for one-stage and two-stage strategies respectively. Among 1,038 individuals with available survival data, comparing two- with one-stage revision, the age-adjusted HR for re-infection was 1.69 (0.58-4.98). The corresponding age- and sex-adjusted HR was 1.70 (0.58-5.00). The association remained consistently absent after further adjustment for potential confounders. Conversely, the HRs were not significant when comparing one- with two-stage revision. HRs did not vary importantly in clinically relevant subgroups

Conclusion Pooled available data suggest no statistically significant increased risk of re-infection comparing the two-stage with one-stage revision strategy and vice versa. The one-stage revision

strategy may be as effective as the two-stage revision strategy in treating PJI of the hip in generally unselected patients.

Keywords: prosthesis related infection; total hip replacement; reoperation; revision; re-infection; one-stage; two-stage; meta-analysis

Systematic review registration: PROSPERO 2015: CRD42015016664

Introduction

Hip replacement is one of the most common surgical procedures. In the UK, over 95,000 primary procedures were performed in 2015 (NJR 2016, Scottish Arthroplasty Project 2016).[1,2] In 2010, it was estimated that 2.5 million Americans were living with a hip replacement.[3] Peri-prosthetic joint infection (PJI) is a serious adverse event affecting approximately one percent of patients with a primary hip joint replacement.[4] PJI has a major negative effect on patients' quality of life,[5-7] and to avoid the need for arthrodesis or amputation, patients and their treating surgeons face complex and protracted treatments.

In 1985, Fitzgerald and Jones described a series of two-stage revisions for the treatment of infected hip implants.[8] With this two-stage strategy, the artificial hip joint is removed and replacement delayed for several months until clear evidence of infection eradication is obtained. An alternative one-stage revision procedure was in use from 1976 at the Endo-Klinik in Hamburg with the implant removed and replaced in one operation;[9] however the two-stage strategy has traditionally been considered the gold standard for PJI treatment.[10]

Given the absence of a robust randomised controlled trial (RCT), the effectiveness of the two strategies have been compared using aggregate data from case series.[11-13] In the most recent review of 98 studies, we reported two-year re-infection rates of about 8% following both one- or two-stage surgical revision for PJI of the hip.[14] Our findings also showed that re-infection outcomes were generally consistent for the revision strategies across important patient characteristics and surgical factors. Some features of our review limited the generalisability of the findings. First, a detailed assessment of the definition of re-infection could not be undertaken as this was not clearly reported in the majority of studies. Second, our aim was to include studies with at least two years of follow-up following revision surgery, but this information was not always available.

In the absence of robust evidence from a carefully designed RCT, access to individual level data from published studies could address the existing uncertainties and enable: i) a consistent approach to the definition of outcomes; ii) a common approach across studies to statistical analyses; and iii) improve generalisability through inclusion of patients from key prospective studies worldwide.

In this context, we established the Global Infection Orthopaedic Management (INFORM) collaboration to: i) compare baseline and clinical characteristics of patients undergoing one-stage and two-stage revision surgery following PJI of the hip; ii) compare the risk of re-infection between the two strategies; and iii) examine the risk of re-infection according to a range of clinically relevant characteristics. This international consortium has allowed central collation and harmonisation of individual participant data (IPD) on 1,856 patients from 44 cohorts based in 13 different countries across 4 continents.

Methods

Data sources

We conducted this systematic review and IPD pooled analysis using a predefined protocol registered in the PROSPERO International prospective register of systematic reviews (CRD42015016664),[15] and in accordance with methods recommended by the IPD Meta-analysis Methods Group of the Cochrane Collaboration,[16] guidance of Riley and colleagues,[17] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Individual Participants Data (PRISMA-IPD) guidelines[18] (**Appendix 1**). We sought IPD from studies identified through systematic searches of MEDLINE, EMBASE, Web of Science, Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, and the WHO International Clinical Trials Registry Platform from March 2011 (date of our search for the previous review[13]) up to August 2016. The computer-based searches combined free and medical subject headings and combination of key words related to hip replacement, infection, and revision with focus on one- and two-stage surgeries. There were no restrictions on language. Studies were also identified from reference lists of all retrieved articles and other relevant publications, including reviews and meta-analyses, and discussions with investigators of unpublished studies. Further details on the search strategy are presented in **Appendix 2**. No separate ethical approval was required for the conduct of this study, as any necessary ethical approval was obtained for each of the individual studies contributing data to this pooled analyses.

Eligibility criteria

Cohort studies were eligible if they met the following inclusion criteria: (i) generally unselected patients with PJI of the hip (i.e., patients' representative of the general patient population); (ii) patients treated exclusively by one-stage or two-stage revision; (iii) and patients with at least two years of follow-up for re-infection outcomes. Studies that reported case series of methods in selected groups of patients (such as subsamples of patients who received revision in one- or two-stages or patients with a specific infection such as fungal infections) were excluded from the review.

Global Infection Orthopaedic Management (INFORM) collaboration

Details of the establishment of the Global INFORM collaboration has been described previously in the published protocol.[15] Briefly, investigators of eligible studies identified by the literature search strategy and well-known investigators in the field, were contacted by email or letter, provided with a summary of the study protocol, and invited to join the collaboration if they had the relevant data available. Investigators expressing interest to collaborate in this effort were then provided with full details of the study protocol.

Data collection

Investigators were provided with a list of relevant study variables that could be used in the analyses (**Appendix 3**). Data from each study were obtained using a standardised spreadsheet, and data dictionaries were also requested. Details of contributing cohorts are presented in **Appendix 4**. The raw data were examined and inconsistencies or irregularities were clarified with the investigators. Individual level data collected was cleaned, coded, and entered into a single database. Additional studies were included where useable data was tabulated in published articles.

Outcome

The primary outcome variable was re-infection, i.e. recurrence of infection by the same organism(s) and/or re-infection with a new organism(s). Patients contributed only the first re-infection recorded

after revision during follow-up. Outcomes were censored if a patient was lost to follow-up or reached the end of the follow-up period.

Statistical analyses

Descriptive statistics were used to summarise baseline characteristics according to type of revision strategy. We report mean, standard deviation (SD), median, and interquartile range (IQR) for continuous variables, and proportions for categorical variables. The risks of re-infection recorded during follow-up comparing the two-stage with the one-stage (reference category) strategy were assessed using Cox proportional shared frailty models.[19] Proportional hazards assumptions were assessed for all models by regressing the scaled Schoenfeld residuals against the log-time.[20] Because the treatment variable (i.e. revision strategy) only varied between studies/cohorts, inferences could only be made based on differences in re-infection rates between studies using either treatment strategy. A stratified Cox model was therefore not suitable in this scenario as the "treatment strategy" did not vary within studies. We employed a shared frailty model, which is an extension of the Cox proportional hazards model and provides a suitable way to introduce random effects in the model to account for unobserved heterogeneity. The random effect (the frailty) has a multiplicative effect on the hazard function of a cluster of individuals (cohort in this case). For each model, we included a frailty term at the cohort level to allow for dependence of individuals within each cohort. Survival curves comparing the one- and two-stage strategies were calculated using unadjusted Kaplan-Meier estimates and compared using the log-rank test. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated with progressive adjustment for age, sex, comorbidities (Charlson comorbidity index[21]), previous hip surgery, and type of infecting organism ("difficult to treat versus "not difficult to treat"[22,23] **Appendix 5**). Subgroup analyses were conducted using interaction tests to assess statistical evidence of any differences in HRs across categories of pre-specified individual level characteristics, specifically: sex, age group, previous hip surgery, and type of infecting organism. A two-sided p-value less than 0.05 was considered statistically significant throughout and all analyses were conducted using Stata version 14 (StataCorp, College Station, Texas, USA).

Results

Study identification and selection

Figure 1 shows the inclusion and exclusion of studies. Our systematic literature search identified 4344 potentially relevant citations. After screening titles and abstracts, 59 articles remained for further evaluation. Following detailed assessments, 35 articles were excluded. The remaining 24 articles (based on 28 unique studies) and 61 articles (based on 70 unique studies) identified from our previous review,[13] were potentially eligible for the pooled analysis. Of this number and in addition to three studies based on our unpublished data, we had access to individual level data from 44 cohort studies. Overall, there were 13 one-stage and 31 two-stage studies based in 13 countries (from North and South America, Europe, and Asia) (**Appendices 4 and 6**).

Baseline and follow-up characteristics

Summary baseline and follow-up characteristics of the 1,856 patients with PJI of the hip treated by one- or two-stage revision that contributed to the analyses are shown in **Table 1**. The mean (SD) age of overall participants at baseline was 65 (13) years and 53% were men. A total of 884 patients received one-stage revision and 972 patients received two-stage revision. The median (interquartile range) follow up time was 4.2 (2.0-8.1) years in the one-stage group and 3.3 (2.0-5.9) years in the two-stage group. During follow-up, 88 (10.0%) participants experienced a re-infection in the one-stage group compared with 134 (13.8%) in the two-stage group. Although the proportion of men, mean BMI, proportion of patients having a previous procedure to treat infection, and median Harris Hip Score (HHS) between the two treatment groups were generally similar, several baseline characteristics and follow-up data were not balanced between one- and two-stage groups. The one-stage revision group had older patients on average and had a higher proportion of patients with previous PJI and previous hip surgery (other than the index surgery) compared with their two-stage counterparts. In addition, the one-stage revision group had higher median levels of blood circulating C-reactive protein (CRP) and a higher proportion of patients presenting with an abscess, sinus,

draining wound, or fistula before revision. In the two-stage group, a higher proportion of patients had a history of smoking and alcohol consumption, cardiometabolic disorders and other comorbidities compared with one-stage patients. The most common indication for the index implantation for both groups was osteoarthritis. This was followed by fractures in the one-stage group and osteonecrosis in the two-stage group (**Figure 2**). The most common cultured microorganism responsible for a PJI after the index operation in the one-stage group was methicillin-sensitive *Staphylococcus (S.) aureus* (MSSA); whereas it was *S. aureus* or coagulase-negative staphylococci (CoNS) in the two-stage group (**Figure 3**). The median times to onset of infection from index implantation and from infection to revision surgery were longer in one-stage revision strategy patients compared with two-stage patients. The median duration of antibiotic use after revision was considerable longer in the one-stage group compared with the two-stage group. However, the median duration of antibiotic therapy in between stages for the two-stage revision group was about two times longer than that after revision therapy in the one-stage group. Thus patients treated with two-stage revision received a longer duration of antibiotics over the entire course of treatment (median, 18.3 weeks) compared with those treated with one-stage (median, 12.6 weeks).

Revision strategy and risk of re-infection

During a median (interquartile range) follow-up of 3.7 (2.0-6.9) years, 222 re-infections were recorded. Cumulative hazard curves demonstrated a greater risk of re-infection among two-stage revision strategy participants compared with one-stage revision strategy participants ($P = 0.0001$ for log-rank test; **Figure 4**). Re-infection rates per 1000 person-years of follow-up across revision strategies were 16.8 (95% CI: 13.6 to 20.7) and 32.3 (95% CI: 27.3 to 38.3) for the one-stage and two-stage strategies respectively. Among 1,038 individuals (113 re-infections) with available survival data, comparing two- with one-stage revision, the age-adjusted HR for re-infection was 1.69 (95% CI: 0.58 to 4.98; P=0.338). The corresponding HR remained consistent 1.70 (95% CI: 0.58 to 5.00; P=0.332) on adjusting for sex; and was attenuated to 1.33 (95% CI: 0.48 to 3.69; P=0.583) after further adjustment for previous hip surgery (**Table 2**). The associations remained absent in analyses

restricted to 439 individuals (41 re-infections) with available data on comorbidities and type of infecting organism (**Table 2**). Similarly, the HRs for re-infection were not significant in analysis that compared one-stage versus two-stage revision (**Table 3**). HRs did not vary importantly by levels or categories of pre-specified patient level characteristics (*P* for interaction > 0.10 for each) (**Figure 5**).

Discussion

Key findings

This study was conducted in an attempt to redress the uncertainties regarding the effectiveness of the one-stage and two-stage revision strategies for treating PJI of the hip, using re-infection as the outcome of interest. In this large-scale study involving pooled analysis of individual level data from 44 observational cohort studies, we have shown that in patients with PJI of the hip, there were generally marked differences in baseline and follow-up characteristics between one- and two-stage revision strategy patients; except for average BMI, proportions of men and patients having a previous procedure to treat infection, and median HHS, which were similar between the two treatment groups. Males were slightly overrepresented in both treatment groups, a finding which was not unexpected given that male sex is an established risk factor for PJI.[24,25] The proportions of patients with a previous hip surgery other than the index surgery as well as a previous PJI were higher in the one-stage revision strategy group compared with the two-stage. Patients in the one-stage revision group seemed to have severe PJI at presentation compared with the two-stage group, given their higher levels of circulating CRP and higher proportion of patients presenting with an abscess, sinus, draining wound, or fistula. These findings were unexpected, as patients with severe PJI usually undergo a two-stage revision to facilitate additional antimicrobial strategies. Given the limited opportunities for additional antibiotic therapy associated with it, the one-stage revision strategy has been traditionally thought to expose patients to a higher risk of re-infection by residual bacteria;[26] and it has been suggested this strategy should only be used in select cases, such as patients with known organisms and sensitivities, non-immunocompromised patients, as well as absence of a sinus tract.[27,28] Our results also showed that MSSA was the most commonly isolated microorganism responsible for a PJI in the one-stage revision

group. Compared with one-stage revision patients, the two-stage group had a higher proportion of patients with a history of smoking and excessive alcohol consumption, as well as the presence of comorbidities (including cardiometabolic disorders). *Staphylococcus* species were the most common causative organisms for PJI in both treatment groups, results which are consistent with the literature.[23,29,30] Results on the time to onset of infection from index implantation suggested that majority of PJIs in the one-stage group were late infections (more than 24 months after surgery), whiles those of the two-stage group were delayed infections (3 to 24 months after surgery).[31] Given that late infections are mostly acquired by haematogenous seeding,[23] this might account for the severity of PJI in the one-stage revision group.

Unadjusted Kaplan-Meier curves suggested a higher re-infection rate for the two-stage revision strategy compared with one-stage revision; however, given the imbalance between several baseline sociodemographic and clinical characteristics, such unadjusted results are likely to be confounded. In multivariate analyses, there was no evidence of a statistically significant increased risk of re-infection, when the two-stage revision strategy was compared with the one-stage revision strategy. However, there was a trend towards a higher risk of re-infection in the two-stage revision group. The statistically non-significant associations remained consistent across clinically relevant subgroups and when the one-stage revision strategy was compared with the two-stage revision strategy (reference comparison).

Comparison with previous work

We are unable to directly compare the current findings with previous work; because this is to our knowledge, the first pooled analysis of individual level data from observational cohort studies based in different countries that have reported re-infection outcomes following one- or two-stage surgical revision for infected hip prosthesis. However, our overall results, which suggest that the one-stage revision strategy may be as effective as the two-stage revision strategy in treating infected hip prostheses, seem to concur and further extend that of previous aggregate reviews conducted on the topic. In an updated review comprising of 38 one-stage and 60 two-stage revision strategy studies, we

demonstrated similar re-infection rates following one- or two-stage surgical revision for infected hip prosthesis.[14] These results confirmed an earlier review by our group, which showed no significant difference in re-infection rates between one- and two-stage revision strategies.[13] Other similar reviews have also reported findings which suggest no significant superiority of either revision strategy over the other. Leonard and colleagues in a review of nine studies comparing re-infection rates between one- and two-stage revision strategies, reported that one-stage revision was associated with similar re-infection rates when compared with two-stage revision with superior functional outcomes.[32] Lange and colleagues in a meta-analysis involving 36 studies, reported results which indicated that there were three additional re-infections per 100 patients with infected hip prosthesis when a one-stage revision was performed compared to a two-stage revision; however, the risk estimates were imprecise with overlapping confidence intervals, demonstrating no clear evidence of a superior revision strategy.[33]

Implications of findings

The current findings, as well as consistent findings from several previous reviews, suggest that the one-stage revision strategy may be as effective as the two-stage strategy in treating many patients with PJI of the hip. These results are very relevant and may have clinical implications for orthopaedic practice. For several decades, the two-stage revision strategy has been presumed to be more effective that the one-stage for treating PJIs.[23,34] However, in the absence of RCTs, several individual observational cohorts, as well as reviews, have consistently failed to show clear supportive evidence for the two-stage strategy being more effective compared to the one-stage strategy. Our finding of a null association is therefore not unexpected as it confirms speculations that the two revision strategies may have comparable effectiveness for treating PJI of the hip in unselected patients. Our findings were also suggestive of a trend towards a higher risk of re-infection for two-stage revision compared with the one-stage revision strategy. Indeed, unadjusted analyses which employed the entire sample in the dataset demonstrated a statistically significant evidence of an association between the two-stage strategy and higher risk of re-infection. Therefore, it is possible that our null results on multivariate

analyses could be attributed to low power, especially given the imprecise estimates (wide confidence intervals). Though claimed to be a more effective revision strategy, the two-stage strategy has several drawbacks. In addition to the significant pain and functional impairment, longer hospitalisation periods, and increased risk of mortality associated with this strategy;[12,34,35] it is known to be associated with higher healthcare costs compared to one-stage revision.[36] For example within the UK National Health System (NHS), the cost of surgical revision of an infected hip replacement is estimated to be about £22,000,[37], with a two-stage costing about 70% more than a one-stage revision.[36] Furthermore, we have shown that those receiving two-stage treatment also receive a longer duration of antibiotics. There has been an increase in the use of the one-stage revision strategy[38-40] after its introduction several decades ago.[9] Despite its drawback of exposing patients to a higher risk of re-infection by any residual bacteria,[26] because of limited opportunities for additional antibiotic therapy; the one-stage strategy has major advantages for unselected patients which include reduced number of surgical procedures, hospitalisation periods, total duration of antibiotic use, and disability, as well as economic benefits. As a result of increasing life expectancy, there is a growing healthcare burden due to osteoarthritis [41] which will result in a projected increase in the numbers of primary THAs as well as those requiring revision surgery for PJI of the hip.[42,43] Indeed, analysis of data for England and Wales using the National Joint Registry suggest that by 2030, the volume of primary and revision THAs will increase by 347% and 31%, respectively between 2012 and 2030.[43] Compared with primary arthroplasty procedures, the cost of revision surgery is higher; with infected being more expensive than aseptic revisions.[37] Given the high financial costs and increased burden on resources associated especially with the two-stage revision strategy, there is a need for optimisation of resources within the current economic climate. The evidence suggests that the two revision strategies have comparable effectiveness in the control of infection in unselected patients with peri-prosthetic hip infection. Our findings also show that the one-stage strategy was an appropriate treatment strategy for patients with characteristics that had previously been thought to be inappropriate for one-stage revision, such as those with sinus tracts at time of presentation. The overall findings suggests that the one-stage

strategy might be a potential preferable strategy for orthopaedic surgeons performing revision surgeries for PJI of the hip.

Strengths and limitations of the study

Several strengths of this study merit consideration. We have conducted the first pooled analysis of individual level data from observational cohort studies, which examines re-infection rates among patients with PJI of the hip who have undergone one- or two stage revision and compared the risk of re-infection between the two revision strategies. Though previous aggregate reviews conducted on the topic have employed a larger number of studies, the current analysis is unique in the following ways: (i) compared with single-country studies, our study pooled individual level data contributed by study investigators across four continents which enhanced generalisability of the findings; (ii) there was a more consistent approach to the definition of re-infection outcomes; (ii) it ensured that participants with at least two years of follow-up were included in the analyses; (iv) there was a common approach across studies to statistical analyses; and (v) analyses included adjustment for relevant confounders which enabled reliable assessment of the treatment effects, given the biases associated with unadjusted results. Despite the novelty and strengths of the current study, there are several limitations which deserve consideration. A main limitation was that because the revision strategy only varied between cohorts, a head-to-head comparison of the two revision strategies could not be made and appropriate inferences could only be made based on differences in re-infection rates between studies using either treatment strategy. However, given the clustered nature of the survival data, we employed a shared frailty Cox proportional model to account for any unobserved heterogeneity. The majority of studies were unable to contribute relevant clinical data, which precluded the ability to adjust for a comprehensive panel of potential confounders, thereby introducing the possibility of residual confounding. We were also unable to conduct detailed subgroup analyses by clinically relevant subgroups such as BMI, duration of antibiotic therapy, and by population (geographical region). Apart from the control of infection, maintenance of joint function is also considered as an important factor for a successful outcome following one- or two stage revision.[44,45] We were unable to compare the two

revision strategies using measures of joint function such as the Western Ontario & McMaster Universities Arthritis Index (WOMAC) Index (a validated patient-reported outcome measure of hip pain, function and stiffness widely used in hip arthroplasty research[46]). A number of qualitative studies (including one by our group) focusing on outcomes after joint surgery, have shown that patients are more concerned with pain and joint function (patient-centred outcome measures) rather than clinical indices such as re-infection rates.[5,47] Because we included populations representative of patients in general clinical practice, the results cannot be generalised to selected patient populations such as immunocompromised patients, culture negative patients, and those with periprosthetic fungal infections. The findings should therefore be interpreted in context of the limitations available. Ideally, to compare the effectiveness of these two revision strategies will require evidence from a carefully designed RCT. Within our INFection ORthopaedic Management (INFORM) Programme, which is involved in developing and establishing optimum management strategies for PJIs, there is an ongoing trial to determine whether there is a difference in patient-reported outcome measures (primary outcome) as well as re-infection rates between one-stage and two-stage revision surgeries for patients with PJI of the hip (INFORM; Current controlled trials ISRCTN10956306).[48] Results from this study may help to elucidate and address any differences in the effectiveness of these two revision strategies.

Conclusions

Pooled available data suggest no significant increased risk of re-infection with the two-stage versus one-stage revision strategy and vice versa. The one-stage revision strategy may be as effective as the two-stage revision strategy in treating PJI of the hip in generally unselected patients.

Contributors

SKK, MRW, AWB, and ADB contributed to study conception and design, data collection, analysis, interpretation, and re-drafting of this paper. SKK, MRW, AWB, and ADB drafted the study protocol and analysis plan. SKK conducted the combined statistical analysis. SKK wrote the first draft of the manuscript.

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Sources of funding

This article presents independent research funded by the National Institute for Health Research (NIHR) under its Programme Grants for Applied Research program (RP-PG-1210-12005). The views expressed in this article are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. *National Joint Registry for England and Wales: 13th Annual Report. Accessed from http://www.hqip.org.uk/resources/national-joint-registry-13th-annual-report-2016/.* 2016.

2. *Scottish Arthroplasty Project 2016. Accessed at http://www.arthro.scot.nhs.uk/docs/2016-08-09-SAP-Report.pdf?1.* 2016.

3. Maradit Kremers H, Larson DR, Crowson CS, et al. Prevalence of Total Hip and Knee Replacement in the United States. *J Bone Joint Surg Am.* 2015;97(17):1386-1397.

4. Huotari K, Peltola M, Jamsen E. The incidence of late prosthetic joint infections: a registry-based study of 112,708 primary hip and knee replacements. *Acta Orthop.* 2015;86(3):321-325.

5. Moore A, Blom A, Whitehouse M, Gooberman-Hill R. Deep prosthetic joint infection: A qualitative study of the impact on patients and their experiences of revision surgery. *BMJ open.* 2015;5:e009495.

6. Andersson AE, Bergh I, Karlsson J, Nilsson K. Patients' experiences of acquiring a deep surgical site infection: An interview study. *Am J Infect Control.* 2010;38(9):711-717.

7. Kunutsor SK, Beswick AD, Peters TJ, et al. Health Care Needs and Support for Patients Undergoing Treatment for Prosthetic Joint Infection following Hip or Knee Arthroplasty: A Systematic Review. *PloS one.* 2017;12(1):e0169068.

8. Fitzgerald RH, Jones DR. Hip implant infection: Treatment with resection arthroplasty and late total hip arthroplasty. *Am J Med.* 1985;78(6):225-228.

9. Buchholz H, Elson R, Engelbrecht E, Lodenkamper H, Rottger J, Siegel A. Management of deep infection of total hip replacement. *J Bone Joint Surg Am.* 1981;63-B(3):342-353.

10. Cooper HJ, Della Valle CJ. The two-stage standard in revision total hip replacement. *Bone Joint J.* 2013;95-B(11 Suppl A):84-87.

11. Gallo J, Smizansky M, Radova L, Potomkova J. Comparison of therapeutic strategies for hip and knee prosthetic joint infection. *Acta Chir Orthop Traumatol Cech.* 2009;76(4):302-309.

12. Wolf CF, Gu NY, Doctor JN, Manner PA, Leopold SS. Comparison of one and two-stage revision of total hip arthroplasty complicated by infection: a Markov expected-utility decision analysis. *J Bone Joint Surg Am.* 2011;93-A(7):631-639.

13. Beswick A, Elvers K, Smith A, Gooberman-Hill R, Lovering A, Blom A. What is the evidence base to guide surgical treatment of infected hip prostheses? Systematic review of longitudinal studies in unselected patients. *BMC Medicine.* 2012;10(1):18.

14. Kunutsor SK, Whitehouse MR, Blom AW, Beswick AD, Inform Team. Re-infection outcomes following one- and two-stage surgical revision of infected hip prosthesis: A systematic review and meta-analysis. *PloS one.* 2015;10(9):e0139166.

15. Kunutsor SK, Whitehouse MR, Webb J, et al. Re-infection outcomes following one- and two-stage surgical revision of infected hip prosthesis in unselected patients: protocol for a

systematic review and an individual participant data meta-analysis. *Systematic reviews.* 2015;4:58.

16. Cochrane Collaboration Individual Patient Data Meta-analysis Methods Group. FAQs (Frequently Asked Questions) on IPD meta-analysis 2007; http://www.ctu.mrc.ac.uk/cochrane/ipdmg/faq.asp#faq26.

17. Riley RD, Lambert PC, Abo-Zaid G. Meta-analysis of individual participant data: rationale, conduct, and reporting. *BMJ.* 2010;340:521-525.

18. Stewart LA, Clarke M, Rovers M, et al. Preferred Reporting Items for Systematic Review and Meta-Analyses of individual participant data: the PRISMA-IPD Statement. *Jama.* 2015;313(16):1657-1665.

19. Hougaard P. Frailty models for survival data. *Lifetime Data Anal.* 1995;1(3):255-273.

20. Therneau TM, Grambsch PM. *Modeling Survival Data: Extending the Cox Model.* New York: Springer; 2000.

21. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis.* 1987;40(5):373-383.

22. Wimmer MD, Randau TM, Friedrich MJ, et al. Outcome Predictors in Prosthetic Joint Infections--Validation of a risk stratification score for Prosthetic Joint Infections in 120 cases. *Acta Orthop Belg.* 2016;82(1):143-148.

23. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med.* 2004;351(16):1645-1654.

24. Kapadia BH, Berg RA, Daley JA, Fritz J, Bhave A, Mont MA. Periprosthetic joint infection. *Lancet.* 2015.

25. Kunutsor SK, Whitehouse MR, Blom AW, Beswick AD, INFORM team. Patient-related risk factors for periprosthetic joint infection after total joint arthroplasty: A systematic review and meta-analysis. *PloS one.* 2016;11(3):e0150866.

26. Buttaro MA, Pusso R, Piccaluga F. Vancomycin-supplemented impacted bone allografts in infected hip arthroplasty. Two-stage revision results. *J Bone Joint Surg Am.* 2005;87-B(3):314-319.

27. Gulhane S, Vanhegan IS, Haddad FS. Single stage revision: regaining momentum. *Journal of Bone & Joint Surgery - British Volume.* 2012;94(11 Suppl A):120-122.

28. Vanhegan IS, Morgan-Jones R, Barrett DS, Haddad FS. Developing a strategy to treat established infection in total knee replacement: a review of the latest evidence and clinical practice. *Journal of Bone & Joint Surgery - British Volume.* 2012;94(7):875-881.

29. Hickson CJ, Metcalfe D, Elgohari S, et al. Prophylactic antibiotics in elective hip and knee arthroplasty: an analysis of organisms reported to cause infections and National survey of clinical practice. *Bone Joint Res.* 2015;4(11):181-189.

30. Stefánsdóttir A, Johansson D, Knutson K, Lidgren L, Robertsson O. Microbiology of the infected knee arthroplasty: Report from the Swedish Knee Arthroplasty Register on 426

surgically revised cases. *Scandinavian Journal of Infectious Diseases.* 2009;41(11-12):831-840.

31. Schafroth M, Zimmerli W, Brunazzi M, Ochsner PE. Infections. In: Ochsner PE, ed. *Total Hip Replacement: Implantation Technique and Local Complications*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2003:65-90.

32. Leonard HA, Liddle AD, Burke O, Murray DW, Pandit H. Single- or two-stage revision for infected total hip arthroplasty? A systematic review of the literature. *Clin Orthop.* 2014;472(3):1036-1042.

33. Lange J, Troelsen A, Thomsen RW, Soballe K. Chronic infections in hip arthroplasties: comparing risk of reinfection following one-stage and two-stage revision: a systematic review and meta-analysis. *Clinical epidemiology.* 2012;4:57-73.

34. Matthews PC, Berendt AR, McNally MA, Byren I. Diagnosis and management of prosthetic joint infection. *BMJ.* 2009;338:1378-1383.

35. Cahill JL, Shadbolt B, Scarvell JM, Smith PN. Quality of life after infection in total joint replacement. *J Orthop Surg.* 2008;16(1):58-65.

36. Klouche S, Sariali E, Mamoudy P. Total hip arthroplasty revision due to infection: A cost analysis approach. *Orthop Traumatol Surg Res.* 2010;96(2):124-132.

37. Vanhegan IS, Malik AK, Jayakumar P, Ul Islam S, Haddad FS. A financial analysis of revision hip arthroplasty: the economic burden in relation to the national tariff. *J Bone Joint Surg Br.* 2012;94(5):619-623.

38. Winkler H. Rationale for one stage exchange of infected hip replacement using uncemented implants and antibiotic impregnated bone graft. *Int J Med Sci.* 2009;6:247-252.

39. Gehrke T, Kendoff D. Peri-prosthetic hip infections: in favour of one-stage. *Hip International.* 2012;22 Suppl 8:S40-45.

40. Klouche S, Leonard P, Zeller V, et al. Infected total hip arthroplasty revision: one- or two-stage procedure? *Orthop Traumatol Surg Res.* 2012;98(2):144-150.

41. Cross M, Smith E, Hoy D, et al. The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. *Ann Rheum Dis.* 2014;73(7):1323-1330.

42. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. *J Bone Joint Surg Am.* 2007;89-A(4):780-785.

43. Patel A, Pavlou G, Mujica-Mota RE, Toms AD. The epidemiology of revision total knee and hip arthroplasty in England and Wales: a comparative analysis with projections for the United States. A study using the National Joint Registry dataset. *Bone Joint J.* 2015;97-B(8):1076-1081.

44. Kendoff D, Gehrke T. Surgical management of periprosthetic joint infection: one-stage exchange. *J Knee Surg.* 2014;27(4):273-278.

45. Rasul AT, Jr., Tsukayama D, Gustilo RB. Effect of time of onset and depth of infection on the outcome of total knee arthroplasty infections. *Clin Orthop.* 1991(273):98-104.

46. Bellamy N, Buchanan W, Goldsmith C, Campbell J, Stitt L. Validation study of WOMAC: a health status instument for measuring clinically-important patient-relevant outcomes following total hip or knee arthroplasty in osteoarthritis. *J Orthop Rheumatol.* 1988;1:95-108.

47. Jeffery AE, Wylde V, Blom AW, Horwood JP. "It's there and I'm stuck with it": patients' experiences of chronic pain following total knee replacement surgery. *Arthritis Care Res.* 2011;63(2):286-292.

48. Strange S, Whitehouse MR, Beswick AD, et al. One-stage or two-stage revision surgery for prosthetic hip joint infection – The INFORM trial: a study protocol for a randomised controlled trial. *Trials.* 2016;17(90).

Figure legends

Figure 1. Selection of studies included in the individual pooled data analysis

Figure 2. Indications for index implantation by type of revision strategy

Figure 3. Type of infecting microorganism after index implantation by type of revision strategy

Figure 4. Cumulative hazard curves for re-infection by type of revision strategy

Figure 5. Hazard ratios for re-infection by participant level characteristics

Hazard ratios were adjusted for age, sex, previous hip surgery other than index surgery (yes/no), and difficult to treat organism (yes/no); CI, confidence interval; HR, hazard ratio; *, *P*-value for interaction
Analysis was limited to 495 participants (comprising 48 re-infections) with available data

Table 1. Summary of baseline characteristics and follow-up data in patients undergoing one- or two-stage revision

	Overall	One-stage revision	Two-stage revision
Total number of participants	1,856	884	972
Socio-demographic characteristics			
Gender	N=1,743	N=864	N=879
Males, n (%)	926 (53.1)	458 (53.0)	468 (53.2)
Females, n (%)	817 (46.9)	406 (47.0)	411 (46.8)
Age at baseline (years), mean (SD)	65.1 (13.0)	66.8 (12.4)	63.4 (13.3)
Smoking	N=365	N=56	N=309
Yes, n (%)	86 (23.6)	9 (16.1)	77 (24.9)
No, n (%)	279 (76.4)	47 (83.9)	232 (75.1)
History of high alcohol consumption	N=110	N=0	N=110
Yes, n (%)	6 (5.5)	0 (0.0)	6 (5.5)
No, n (%)	104 (94.6)	0 (0.0)	104 (94.6)
Physical measurements			
Body mass index in kg/m^2, mean (SD)	27.6 (6.6)	27.5 (5.9)	27.8 (7.0)
Medical and surgical history			
History of diabetes	N=803	N=282	N=521
Yes, n (%)	131 (16.3)	35 (12.4)	96 (18.4)
No, n (%)	676 (83.7)	247 (87.6)	425 (81.6)
History of hypertension	N=340	N=157	N=183
Yes, n (%)	119 (35.0)	52 (33.1)	67 (36.6)
No, n (%)	221 (65.0)	105 (66.9)	116 (63.4)
History of CVD	N=403	N=161	N=242
Yes, n (%)	99 (24.6)	38 (23.6)	61 (25.2)
No, n (%)	304 (75.4)	123 (76.4)	181 (74.8)
Comorbidity Index	N=785	N=282	N=503
No previously recorded disease categories, n (%)	256 (32.6)	45 (16.0)	211 (42.0)
One or two disease categories, n (%)	433 (55.2)	212 (75.2)	221 (43.9)
More than two disease categories, n (%)	96 (12.2)	25 (8.9)	71 (14.1)
History of previous PJI	N=321	N=120	N=201
Yes, n (%)	62 (19.3)	47 (39.2)	15 (7.5)
No, n (%)	259 (80.7)	73 (60.8)	186 (92.5)
Previous hip surgery	N=1,060	N=809	N=251
Yes, n (%)	825 (77.8)	748 (92.5)	77 (30.7)
No, n (%)	235 (22.2)	61 (7.5)	174 (69.3)
Hip involved in index implantation	N=1,233	N=632	N=601
Right, n (%)	676 (54.8)	348 (55.1)	328 (54.6)
Left, n (%)	557 (45.2)	284 (44.9)	273 (45.4)
Characteristics of infection before revision procedure			

Previous procedure performed to treat infection	N=541	N=277	N=264
Yes, n (%)	137 (25.3)	70 (25.3)	67 (25.4)
No, n (%)	404 (74.7)	207 (74.7)	197 (74.6)
Presence of abscess, sinus, draining wound, or fistula at presentation	N=588	N=278	N=310
Yes, n (%)	160 (27.2)	87 (31.3)	73 (23.6)
No, n (%)	428 (72.8)	191 (68.7)	237 (76.5)
Time from index implantation to infection (weeks), median (IQR)	102.7 (36.6-299.2)	154.3 (51.4-350.1)	102.6 (32.6-268.5)
Time from infection to revision procedure (weeks), median (IQR)	20.6 (8.4-51.4)	30.0 (10.2-94.2)	12.9 (6.4-34.3)
Baseline data before revision			
C-reactive protein (mg/l), median (IQR)	18.9 (6.1-54.0)	22.5 (9.0-56.5)	17.1 (5.8-50.5)
Erythrocyte sedimentation rate (mm/hr), median (IQR)	47 (26-73)	41 (28-55)	51 (25-76)
Neutrophils /µl, median (IQR)	4520 (2800-6000)	4800 (4100-6000)	3835 (99-5980)
WBC /µl, median (IQR)	7380 (6020-9090)	7100 (5920-8580)	8030 (6630-10860)
Harris Hip Score, median (IQR)	55.0 (48.0-60.0)	55.5 (43.5-63.5)	55.0 (48.0-60.0)
Characteristics of revision procedure and management			
Type of re-implantation	N=122	N=89	N=33
Cemented, n (%)	91 (74.6)	65 (73.0)	26 (78.8)
Cementless, n (%)	23 (18.9)	16 (18.0)	7 (21.2)
Hybrid, n (%)	8 (6.6)	8 (9.0)	0 (0.0)
Antibiotics in cement	N=1,092	N=758	N=334
Yes, n (%)	750 (68.7)	584 (77.0)	166 (49.7)
No, n (%)	342 (31.3)	174 (23.0)	168 (50.3)
Nature of spacer used	-	-	N=293
Unknown, n (%)	-	-	2 (0.7)
Articulated, n (%)	-	-	287 (98.0)
Static, n (%)	-	-	4 (1.4)
Type of spacer	-		N=183
Unknown, n (%)	-	-	1 (0.6)
Handmade, n (%)	-	-	167 (91.3)
Commercial, n (%)	-	-	15 (8.2)
Antibiotics in spacer	-	-	N=183
Yes, n (%)	-		180 (98.4)
No, n (%)	-		3 (1.6)
Duration between stages (weeks), median (IQR)	-	-	14.5 (11.0-24.0)
Duration of antibiotics use between stages (weeks), median (IQR)	-	-	24.0 (4.5-24.0)
After revision (Follow-up)			
Duration of antibiotic use after revision surgery (weeks), median (IQR)	12.1 (6.1-12.6)	12.6 (12.0-12.6)	1.3 (0.5-5.5)
Duration of follow-up (years), median (IQR)	3.7 (2.0-6.9)	4.2 (2.0-8.1)	3.3 (2.0-5.9)
Harris Hip Score at follow up, median (IQR)	86.0 (73.0-93.0)	80.0 (52.0-90.0)	87.0 (78.0-95.0)
Number of re-infections	222	88	134

CVD, cardiovascular disease; IQR=interquartile range; MR, methicillin resistant; MS, methicillin sensitive; PJI, periprosthetic joint infection; SD, standard deviation; WBC, white blood cells

Table 2. Hazard ratios for re-infection comparing two-stage revision versus one-stage revision adjusted progressively for risk factors

Model	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
	1,038 participants (113 re-infections) with available data		439 participants (41 re-infections) with available data	
Model 1	1.69 (0.58 to 4.98)	0.338	1.65 (0.44 to 6.20)	0.460
Model 2	1.70 (0.58 to 5.00)	0.332	1.66 (0.44 to 6.24)	0.454
Model 3	1.33 (0.48 to 3.69)	0.583	1.57 (0.45 to 5.51)	0.484
Model 4	-	-	1.59 (0.39 to 6.55)	0.520
Model 5	-	-	1.71 (0.39 to 7.50)	0.479

Model 1: adjusted for age

Model 2: model 1 plus sex

Model 3: model 2 plus previous hip surgery other than index surgery (yes/no)

Model 4: model 3 plus Charlson comorbidity index (no previous disease/one or two disease categories/more than two disease categories)

Model 5: model 4 plus difficult to treat organism (yes/no)

Table 3. Hazard ratios for re-infection comparing one-stage revision versus two-stage revision adjusted progressively for risk factors

Model	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
	1,038 participants (113 re-infections) with available data		439 participants (41 re-infections) with available data	
Model 1	0.59 (0.20 to 1.74)	0.338	0.61 (0.16 to 2.28)	0.460
Model 2	0.59 (0.20 to 1.72)	0.332	0.60 (0.16 to 2.27)	0.454
Model 3	0.75 (0.27 to 2.08)	0.583	0.64 (0.18 to 2.25)	0.484
Model 4	-	-	0.63 (0.15 to 2.59)	0.520
Model 5	-	-	0.58 (0.13 to 2.58)	0.479

Model 1: adjusted for age

Model 2: model 1 plus sex

Model 3: model 2 plus previous hip surgery other than index surgery (yes/no)

Model 4: model 3 plus comorbidities (no previous disease/one or two disease categories/more than two disease categories)

Model 5: model 4 plus difficult to treat organism (yes/no)

Clinical Infectious Diseases

MAJOR ARTICLE

The Not-So-Good Prognosis of Streptococcal Periprosthetic Joint Infection Managed by Implant Retention: The Results of a Large Multicenter Study

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Background. Streptococci are not an infrequent cause of periprosthetic joint infection (PJI). Management by debridement, antibiotics, and implant retention (DAIR) is thought to produce a good prognosis, but little is known about the real likelihood of success.

Methods. A retrospective, observational, multicenter, international study was performed during 2003–2012. Eligible patients had a streptococcal PJI that was managed with DAIR. The primary endpoint was failure, defined as death related to infection, relapse/persistence of infection, or the need for salvage therapy.

Results. Overall, 462 cases were included (median age 72 years, 50% men). The most frequent species was *Streptococcus agalactiae* (34%), and 52% of all cases were hematogenous. Antibiotic treatment was primarily using β-lactams, and 37% of patients received rifampin. Outcomes were evaluable in 444 patients: failure occurred in 187 (42.1%; 95% confidence interval, 37.5%–46.7%) after a median of 62 days from debridement; patients without failure were followed up for a median of 802 days. Independent predictors (hazard ratios) of failure were rheumatoid arthritis (2.36), late post-surgical infection (2.20), and bacteremia (1.69). Independent predictors of success were exchange of removable components (0.60), early use of rifampin (0.98 per day of treatment within the first 30 days), and long treatments (≥21 days) with β-lactams, either as monotherapy (0.48) or in combination with rifampin (0.34).

Received 28 November 2016; editorial decision 12 February 2017; accepted 14 March 2017.

[a]See listing in acknowledgments.

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Clinical Infectious Diseases® 2017;00(00):1–11

DOI: 10.1093/cid/cix227

Conclusions. This is the largest series to our knowledge of streptococcal PJI managed by DAIR, showing a worse prognosis than previously reported. The beneficial effects of exchanging the removable components and of β-lactams are confirmed and maybe also a potential benefit from adding rifampin.

Keywords. biofilm; bone and joint infection; DAIR; rifampin.

Periprosthetic joint infection (PJI) is a dreaded complication of joint replacement [1, 2]. Removal of the infected foreign body is the rule for any given device-associated infection. However, debridement, antibiotics, and implant retention (DAIR) may be attempted in some acute cases of PJI [2–4]. When strict selection of patients is followed, the success rate may reach >85% [4–7].

Streptococci are responsible for PJI in 4–12% of cases [8, 9] especially in hematogenous infections [10, 11]. Some studies have suggested that streptococcal PJI may have a more favorable outcome compared with other etiologies [12–14], but this has been contested by others [15]. In fact, the success rate of streptococcal PJI (mostly *Streptococcus agalactiae*) treated with DAIR varies from 22% to 100%, presumably depending on the selection criteria used [6, 13, 15–18] (Supplementary Table 1). Thus, the real success rate for patients managed by DAIR remains uncertain.

The optimal antimicrobial treatment for streptococcal PJI is also unknown. Current guidelines recommend the use of β-lactams [2, 4], but these antibiotics may have a very high

Table 1. Baseline Features, Clinical Presentation, Surgical Management and Outcome and Comparative Analysis of Hematogenous and Nonhematogenous Cases

	All Patients (*n* = 462)	Nonhematogenous Cases (*n* = 220)	Hematogenous Cases (*n* = 242)	*P*
Baseline features				
Sex (men)	232 (50%)	121 (45%)	111 (54%)	**.050**
Age (years)[a]	72 (65–78)	72 (64–78)	72 (65–78)	.986
Diabetes	111 (24%)	50 (23%)	61 (25%)	.533
Renal chronic disease	45 (10%)	20 (9%)	25 (10%)	.654
Rheumatoid arthritis	37 (8%)	15 (7%)	22 (9%)	.369
Immunosuppressive therapy	49 (11%)	22 (10%)	27 (11%)	.687
Malignancy	29 (6%)	7 (3%)	22 (9%)	**.009**
Liver cirrhosis	19 (4%)	9 (4%)	10 (4%)	.982
Chronic lung disease	56 (12%)	27 (12%)	29 (12%)	.924
Chronic heart disease	128 (28%)	54 (25%)	74 (31%)	.148
Prosthesis location (knee)	273 (59%)	117 (53%)	156 (65%)	**.014**
Revision prosthesis	114 (25%)	48 (22%)	66 (27%)	.174
Clinical presentation and microbiological data				
Temperature > 37°C	300 (66%)	110 (51%)	190 (80%)	**<.001**
Sinus tract	62 (14%)	46 (21%)	16 (7%)	**<.001**
Leukocyte count (×10E9/L)[a]	12.0 (8.5–15.4)	11.0 (7.3–14.6)	13.0 (9.6–16.0)	**.001**
C-reactive protein at diagnosis (mg/L)[a]	186 (85–283)	135 (55–230)	234 (130–305)	**<.001**
Rx signs of infection	85 (18%)	41 (19%)	44 (18%)	.900
Bacteremia	138 (31%)	35 (17%)	103 (45%)	**<.001**
Penicillin MIC > 0.125 mg/L §	24/425 (6%)	15 (8%)	9 (4%)	.113
Polymicrobial infection	63 (14%)	52 (24%)	11 (5%)	**<.001**
Surgical management				
Time to debridement (days)[ab]	5 (2–13)	5 (2–16)	5 (2–12)	.688
Exchange of removable components [c]	220/418 (53%)	100/200 (50%)	120/218 (55%)	.302
Need for ≥2 debridements	42 (9%)	21 (10%)	21 (9%)	.797
Outcome[d]				
Overall failure	187/444 (42%)	92/210 (44%)	95/234 (41%)	.494
Early failure[d]	55/187 (29%)	25/92 (27%)	30/95 (32%)	.509
Late failure[d]	71/187 (38%)	34/92 (37%)	37/95 (39%)	.779
	61/187 (33%)	33/92 (36%)	28/95 (30%)	.351

Abbreviation: MIC, minimal inhibitory concentration.

Data expressed as count and (percentage) except for [a]continuous variables (median and interquartile range).

[b]Time from onset of symptoms to surgical debridement.

[c]Data available in 418 cases.

[d]444 patients evaluable for outcome, percentages given over the whole of failures.

minimal biofilm eradication concentration [19, 20]. The role of alternative compounds with a better antibiofilm profile [21] has not been consistently explored in clinical studies.

Our aim was to analyze the clinical presentations and outcomes of a large cohort of patients with streptococcal PJI managed by DAIR, focusing on the impact of antimicrobial therapy.

METHODS

Setting and Patients

This was a multicenter retrospective study performed in 52 hospitals from 15 nations between 2003 and 2012. Patients were included if they had suffered a PJI that was caused by streptococci and initially managed by DAIR. Eighty-one cases included here have previously been published [6, 15, 22].

PJI was defined according to Infectious Diseases Society of America (IDSA) guidelines as the presence of a sinus tract communicating with the prosthesis, acute inflammation on histologic examination, purulence surrounding the prosthesis, and/or ≥2 evaluable samples yielding the same organism [4]. Polymicrobial cases were also included if streptococci were isolated from the beginning, but we excluded cases of streptococcal superinfection. Microorganisms were identified following standard criteria [23], after samples had been inoculated in liquid and solid media and incubated for ≥7 days. Enterococci, obligate anaerobes (i.e., *Peptostreptococcus* spp.) or nutritionally variant streptococci (i.e., *Abiotrophia* spp.) were not included.

PJI was classified as early postoperative, if the symptoms began within the first 3 months after the prosthesis was placed, and late post-surgical, if they started thereafter. The episode was considered acute hematogenous, if it occurred after an uneventful postoperative course and after microbiologically confirmed or clinically suspected streptococcal bacteremia. A contiguous spread was considered, if the PJI occurred in a limb with either infectious cellulitis, or a soft tissue abscess. New radiographical signs of infection were taken as a surrogate marker of chronicity (i.e., periprosthetic radiolucency, bone sclerosis, or osteolytic lesions). Chronic renal failure was defined as a baseline creatinine >150 µmol/L; immunosuppressant therapy was recorded if the patient received, was receiving glucocorticoid, or other immunosuppressant drug therapy.

Data were recorded in a Microsoft-Access database. All cases were critically reviewed by one author (J. L.-T.), and any doubts or inconsistencies were double-checked by the investigator at each hospital.

Clinical and Surgical Management

DAIR has been described elsewhere [2, 3, 24]. Briefly, it comprises thorough surgical debridement of all purulent collections and necrotic tissues surrounding the prosthesis. Mobile parts of the device (i.e., the polyethylene liner) are exchanged if feasible. DAIR is recommended in patients who meet the criteria proposed by the IDSA guidelines [4]. Patients with early postoperative (<1 month) or acute hematogenous PJI with ≤3 weeks of symptoms qualify for DAIR if they have a soundly fixed prosthesis, good periprosthetic soft tissues condition, and antibiotics are available with a reasonable activity against biofilm-embedded bacteria. In the present study, these criteria were not strictly met by many patients, and the decision to undergo DAIR was taken by individual medical group on a case by case basis.

Table 2. Etiology of 462 Episodes of Streptococcal Periprosthetic Joint Infection

Streptococcus		
S. agalactiae		**159 (34.4%)**
S. pyogenes		**36 (7.8%)**
S. pneumoniae		**21 (4.5%)**
Other large-colony β-haemolytic streptococci		**121 (26.2%)**
S. dysagalactiae	49 (10.6%)	
Group G streptococci	40 (8.7%)	
Other β-haemolytic streptococci	28 (6.1%)	
S. equisimilis	4 (0.9%)	
S. anginosus group		**32 (6.9%)**
S. anginosus	17 (3.7%)	
S. constellatus	8 (1.7%)	
S. milleri	4 (0.9%)	
S. intermedius	3 (0.6%)	
Viridans group		**86 (18.6%)**
Unspecified viridans streptococci	25 (5.4%)	
S. mitis	25 (5.4%)	
S. oralis	17 (3.7%)	
S. sanguis	10 (2.2%)	
S. salivarius	4 (0.9%)	
S. gordonii	2 (0.4%)	
S. mutans	2 (0.4%)	
S. parasanguis	1 (0.2%)	
Other streptococci		**7 (1.5%)**
S. bovis	6 (1.3%)	
S. canis	1 (0.2%)	
Other microorganisms (polymicrobial episodes)		
Gram positive microorganisms		59
Staphylococcus aureus	29	
Coagulase-negative staphylococci^	15	
Enterococcus faecalis	7	
Corynebacterium striatum^	2	
Other Gram-positive microorganisms[a]	6	
Gram negative microorganisms		19
Enterobacteriaceae[c]	15	
Nonfermentative Gram-negative bacilli[b]	2	
Anaerobe Gram-negative microorganisms[d]	2	

[a]Includes *Aerococcus viridans* (n = 1), *Arcanobacterium haemolyticus* (*n* = 1), *Bacillus* spp (*n* = 2), *Lactobacillus acidophilus* (*n* = 1) and *Peptostreptococcus* spp (*n* = 1).

[b]Includes *Pseudomonas aeruginosa* (*n* = 1), *Acinetobacter baumannii* (*n* = 1).

[c]Includes *Escherichia coli* (*n* = 5), *Klebsiella pneumoniae* (*n* = 1), *Enterobacter cloacae* (*n* = 4), *Proteus mirabilis* (*n* = 3), *Serratia* sp. (*n* = 1), and *Citrobacter* sp. (*n* = 1).

[d]Includes *Veillonella* spp. and *Prevotella* spp.

Outcome and Follow-up

Patients were followed until death, treatment failure, removal or replacement of the prosthesis, or until loss to follow-up. *Overall*

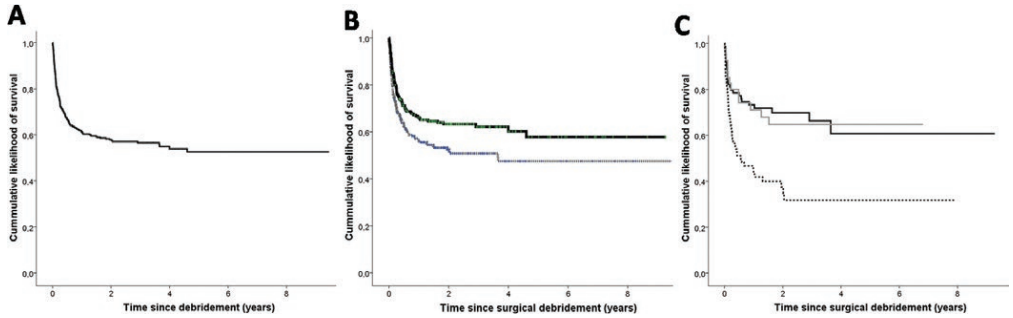

Figure 1. Kaplan-Meier curves of patients with streptococcal periprosthetic joint infection according to the criteria for indicating debridement and implant retention. A, Kaplan-Meier curve of all evaluable patients (n = 444, 187 failures). Causes of failure were due to the streptococcal infection in 147 cases (79%), the other reasons being prosthesis removal due to orthopedic causes (15 patients [8%]), and superinfection by other microorganisms (25 cases [13%]). Death related to PJI was observed in 11 cases (2%). B, Black continuous line: patients meeting IDSA criteria for DAIR (see text): 81 failures in 221 episodes of infection; grey dotted line: patients not meeting IDSA criteria for DAIR: 106 failures in 223 episodes of infection; long-rank test, $P = .017$. Reasons for not fulfilling the IDSA criteria were (more than 1 motive per patient is possible): in 67 patients (30%) symptoms duration was longer than 21 days; 90 patients (40%) had a post-surgical infection with symptoms beginning beyond the first month after the placement of the prosthesis; 61 patients (27%) presented with a sinus tract; and in 80 cases (36%) there were radiographic signs of prosthesis loosening and/or chronic infection. C, Post-surgical cases (i.e., nonhematogenous cases) (n = 189, 82 failures): black continuous line: cases with symptoms beginning within the first 30 days after the placement of the prosthesis (n = 78, 25 failures); grey continuous line: cases with symptoms beginning within 31 and 90 days after the placement of the prosthesis (n = 41, 13 failures); black dotted line: cases with symptoms beginning beyond 90 days after the placement of the prosthesis (n = 70, 44 failures). Long-rank test, $P < .001$. Abbreviations: DAIR, debridement, antibiotics, and implant retention; IDSA, Infectious Diseases Society of America.

Failure was the primary endpoint and was considered in cases of: (i) death related to the infection; (ii) need for salvage therapy to control the infection, including supplementary surgical debridements >30 days after the first debridement, prosthesis removal (due to any cause during the first year after debridement, or due to streptococcal persistence or relapse, or superinfection by other microorganisms), or the need for supplementary courses of antibiotics beyond the initially scheduled treatment (including chronic suppressive antimicrobial therapy); and/or (iii) persistent signs of infection at the last visit or follow-up appointment.

Given the retrospective nature of this study, and to avoid a survivor bias when analyzing the impact of antimicrobial therapy, several failure dynamics were studied:

- *Early Failure* was considered to have occurred in patients who met the failure criteria within the first 30 days after surgical debridement.
- *Late Failure* was considered to have occurred in patients who met the failure criteria beyond the first 30 days after debridement but who were still under antimicrobial therapy. In this group, only antimicrobials received during the first 30 days were analyzed.
- *Failure after Therapy* was considered to have occurred in patients who met the failure criteria once they had finished the scheduled therapy. In this analysis, the antibiotics received throughout treatment were included.

Statistical Analysis

Categorical parameters were compared with the χ^2 test or Fisher exact test, and continuous variables were compared with the Mann–Whitney *U* test or Kruskal–Wallis test. Parameters associated with *Overall Failure*, *Late Failure*, and *Failure after Therapy* were identified by Kaplan–Meier curves (long-rank test), univariate, and multivariate Cox regression. For the analysis of *Early Failure*, logistic regression were performed. All analyses were 2-tailed, and a *P* value < .05 was considered statistically significant.

RESULTS

Description of the Series

Overall, 922 cases of PJI were recorded, of which 92 (10.0%) were excluded for various reasons, leaving a cohort of 830 cases. We initially managed 462 (55.7%) by DAIR, and these cases were used as the focus of this analysis (Supplementary Figure 1).

The median age was 72 years (interquartile range [IQR], 65–78 years), and 50% were men. The most frequent type of PJI was hematogenous (52%), which occurred more frequently in men, in patients with malignancy and in those with knee prostheses. Patients with hematogenous PJI more frequently presented with bacteremia and elevated temperature, along with higher leukocyte counts and C-reactive protein (CRP) levels (Table 1).

The most frequent species was *S. agalactiae* (159 cases [34.4%]) (Table 2). There were 63 (14%) polymicrobial infections that were typically postoperative (83%), presented less frequently with fever (51% vs. 68%, $P = .007$) and more frequently with a sinus tract (34% vs. 10%, $P < .001$), and had lower CRP levels (80 mg/L [IQR 41–150] vs. 202 mg/L [IQR 110–291], $P < .001$).

Baseline features, clinical presentation, and management were similar among the streptococcal species (Supplementary Table 2). Exceptions to this were the higher rate of patients

Table 3. Predictors of Overall Failure and Influence of Early Antibiotic

		All Evaluable Cases—Overall Failure (n = 444, 187 Failures)					Evaluable Cases Not Failing within the First 30 days (n = 389, 132 Failures)				
Variable	Categories	Failures/n	HR (95%CI)	P	aHR (95%CI)	P	Failures/n	HR (95%CI)	P	aHR (95%CI)	P
Sex	Female	90/225	0.86 (0.65–1.14)	.30			60/195	0.75 (0.53–1.06)	**.10**	…	…
	Male[a]	97/219					72/194				
Age (per year)	…	…	1.00 (0.99–1.01)	.93			…	0.99 (0.98–1.01)	.32		
Diabetes	Yes	50/108	1.16 (0.84–1.60)	.38			36/94	1.20 (0.82–1.76)	.36		
	No[a]	137/336					96/295				
Renal Chronic Disease	Yes	24/44	1.58 (1.03–2.43)	**.05**	1.55 (0.97–2.48)	.07	16/36	1.57 (0.93–2.65)	**.09**	…	…
	No[a]	163/400					116/353				
Rheumatoid arthritis	Yes	24/37	2.23 (1.45–3.43)	**<.01**	2.36 (1.50–3.72)	**<.01**	14/27	2.04 (1.17–3.54)	**.02**	…	…
	No[a]	163/407					118/362				
Immunosuppressive therapy	Yes	29/48	1.86 (1.25–2.76)	**<.01**	…	…	21/40	2.08 (1.31–3.32)	**<.01**	1.66 (0.99–2.18)	.055
	No[a]	158/396					111/349				
Malignancy	Yes	11/28	0.90 (0.49–1.66)	.73			10/27	1.20 (0.63–2.29)	.59		
	No[a]	176/416					122/362				
Prosthesis location	Knee	116/263	1.05 (0.95–1.16)	.31			82/229	1.09 (0.91–1.29)	.36		
	Other[a]	71/181					50/160				
Revision prosthesis	Yes	60/112	1.60 (1.18–2.17)	**<.01**	1.37 (0.98–1.90)	.06	42/94	1.66 (1.15–2.40)	**<.01**	1.47 (0.99–2.18)	.06
	No[a]	127/332					90/295				
Hematogenous infection	Yes	95/234	0.90 (0.68–1.20)	.48			65/204	0.84 (0.60–1.18)	.32		
	No[a]	92/210					67/185				
Late post-surgical infection	Yes	44/70	1.41 (1.19–1.67)	**<.01**	2.20 (1.51–3.20)	**<.01**	31/57	1.28 (1.12–1.46)	**<.01**	1.69 (1.10–2.60)	**.02**
	No[a]	143/374					101/332				
Temperature >37°C	Yes	122/288	1.08 (0.79–1.46)	.65			85/251	1.05 (0.73–1.52)	.78		
	No[a]	60/149					42/132				
Sinus tract	Yes	27/61	1.12 (0.75–1.69)	.58			21/55	1.29 (0.81–2.06)	.30		
	No[a]	155/378					107/330				
Rx signs of infection	Yes	39/80	1.08 (0.99–1.19)	.11			25/66	1.21 (0.77–1.91)	.42		
	No[a]	98/251					72/225				
Leukocytes (per unit/μL)		…	1.00 (1.00–1.00)	.21			…	1.00 (1.00–1.00)	.11		
C-reactive protein	Per mg/L	…	1.00 (1.00–1.00)	.91			…	1.00 (1.00–1.00)	.76		
Penicillin MIC	>0.125 mg/L	8/23	0.80 (0.40–1.63)	.53			4/19	0.58 (0.21–1.56)	.24		
	≤0.125 mg/L[a]	161/384					111/334				
Bacteriemia	Yes	63/132	1.44 (1.06–1.96)	**.02**	1.69 (1.19–2.40)	**<.01**	39/108	1.23 (0.84–1.79)	.30		
	No[a]	110/290					83/263				
Polymicrobial infection	Yes	28/59	1.17 (0.78–1.74)	.46			21/52	1.27 (0.80–2.03)	.32		
	No[a]	159/385					111/337				
Time to debridement[b]	Per day	…	1.00 (1.00–1.00)	**.06**	…	…	…	1.00 (1.00–1.00)	**.01**	1.00 (1.00–1.00)	**.05**
	>7 days	82/173	1.28 (0.96–1.71)	**.09**			61/152	1.45 (1.03–2.05)	**.03**		
	≤7 days[a]	105/271					71/237				
	>21 days	35/67	1.33 (0.92–1.92)	.14			27/59	1.51 (0.99–2.31)	**.07**		
	≤21 days[a]	152/377					105/330				
Polyethylene exchange	Yes	73/211	0.59 (0.44–0.80)	**<.01**	0.60 (0.44–0.81)	**<.01**	53/191	0.60 (0.42–0.86)	**<.01**	0.65 (0.50–0.93)	**.02**
	No[a]	98/190					68/160				
Need for ≥2 debridements	Yes	41/80	1.41 (1.00–2.00)	**.05**	1.38 (0.96–1.99)	.08	30/69	1.53 (1.02–2.30)	**.05**	1.68 (1.10–2.57)	**.02**
	No[a]	146/364					102/320				
Treatment with rifampin[c]	Per day	…	…				…	0.99 (0.97–1.00)	**.05**	0.98 (0.96–0.998)	**.03**
	>14 days	…	…				33/116	0.72 (0.48–1.06)	**.09**		
	≤14[a]days	…	…				99/273				
Treatment with β-lactams[c]	Per day	…	…				…	0.99 (0.98–1.01)	.99		
	>14 days	…	…				87/270	0.85 (0.59–1.22)	.39		
	≤14[a] days	…	…				45/119				
Treatment with glycopeptides[c]	Days	…	…				…	1.04 (1.02–1.06)	**<.01**	1.04 (1.02–1.06)	**<.01**
	>14 days	…	…				16/29	2.37 (1.40–4.00)	**<.01**		
	≤14[a] days	…	…				116/360				

Table 3. Continued

		All Evaluable Cases—Overall Failure (n = 444, 187 Failures)					Evaluable Cases Not Failing within the First 30 days (n = 389, 132 Failures)				
Variable	Categories	Failures/n	HR (95%CI)	P	aHR (95%CI)	P	Failures/n	HR (95%CI)	P	aHR (95%CI)	P
Treatment with co-trimoxazole[c]	Days	…	…				…	1.03 (1.00–1.06)	**.04**	1.04 (1.002–1.08)	**.04**
	>14 days	…	…				6/9	2.33 (1.03–5.30)	**.04**		
	≤14[a] days	…	…				126/380				

Abbreviations: aHR, adjusted hazard ratio. CI, confidence interval; CPR, C-reactive protein; HR, hazard ratio; MIC, minimal inhibitory concentration.

[a]Reference category.

[b]Time from onset of symptoms to surgical debridement. [c]Treatments considered are those received within the first 30 days after surgical debridement. Overall analysis does not include the influence of antibiotics in order to avoid survivors bias. The initial model of the multivariate analyses was built with variables with a P value ≤ .10 in the univariate analysis, and then selected with a stepwise backward process (variables excluded during this process are marked as "–").

with rheumatoid arthritis among episodes caused by *S. pyogenes*, and the higher rate of chronic lung disease and malignancy in PJI due to *S. pneumoniae*. Pneumococcal PJI was also more frequently hematogenous, occurred more frequently with knee prostheses, and presented with a higher leukocyte count. Penicillin minimum inhibitory concentration (MIC) was >0.125 mg/L in 24/425 cases (6%).

DAIR Management

Patients underwent debridement after a median of 5 days (IQR 2–13) from the onset of symptoms. Removable components were exchanged in 53% of cases, this being highly variable across participating centers (Supplementary Figure 2). The median number of different antimicrobial classes prescribed per patient was 2 (range 1–6). Patients were usually treated with β-lactams, which were given intravenously for a mean time of 21 days ± 20 days. Rifampin-based combinations were significantly used (i.e., during >21 days) in 37% of patients, but this fraction was also highly variable across the participating hospitals (in those recruiting >10 patients, it ranged from 18% to 88%) (Supplementary Figure 2). Alternative antimicrobials such as fluoroquinolones, clindamycin, or linezolid were used less often (Supplementary Table 3). In patients not failing while on treatment, antimicrobial therapy was continued for a median of 91 days (IQR, 58–171 days).

Outcome

The primary endpoint was evaluable in 444 patients (96.1%). *Overall Failure* occurred in 187 patients (42.1%; 95% confidence interval [CI], 37.5%–46.7%) after a median of 62 days from debridement (IQR, 25–160 days); by contrast, 257 patients (57.1%) did not fail and were followed up for a median of 802 days (IQR, 507–1339 days) (Figure 1A). Success rates were highly variable among the participating centers (Supplementary Figure 2), with it ranging from 44% to 91% among hospitals recruiting >10 patients.

Independent predictors of a poor outcome were rheumatoid arthritis (hazard ratio [HR], 2.36), late post-surgical infection (HR, 2.20), and bacteremia (HR, 1.69). The exchange of removable components was independently associated with a favorable outcome (HR, 0.60) (Table 3). No one streptococcal species was associated with a higher likelihood of *Overall Failure*, although a nonsignificant better prognosis was observed for *S. pneumoniae* (24% failure). A high penicillin MIC (>0.125 mg/L) was also not associated with failure. Also, polymicrobial cases were not associated with a higher likelihood of failure, even when *S. aureus* was involved (data not shown).

Late post-surgical infection was indeed a predictor of bad prognosis, when defined as onset of symptoms beginning >3 months after the prosthesis placement (Figure 1C). Cases with symptoms beginning within the first and third month had a similar prognosis to that of cases with symptoms beginning within the first month after prosthesis placement. No relevant differences were observed in these 2 groups of patients (data not shown).

The failure rate was higher in patients not fulfilling the IDSA criteria for DAIR, namely, 106/223 (48%) versus 81/221 (37%) (long-rank test, P = .017) (Figure 1B). Again, indication of DAIR according to the IDSA criteria was highly variable among participating centers (Supplementary Figure 2), ranging from 33% to 83% in those recruiting >10 patients. Independent predictors of failure among patients meeting the IDSA criteria were rheumatoid arthritis (HR, 2.46 [95% CI, 1.34–4.53]), bacteremia (HR, 1.92 [95% CI, 1.22–3.02]), and male sex (HR, 1.85 [95% CI, 1.18–2.91]). Interestingly, the exchange of removable components during debridement was especially beneficial in patients not meeting the IDSA criteria (37% failures vs. 62%, P < .001), in comparison with patients fulfilling them (failures 33% vs. 39%, P = .286).

Failure Dynamics and Antimicrobial Therapy

Among the 187 patients who failed, 55 (29%) developed *Early Failure*, 71 (38%) developed *Late Failure*, and 61 developed *Failure after Therapy* (33%). Variables independently associated with *Early Failure* were age, rheumatoid arthritis, late post-surgical infection, bacteremia, and infection by *S. pyogenes* (Table 4).

Characteristics associated with *Late Failure* were male sex, immunosuppressant therapy, revision prosthesis, debridement

Table 4. Univariate and Multivariate Analysis of Parameters Predicting Early Failure, Late Failure and Failure After Therapy

	Early Failure (*n* = 444, 55 Failures)[b]				Late Failure (n = 389, 71 Failures)[c]				Failure After Therapy (N = 318, 61 Failures)[d]			
	OR (95% CI)	*P*	aOR (95%CI)	*P*	HR (95%CI)	*P*	aHR (95% CI)	*P*	HR (95%CI)	*P*	aHR (95% CI)	*P*
Sex (female)	1.19 (0.68–2.10)	.540			0.50 (0.31–0.81)	**.004**	0.51 (0.30–0.85)	**.009**	1.16 (0.69–1.92)	.572		
Age (per year)	1.03 (0.99–1.01)	**.076**	1.04 (1.00–1.07)	**.027**	1.00 (0.98–1.02)	.995			0.99 (0.97–1.01)	.348		
Rheumatoid arthritis	2.98 (1.35–6.56)	**.007**	3.33 (1.40–7.93)	**.007**	2.95 (1.55–5.62)	**.004**	…	…	1.19 (0.37–3.81)	.772		
Immunosuppressive therapy	1.49 (0.66–3.66)	.343			2.76 (1.56–4.89)	**.002**	2.64 (1.46–4.79)	**.001**	1.51 (0.65–3.51)	.363		
Renal chronic disease	1.67 (0.73–3.81)	.223			1.99 (1.05–3.79)	**.053**	…	…	1.17 (0.47–2.91)	.746		
Prosthesis location (knee)	1.04 (0.86–1.26)	.677			0.98 (0.83–1.14)	.753			1.18 (0.98–1.41)	**.073**	…	…
Revision prosthesis	1.53 (0.83–2.81)	.173			1.78 (1.09–2.91)	**.027**	1.77 (1.07–2.93)	**.027**	1.56 (0.90–2.70)	.129		
Chronic post-surgical inf.	1.212 (0.97–1.23)	**.091**	1.41 (1.10–1.81)	**.007**	1.12 (0.92–1.37)	.256			1.47 (1.22–1.77)	**<.001**	2.24 (1.24–4.05)	**.008**
Sinus tract	0.75 (0.31–1.84)	.529			1.05 (0.54–2.06)	.881			1.61 (0.84–3.11)	.175		
Bacteremia	2.17 (1.20–3.92)	**.011**	2.23 (1.80–4.20)	**.014**	1.24 (0.74–2.06)	.420			1.23 (0.70–2.19)	.478		
Rx signs of infection	1.16 (0.98–1.39)	**.091**	…	…	0.77 (0.40–1.48)	.421			2.21 (1.14–4.30)	**.025**	…	…
Infection by *S. pyogenes*	3.10 (1.41–6.85)	**.005**	3.31 (1.41–7.77)	**.006**	0.60 (0.19–1.92)	.357			1.11 (0.45–2.78)	.821		
Infection by virdidans streptococci	0.71 (0.32–1.57)	.401			1.60 (0.94–2.70)	**.094**	…	…	1.01 (0.51–1.98)	.987		
Polymicrobial infection	0.95 (0.41–2.20)	.896			1.33 (0.71–2.47)	.385			1.23 (0.61–2.49)	.579		
Time to debridement (>7 days)[a]	0.96 (0.54–1.72)	.899			1.60 (1.00–2.54)a	**.050**	1.70 (1.05–2.75)	**.033**	1.33 (0.80–2.20)	0.281		
Exchange of polyethylene	0.56 (0.31–1.02)	**.059**	…	…	0.75 (0.46–1.21)	.234			0.45 (0.26–0.77)	**.033**	0.44 (0.26–0.76)	**.003**
Need for ≥2 debridements	1.16 (0.57–2.36)	.683			2.26 (1.63–4.36)	**<.001**	2.45 (1.45–4.15)	**.001**	0.60 (0.26–1.40)	.206		
Antimicrobial therapy‡												
B-lactams (without rifampin)	…	…			1.41 (0.88–2.27)	.155			0.62 (0.37–1.03)	**.061**	0.48 (0.28–0.84)	**.010**
β-lactams + rifampin	…	…			0.89 (0.47–1.70)	.724			0.42 (0.18–0.98)	**.025**	0.34 (0.12–0.96)	**.041**
Quinolones + rifampin	…	…			0.19 (0.03–1.36)	**.082**	0.21 (0.03–1.54)	.125	1.03 (0.45–2.40)	.940		
Glycopeptides without rifampin	…	…			3.97 (2.08–7.58)	**<.001**	2.82 (1.43–5.53)	**.003**	4.25 (1.32–13.7)	**.015**	…	…
Duration of therapy >120 days	…	…			…	…			0.54 (0.29–0.90)	**.046**	…	…

Abbreviations: aHR, adjusted hazard ratio; aOR, adjusted odds ratio; CI, confidence interval; HR, hazard ratio; OR, odds ratio.

[a]Time to debridement: time from onset of symptoms to the first surgical debridement. Initial models of multivariate analyses were built with variables with a *P* value < .10 in the univariate analysis and then selected with a stepwise backward process.

[b]*Early Failure*: the initial multivariate model included age, rheumatoid arthritis, late post-surgical infections, Rx signs of infection, infection by *S. pyogenes,* and bacteremia.

[c]*Late Failure*: the initial multivariate model included sex, rheumatoid arthritis, immunosuppressant therapy, chronic renal disease, infection by *S. viridians*, time to debridement, need for ≥2 debridements, treatment with quinolones plus rifampin, and treatment with glycopeptides without rifampin.

‡Treatments included in this analysis are those received during the first 30 days after debridement and are considered if they were administered for at least 15 days.

[d]*Failure After Therapy*: the initial multivariate model included prosthesis location, late post-surgical infection, Rx signs of infection, exchange of removable components (i.e., polyethylene liner), treatment with beta-lactams (without rifampin), treatment with beta-lactams plus rifampin, and treatment with glycopeptides without rifampin.

‡Treatments included in this analysis are those received during the whole period of treatment, both orally and intravenously, and are considered if they were administered for at least 22 days.

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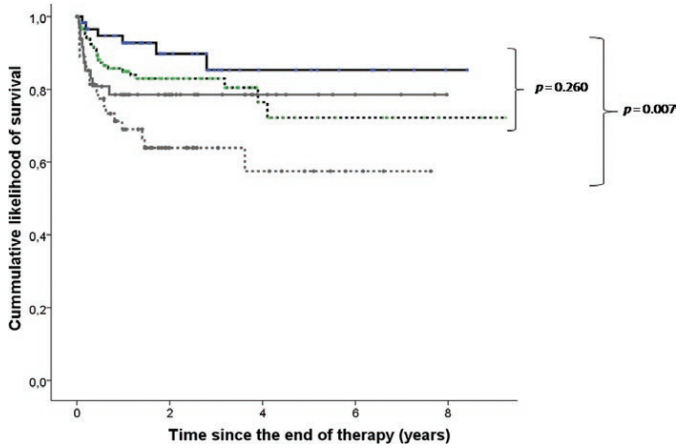

Figure 2. Prognostic after the end of therapy according to the antibiotic treatment.Analysis performed in cases that did not fail during treatment (n = 318, failures = 61). Black continuous line: patients treated during >21 days with β-lactams + rifampin (n = 60, failures = 6); black dotted line: patients treated during >21 days with β-lactams, but no rifampin (n = 154, failures = 26); gray continuous line: patients treated >21 days with a rifampin-based combination other than β-lactams plus rifampin (n = 48; failures = 10); gray dotted line: patients who did not receive either β-lactams or rifampin for >21 days (n = 56; failures = 19). Comparisons calculated with the Long-rank test. The comparison of these 4 treatment regimes showed similar trends when the analysis was stratified for patients meeting and not meeting IDSA criteria and for patients who did and did not undergo exchange of removable components during debridement. Abbreviation: IDSA, Infectious Diseases Society of America.

delay >7 days, and the need for >1 debridement to control the infection. Failure was also associated with the early use of glycopeptides during >14 days. However, the addition of rifampin to treatment with glycopeptides neutralized this poor prognosis. The early use of rifampin plus fluoroquinolones also showed a trend toward a favorable outcome in the univariate analysis (HR, 0.19; $P = .082$).

Late post-surgical infection was an independent predictor of *Failure after Therapy*, whereas the exchange of removable components was associated with a favorable outcome. The use of β-lactams for >21 days, both alone and combined with rifampin, were independently associated with better outcomes (HR, 0.48 and 0.34, respectively) (Figure 2).

The benefits of early treatment with rifampin were also observed for patients when treatment did not fail within the first 30 days after debridement (HR, 0.98 per day of treatment, $P = .034$) (Table 3).

DISCUSSION

This is the largest series to our knowledge assessing the management of streptococcal PJI by DAIR. Our results show an overall long-term likelihood of curing the infection and keeping the prosthesis of 57%. The large sample used in our study, the diversity of streptococcal species, and the high number of participating hospitals increase the external validity of our results.

Predictors of a poor outcome in this series were similar to those found in previous studies of PJI by staphylococci and GNB managed by DAIR. In previous reports, patients with bacteremia, needing >1 debridement, or with high CRP levels have shown to have a bad prognosis [24–29]. In our series, bacteremia and infection by *S. pyogenes* were independent predictors of *Early Failure*.

Otherwise, the streptococcal species presented a very similar pattern regarding clinical presentation and outcome, though *S. pneumoniae* presented more frequently as a hematogenous infection, and was usually associated with a better prognosis (nonsignificant).

The percentage of hematogenous infection in this series was notably high, when compared with PJI by *S. aureus* (52% vs. 15%) [25]. Moreover, we cannot rule out that some late post-surgical infections were actually hematogenous. Although staphylococcal hematogenous PJI has been reported to carry a poor prognosis [25, 30, 31], in this study we did not find an association with failure, despite the higher association of hematogenous infection with bacteremia, fever, high levels of CRP, and a high leukocyte count. It is possible that the ability of β-lactams to clear bacteremia and planktonic infection in hematogenous PJI could be higher for streptococci than for staphylococci.

Univariate and multivariate analyses have shown that some debilitating baseline conditions are associated with a worse outcome. Taken together with our previous large series, rheumatoid arthritis, immunosuppressant therapy, and chronic renal insufficiency seem to be associated with a higher risk of treatment failure when attempting DAIR [25, 27]. The exchange of removable components was associated with a favorable outcome, something that has also been observed in previous studies [25, 32]. This is consistent with the physical removal of the biofilm and probably stands as a surrogate marker of an exhaustive surgical debridement. Of note, this benefit was particularly observed in patients not fulfilling IDSA criteria for DAIR.

Unfortunately, the possibility of performing an accurate analysis of antimicrobial efficacy is impaired by the retrospective nature of this study, along with the heterogeneity of the therapeutic schedules. Still, the large size of our series allows for some interesting considerations.

B-lactams have classically been the preferred therapy for streptococcal infections, including PJI, providing very good activity for the initial planktonic phase of these infections [33]. However, once this initial phase has passed, the antibiofilm profile of these antimicrobials is questionable because, as with any antibiotic with a mechanism of action dependent on cell wall synthesis, they will become less effective against biofilm-embedded bacteria [34]. There is now strong evidence that β-lactams have poor efficacy for staphylococcal and GNB PJI, especially when contrasted with other antibiotics that have superior antibiofilm profiles, such as rifampin against staphylococci or fluoroquinolones against GNB [25–27, 35, 36]. However, these findings have not been demonstrated in streptococcal PJI, which haves been disregarded in those studies.

Our patients were mostly treated with β-lactams, in line with classic recommendations and routine clinical practice. The multivariate analysis concerning *Failure after Therapy* showed that this therapy was beneficial, with superiority over less effective alternatives like glycopeptides. This beneficial effect probably depended, in part, on the activity of β-lactams against planktonic bacteria in the first weeks of treatment [37]. Therefore, this contribution may be relevant to the outcome of PJI.

However, other data could indicate the suboptimal antibiofilm activity of β-lactams in our series, along with some evidence of a possible beneficial effect of rifampin. Among patients who completed a long course of treatment with β-lactams, we did not observe statistical differences among those also receiving rifampin or not, but a tendency toward a better prognosis was found in those treated with combined therapy (10.0% failure rate vs. 16.8%, Figure 2). In addition, the initial treatment with rifampin was also proved as an independent predictor of a favorable outcome (Table 4).

IDSA criteria for instituting DAIR were not met by all cases in this study. Consistent with previous studies, this allowed us to confirm the usefulness of these criteria for selecting suitable candidates for DAIR [6, 7, 25, 27]. We were also able to test the effect of each of these criteria on the outcomes. In this regard, the duration of symptoms may be difficult to establish, especially in postoperative cases where pain and inflammation may overlap those of the post-surgical period. The age of the prosthesis may therefore be a more objective measure in such cases, consistent with the IDSA recommendation that patients undergo DAIR only if there is a short time between the prosthesis placement and debridement [4]. The definition of early postoperative PJI has varied over time in several landmark publications, ranging from 1 to 3 months [2, 11, 36], with the IDSA recommending that DAIR should be performed within 1 month after placing the prosthesis [4]. However, we have observed a similar prognosis for patients with postoperative infection whose symptoms began within the first month after prosthesis placement and those whose symptoms started between the first and third month (Figure 2). A similar finding was also observed for staphylococcal PJI [25], and it would emphasize this 3-month time limit over a more strict cutoff.

As mentioned, our analysis has the inherent limitations of retrospective studies. For instance, the influence of antibiotics was evaluated with continuous variables (i.e., days of antibiotics) but also after arbitrarily categorizing these parameters (i.e., >21 days of treatment). Also, the possible relevance of endocarditis was not evaluated in this study. Finally, it has been already mentioned the significant heterogeneity of patients included across the participating institutions, especially regarding their management: the fulfillment of the IDSA criteria, the participation of different surgical teams, or the decision on whether to use or not rifampin are all examples of this variability (supplementary Figure 2). Still, these cases form a large cohort of patients with streptococcal PJI, all treated by DAIR. This has given us the opportunity to study their prognosis in the best and the worst possible clinical scenario, thus providing an overall perspective of the clinical problem.

In summary, we analyzed the largest series of streptococcal PJI managed by DAIR to date and showed a modest prognosis of curing the infection and retaining the prosthesis. We conclude that classical treatment with β-lactams is probably ideal for fighting the planktonic component of the infection. We found a piece of evidence suggesting that addition of rifampin some days or weeks after debridement could improve the outcome, but this should be confirmed in further studies. IDSA criteria are a valid clinical tool for deciding DAIR, late post-surgical infection (i.e., symptoms beginning >3 months since prosthesis placement) being the most important contra-indication. The exchange of removable components during debridement stands as an independent predictor of a favorable outcome.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank Michael Maudsley (University of Barcelona) for reviewing the English manuscript.

List of collaborators/Group of Investigators for Streptococcal Prosthetic Joint Infection

This is a multicenter study. In each institution there are many researchers that have helped to make this study possible. We are deeply indebted to these collaborators, who are:

- Fernando Chaves, José Alberto Moreno-Beamud, Rafael Navarro Arribas (Hospital Universitario 12 de Octubre, Madrid, Spain).
- Sophie Nguyen (Gustave Dron Hospital of Tourcoing, France).
- Oscar Murillo, Xavier Cabo, Salvador Pedrero (Hospital Universitari de Bellvitge, Barcelona, Spain).

- Frédéric Dauchy, Hervé Dutronc, Bertille de Barbeyrac (Centre correspondant de prise en charge des Infections Ostéo-articulaires Complexes Du Grand Sud-Ouest—CHU Bordeaux—France.
- Matthew Scarborough, Martin McNally, Bridget Atkins (Bone Infection Unit, Nuffield Orthopaedic Centre, Oxford, United Kingdom.
- Pierre Tattevin, Marie Ghéno, Enora Ouamara-Digue (Rennes University Hospital, Rennes, France).
- Bernhard Kessler (Kantosspital Baselland, Liestal, Switzerland).
- Sébastien Lustig, Florent Valour, Christian Chdiac (Hôpital de la Croix-Rousse, Hospices Civils de Lyon. Lyon, France).
- Miguel Ángel Goenaga, Asier Mitxelena, Enrique Moreno (Hospital Universitario Donostia, San Sebastián, Spain).
- Maja Bombek Ihan, Zmago Krajnc (University Clinical Center, Maribor, Slovenia).
- Carles Pigrau, Pablo S. Corona Pérez-Cardona (Hospital Universitari Vall d'Hebron, Barcelona, Spain).
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- Markku Vuorinen, Jarkko Leskinen (Helsinki University Hospital, Helsinki, Finland).
- Tristan Yolland, Mark Lowenthal (John Hunter Hospital, Newcastel, NSW, Australia).
- Julia Praena, Salvador Fornell, María-José Gómez (Hospital Universitario Virgen del Rocío, Sevilla, Spain).
- Paul C. Jutte (University Medical Center Groningen, Groningen, The Netherlands).
- Anže Mihelič, Rene Mihalič (Valdoltra Orthopaedic Hospital, Ankaran, Slovenia).
- Guillem Bori, Laura Morata, Eduard Tornero (Hospital Clínic, Barcelona, Spain).
- Carlos Fuster Foz, Susana García Villabrille, Marta Novoa (Hospital de El Bierzo, Ponferrada, Spain).
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- Antonio Blanco, Joaquín García-Cañete, Raúl Parrón (Fundación Jiménez Díaz, Madrid, Spain).
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- Guido Grappiolo, Mattia Loppini, Marco Scardino (Humanitas Research Hospital, Milan, Italy).
- Elaine Cheong, Genevieve McKew, Amarita Ronnachit (Concord Hospital, Concord, NSW, Australia).

Financial support. J. L.-T holds a clinical research contract "Sara Borrell" (CD14/00176) from the Instituto de Salud Carlos III (Spanish Ministry of Economy and Competitiviness). CRIOGO is funded by the French Ministry of Health. A. R. was supported by a research grant from the Bellvitge Biomedical Research Institute (IDIBELL). REIPI is supported by the Spanish Ministry of Economy and Competititviness, Instituto de Salud Carlos III, and by the European Development Regional Fund "A way to achieve Europe."

Potential conflicts of interest. Author certifies no potential conflicts of interest. No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Darouiche RO. Treatment of infections associated with surgical implants. N Engl J Med **2004**; 350:1422–9.
2. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med **2004**; 351:1645–54.
3. Byren I, Bejon P, Atkins BL, et al. One hundred and twelve infected arthroplasties treated with 'DAIR' (debridement, antibiotics and implant retention): antibiotic duration and outcome. J Antimicrob Chemother **2009**; 63:1264–71.
4. Osmon DR, Berbari EF, Berendt AR, et al.; Infectious Diseases Society of America. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis **2013**; 56:e1–e25.
5. Laffer RR, Graber P, Ochsner PE, Zimmerli W. Outcome of prosthetic knee-associated infection: evaluation of 40 consecutive episodes at a single centre. Clin Microbiol Infect **2006**; 12:433–9.
6. Sendi P, Christensson B, Uçkay I, et al.; GBS PJI study group. Group B streptococcus in prosthetic hip and knee joint-associated infections. J Hosp Infect **2011**; 79:64–9.
7. Tschudin-Sutter S, Frei R, Dangel M, et al. Validation of a treatment algorithm for orthopaedic implant-related infections with device-retention-results from a prospective observational cohort study. Clin Microbiol Infect **2016**; 22:457.e1–9.
8. Benito N, Franco M, Ribera A, et al. Time trends in the aetiology of prosthetic joint infections: a multicentre cohort study. Clin Microbiol Infect **2016**; 22:732.e1–8.
9. Peel TN, Cheng AC, Buising KL, Choong PF. Microbiological aetiology, epidemiology, and clinical profile of prosthetic joint infections: are current antibiotic prophylaxis guidelines effective? Antimicrob Agents Chemother **2012**; 56:2386–91.
10. Marculescu CE, Berbari EF, Hanssen AD, et al. Outcome of prosthetic joint infections treated with debridement and retention of components. Clin Infect Dis **2006**; 42:471–8.
11. Tsukayama DT, Estrada R, Gustilo RB. Infection after total hip arthroplasty: a study of the treatment of one hundred and six infections. J Bone Joint Surg Am **1996**; 78:512–23.
12. Betz M, Abrassart S, Vaudaux P, et al. Increased risk of joint failure in hip prostheses infected with *Staphylococcus aureus* treated with debridement, antibiotics and implant retention compared to *Streptococcus*. Int Orthop **2015**; 39:397–401.
13. Everts RJ, Chambers ST, Murdoch DR, Rothwell AG, McKie J. Successful antimicrobial therapy and implant retention for streptococcal infection of prosthetic joints. ANZ J Surg **2004**; 74:210–4.
14. Zürcher-Pfund L, Uçkay I, Legout L, Gamulin A, Vaudaux P, Peter R. Pathogen-driven decision for implant retention in the management of infected total knee prostheses. Int Orthop **2013**; 37:1471–5.
15. Zeller V, Lavigne M, Biau D, et al. Outcome of group B streptococcal prosthetic hip infections compared to that of other bacterial infections. Joint Bone Spine **2009**; 76:491–6.
16. Corvec S, Illiaquer M, Touchais S, et al.; Bone and Joint Infection Study Group. Clinical features of group B Streptococcus prosthetic joint infections and molecular characterization of isolates. J Clin Microbiol **2011**; 49:380–2.
17. Duggan JM, Georgiadis G, VanGorp C, Kleshinski J. Group B streptococcal prosthetic joint infections. J South Orthop Assoc **2001**; 10:209–14; discussion 14.
18. Meehan AM, Osmon DR, Duffy MC, Hanssen AD, Keating MR. Outcome of penicillin-susceptible streptococcal prosthetic joint infection treated with debridement and retention of the prosthesis. Clin Infect Dis **2003**; 36:845–9.
19. del Prado G, Ruiz V, Naves P, Rodríguez-Cerrato V, Soriano F, del Carmen Ponte M. Biofilm formation by *Streptococcus pneumoniae* strains and effects of human

serum albumin, ibuprofen, N-acetyl-l-cysteine, amoxicillin, erythromycin, and levofloxacin. Diagn Microbiol Infect Dis **2010**; 67:311–8.
20. Olson ME, Ceri H, Morck DW, Buret AG, Read RR. Biofilm bacteria: formation and comparative susceptibility to antibiotics. Can J Vet Res **2002**; 66:86–92.
21. García-Castillo M, Morosini MI, Valverde A, et al. Differences in biofilm development and antibiotic susceptibility among *Streptococcus pneumoniae* isolates from cystic fibrosis samples and blood cultures. J Antimicrob Chemother **2007**; 59:301–4.
22. Fiaux E, Titecat M, Robineau O, et al.; G4 bone and joint infection study group (G4BJIS). Outcome of patients with streptococcal prosthetic joint infections with special reference to rifampicin combinations. BMC Infect Dis **2016**; 16:568.
23. Murray PR, Jo Baron E, Jorgesen JH, Landry ML, Pfaller MA. Manual of Clinical Microbiology. 9th ed. Washington, DC: ASM Press, **2007**.
24. Brandt CM, Sistrunk WW, Duffy MC, et al. *Staphylococcus aureus* prosthetic joint infection treated with debridement and prosthesis retention. Clin Infect Dis **1997**; 24:914–9.
25. Lora-Tamayo J, Murillo O, Iribarren JA, et al.; REIPI Group for the Study of Prosthetic Infection. A large multicenter study of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* prosthetic joint infections managed with implant retention. Clin Infect Dis **2013**; 56:182–94.
26. Martínez-Pastor JC, Muñoz-Mahamud E, Vilchez F, et al. Outcome of acute prosthetic joint infections due to Gram-negative bacilli treated with open debridement and retention of the prosthesis. Antimicrob Agents Chemother **2009**; 53:4772–7.
27. Rodríguez-Pardo D, Pigrau C, Lora-Tamayo J, et al.; REIPI Group for the Study of Prosthetic Infection. Gram-negative prosthetic joint infection: outcome of a debridement, antibiotics and implant retention approach. A large multicentre study. Clin Microbiol Infect **2014**; 20:O911–9.
28. Vilchez F, Martínez-Pastor JC, García-Ramiro S, et al. Outcome and predictors of treatment failure in early post-surgical prosthetic joint infections due to *Staphylococcus aureus* treated with debridement. Clin Microbiol Infect **2011**; 17:439–44.
29. Tornero E, Martínez-Pastor JC, Bori G, et al. Risk factors for failure in early prosthetic joint infection treated with debridement. Influence of etiology and antibiotic treatment. J Appl Biomater Funct Mater **2014**; 12:129–34.
30. Sendi P, Banderet F, Graber P, Zimmerli W. Clinical comparison between exogenous and haematogenous periprosthetic joint infections caused by *Staphylococcus aureus*. Clin Microbiol Infect **2011**; 17:1098–100.
31. Vilchez F, Martínez-Pastor JC, García-Ramiro S, et al. Efficacy of debridement in hematogenous and early post-surgical prosthetic joint infections. Int J Artif Organs **2011**; 34:863–9.
32. Choi HR, von Knoch F, Zurakowski D, Nelson SB, Malchau H. Can implant retention be recommended for treatment of infected TKA? Clin Orthop Relat Res **2011**; 469:961–9.
33. Baker CN, Thornsberry C, Facklam RR. Synergism, killing kinetics, and antimicrobial susceptibility of group A and B streptococci. Antimicrob Agents Chemother **1981**; 19:716–25.
34. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science **1999**; 284:1318–22.
35. Senneville E, Joulie D, Legout L, et al. Outcome and predictors of treatment failure in total hip/knee prosthetic joint infections due to *Staphylococcus aureus*. Clin Infect Dis **2011**; 53:334–40.
36. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. JAMA **1998**; 279:1537–41.
37. Sendi P, Zimmerli W. Antimicrobial treatment concepts for orthopaedic device-related infection. Clin Microbiol Infect **2012**; 18:1176–84.

Clinica Chimica Acta 468 (2017) 215–224

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Development and validation of a measurement procedure based on ultra-high performance liquid chromatography-tandem mass spectrometry for simultaneous measurement of β-lactam antibiotic concentration in human plasma

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ARTICLE INFO

Article history:
Received 9 December 2016
Received in revised form 10 February 2017
Accepted 9 March 2017
Available online 10 March 2017

Keywords:
β-Lactam antibiotics
Human plasma
Protein precipitation
UHPLC-MS/MS
Therapeutic drug monitoring

ABSTRACT

Background: The administration of β-lactam antibiotics in continuous infusion could let optimize the pharmacokinetic/pharmacodynamic parameters, especially in the treatment of serious bacterial infections. In this context, and also due to variability in their plasmatic concentrations, therapeutic drug monitoring (TDM) may be useful to optimize dosing and, therefore, be useful for the clinicians.
Material and methods: We developed and validated a measurement procedure based on ultra-high performance liquid chromatography-tandem mass spectrometry for simultaneous measurement of amoxicillin, ampicillin, cloxacillin, piperacillin, cefepime, ceftazidime, cefuroxime, aztreonam and meropenem concentrations in plasma. The chromatographic separation was achieved using an Acquity®-UPLC® BEH™ (2.1 × 100 mm id, 1.7 μm) reverse-phase C_{18} column, with a water/acetonitrile linear gradient containing 0.1% formic acid at a 0.4 mL/min flow rate. β-Lactam antibiotics and their internal standards were detected by electrospray ionization mass spectrometry in multiple reaction monitoring mode.
Results: Chromatography run time was 7.0 min and β-lactam antibiotics eluted at retention times ranging between 1.08 and 1.91 min. The lower limits of quantification were between 0.50 and 1.00 mg/L. Coefficients of variation and relative bias absolute values were <13.3% and 14.7%, respectively. Recovery values ranged from 55.7% to 84.8%. Evaluation of the matrix effect showed ion enhancement for all antibiotics. No interferences or carry-over were observed.
Conclusions: Our measurement procedure could be applied to daily clinical laboratory practice to measure the concentration of β-lactam antibiotics in plasma, for instance in patients with bone and joint infections and critically ill patients.

Abbreviations: AMX, amoxicillin; AMP, ampicillin; ASP, antimicrobial stewardship program; ATM, aztreonam; β-LA, β-lactam antibiotics; CAZ, ceftazidime; CI, continuous infusion; CLO, cloxacillin; CLSI, Clinical and Laboratory Standards Institute; *CV*, coefficient of variation; CXM, cefuroxime; δ_r, relative bias; DMSO, dimethyl sulfoxide; EI, extended infusion; EMA, European Medicines Agency; FEP, cefepime; ESI, electrospray ionization; HPLC, high-performance liquid chromatography; IFCC, International Federation of Clinical Chemistry; IUPAC, International Union of Pure and Applied Chemistry; IS, internal standard; LC, liquid chromatography; LLOQ, lower limit of quantification; MEM, meropenem; MIC, minimal inhibitory concentration; MRM, multiple reaction monitoring; MS, mass spectrometer; MS/MS, tandem mass spectrometry; *m/z*, mass-to-charge; PD, pharmacodynamic; PIP, piperacillin; PK, pharmacokinetic; QC, quality control; *S/N*, signal-to-noise; UHPLC, ultra-high performance liquid chromatography; UV, ultraviolet; ULOQ, upper limit of quantification; LLOD, lower limit of detection; %D, percent deviation; TDM, therapeutic drug monitoring; T > MIC, time the drug concentration remains above the MIC.

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http://dx.doi.org/10.1016/j.cca.2017.03.009
0009-8981/

1. Introduction

β-Lactam antibiotics (β-LA) are widely used in clinical practice, mainly with the administration of fixed dosing regimens by intermittent boluses. They have a time-dependent activity, meaning that their bacterial killing is determined by the time the drug concentration remains above the minimal inhibitory concentration (MIC) of the organism (T > MIC) [1–5]. In order to avoid clinical failure or development of resistance in particular situations, their administration could be optimized in terms of drug pharmacokinetics/pharmacodynamics (PK/PD) by using β-LA in continuous (CI) or extended infusion (EI) [1,6–8]. Particularly, in the last years, the worldwide emergence of multidrug resistant microorganisms, together with the limited pipeline of new antibiotics, has led to a difficult-to-treat scenario [9–11]. In this setting, there is a need for an optimized use of antibiotics, and this has renewed the interest for using β-LA in CI or EI mainly in combination with other drugs [1,6–9].

It seems that an individualized approach with therapeutic drug monitoring (TDM) of β-LA is mandatory when clinicians face up these difficult-to-treat infections [12]. However, there are no available commercial procedures for routine measurement of β-LA concentration in human plasma and thus, several measurement procedures have to be developed and validated in-house. Among these, several high-performance liquid chromatography (HPLC) procedures for simultaneous measurement of β-LA concentrations in plasma using ultraviolet (UV) detection have been described [13–18]. They usually present low detection capabilities and are not very selective, owing to the presence of endogenous interferences as well as the limited UV absorption characteristics of the β-lactam moiety (see Supplementary material) and the low wavelengths required to measure β-LA concentrations. Greater detection capabilities and more selective HPLC procedures have been developed using HPLC coupled with tandem mass spectrometry (MS/MS) [19–26]. Nevertheless, to our knowledge, only some of them were used to measure β-LA concentration in human plasma using ultra-high performance liquid chromatography (UHPLC)-MS/MS procedures [19,21–23,26]. These measurement procedures provide more resolution and shorter retention times [27–29]. Among the UHPLC or HPLC-MS/MS procedures reported previously, none of them have been used for simultaneous measurement of β-LA plasma concentration that may be used in CI or EI, and moreover, they presented some limitations (e.g. time-consuming sample extraction procedures, did not study some performance characteristics as carry over or dilution integrity).

In this study, we aimed to develop and to validate (following international guidelines) an easy-to-use UHPLC-MS/MS procedure for simultaneous measurement of concentration of nine β-LA in human plasma that may be used in CI or EI: amoxicillin (AMX), ampicillin (AMP), cloxacillin (CLX), piperacillin (PIP), cefepime (FEP), ceftazidime (CAZ), cefuroxime (CXM), aztreonam (ATM) and meropenem (MEM).

2. Material and methods

2.1. Chemicals and reagents

Certified reference materials of amoxicillin trihydrate (purity of 93.5%), ampicillin trihydrate (purity of 99.8%), cloxacillin sodium (purity of 93.9%), piperacillin (purity of 94.4%), cefepime dihydrochloride monohydrate (purity of 93.1%), ceftazidime (purity of 85.3%), cefuroxime sodium (purity of 96.7%), meropenem trihydrate (purity of 87.0%) were purchased from European Pharmacopeia (European Directorate for the Quality of Medicines-Council of Europe, Strasburg, France). Certified reference material of aztreonam (purity of 99.8%) was obtained from United States Pharmacopeia (Rockville, MD, USA). The labeled internal standards *amoxicillin-d_4* (IS for AMX), *ampicillin-d_5* (IS for AMP), *cloxacillin-$^{13}C_4$* (IS for CLX), *piperacillin-d_5* (IS for PIP), *cefepime-d_3* (IS for FEP), *ceftazidime-d_5* (IS for CAZ), *cefuroxime-d_3* (IS for CXM) and *meropenem-d_6* (IS for MEM), were supplied by Toronto Research Chemicals (Ontario, Canada). Carumonam disodium salt (purity of 97.0%; IS for ATM) and LC-MS-grade acetonitrile, dimethyl sulfoxide (DMSO), formic acid and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). LC-MS-grade water was supplied by Merck Biosciences (Danvers, MA, USA). Drug-free human plasma was obtained from patients not treated with any of the β-LA in study.

2.2. Calibration samples, quality control samples and internal standards

Two stock solutions from independent weighing were prepared at a concentration of 2.00 g/L. One set of stock solutions was used for the preparation of calibrator samples, while the other set was used for quality control (QC) sample preparation. The stock solutions were prepared by weighing an appropriated amount of each certified reference material and dissolving these materials altogether in 20 mL water:metanol:DMSO (50:25:25, *v*/*v*/*v*). The stock solution for calibrator samples was used to prepare nine working standards (0.00, 5.00, 10.0, 50.0, 150, 450, 750, 1250 and 1750 mg/L) in water. These solutions were stored light-protected for up to 6 months at (−75 ± 3) °C as 100 μL aliquots in 1.50 mL-polypropylene microcentrifuge tubes. Plasma calibration samples at 0.00, 0.50, 1.00, 5.00, 15.0, 45.0, 75.0, 125 and 175 mg/L were prepared on the day of analysis by diluting these working standards in human drug-free plasma in a ratio of 1:9. Working QC were similarly prepared and conserved, using a separate stock solution. Plasma QC samples were ready-made at concentrations of 3.00, 30.0 and 120 mg/L.

Stock solutions of labeled IS were prepared by diluting 1 mg of each IS in 10.0 mL of the appropriate solvent (DMSO, methanol or water according to the manufacturer's certificate of analysis). Carumonam IS stock solution was ready-made weighing 10.0 mg in 100 mL of methanol. All IS stock solutions were stored for up to 6 months at (−75 ± 3) °C as 150 μL aliquots in 1.50 mL-polypropylene microcentrifuge tubes. A working solution of IS was prepared freshly for 20 samples analysis by adding 150 μL of each stock solution to 4.5 mL of acetonitrile.

2.3. Sample preparation

One hundred microliters of either calibration, QC or plasma samples were transferred to 1.50 mL-polypropylene microcentrifuge tubes and 300 μL of IS working solution were added for protein precipitation. After vortexing for 3 min, the tubes were centrifuged for 10 min at 11000*g* at (4.00 ± 1.00) °C. One hundred microliters of the supernatant was transferred into a new 1.50 mL-polypropylene microcentrifuge tube containing 400 μL of 0.1% (*v*/*v*) formic acid in water. Tubes were vortexed for 10 s and the whole volume was transferred into specific screw neck glass vials with silicon septa caps (Waters, Milford, MA, USA) and placed in the autosampler ready for injection.

2.4. Instrumentation

Analyses were conducted using an Acquity® UPLC® integrated system (Waters, Milford, MA, USA) consisting of a thermostatic autosampler, a binary solvent delivery manager and a column over a thermostated compartment. Chromatographic separation was performed on an Acquity® UPLC® BEH™ C_{18} reverse-phase column (100 mm × 2.1 mm) with a 1.7 μm particle size and 130 Å pore diameter equipped with a 0.2 μm pre-column filter unit, and an Acquity® UPLC® BEH™ C_{18} VanGuard Pre-column (5 mm × 2.1 mm; 130 Å, 1.7 μm) (Waters, Milford, MA, USA). The column chamber was held at a temperature of 30 °C. Mobile phase A consisted in 0.1% formic acid (*v*/*v*) in water, which was also used as a weak wash solvent. Mobile phase B consisted of 0.1% formic acid (*v*/*v*) in acetonitrile, which was also used as a strong wash solvent. A water:methanol solution (80:20 *v*/*v*) was used as a seal wash. The mobile phase flow rate was maintained at 0.4 mL/min. From 0.0 to 0.5 min, isocratic conditions were run with 2% of B. Solvent B was

increased linearly from 5 to 50% in the time range from 0.5 to 2.0 min. Thereafter, from 2.0 to 2.5 min, a column cleaning procedure was performed to remove interfering plasma components by increasing non-linearly solvent B to 98%. Re-equilibration was performed from 2.5 to 3.5 min at 2% B using a non-linear gradient. The injection volume was 10 μL in a 50 μL loop (partial loop with needle overfill injection mode) and the autosampler temperature was held at (4 ± 1) °C.

Detection was carried out using an Acquity® TQD® tandem-quadrupole mass spectrometer equipped with a Z-spray electrospray ionization source (Waters, Milford, MA, USA). The mass spectrometer operated in multiple reaction monitoring (MRM) and in positive and negative electrospray ionization (ESI) modes. Nitrogen was used as the nebulizing and desolvation gas, and argon was used as the collision gas. For each β-LA, two transitions were followed: one of them was used for quantification (*the quantifier*), and the other was monitored for identification or confirmation (*the qualifier*). For each of the IS, only one MRM transition was used. Precursor and product ions, cone voltage and collision energy were optimized by infusion of 10.0 mg/L in a mixture of water:acetonitrile 50:50 *v/v* containing 0.1% formic acid. Due to the large number of MRM mass transitions which were followed, they were distributed in six overlapping acquisitions functions (see Table 1). The optimized MRM transitions, the number of MRM acquisitions used by β-LA, ESI mode used, cone voltages and collision energies are listed in Table 1. For all β-LA and their IS, the optimized mass spectrometer settings were identical for ESI + and ESI − as follows: capillary voltage 1.2 kV, extractor voltage 3 V, RF lens voltage 0.1 V, source temperature 130 °C, desolvation temperature 450 °C, desolvation gas flow rate 800 L/h, collision gas flow 0.20 mL/min. The dwell time was set to 50 ms for every channel.

2.5. Validation

The validation was carried out, mostly, according to the current European Medicines Agency (EMA) guideline [30]. The developed procedure was validated in terms of selectivity, carry-over, calibration curve, lower limit of quantification, imprecision, bias, dilution integrity, recovery, matrix effect and stability.

2.5.1. Selectivity

Ten different batches of plasma were used from patients not treated with the β-LA in study but receiving other drugs such as digoxin, mycophenolic acid, anticonvulsants (valproic acid, phenytoin, phenobarbital, carbamazepine), or other antibiotics (amikacin, gentamycin, tobramycin, vancomycin). Concentrations of drug in plasma were maintained in their respective therapeutic intervals.

According to the EMA guideline, the absence of interfering components is accepted when the peak area response of interfering peak at the retention time of analyte (each β-LA in our case) is <20% of the lower limit of quantification (LLOQ) for the analyte and 5% for the IS.

2.5.2. Carry-over

In accordance with the EMA guideline, carry-over was assessed by injecting blank calibration sample (0.00 mg/L) after the highest calibration sample (175 mg/L). Carry-over is acceptable if the peak area response in the blank sample obtained after measurement of the high-concentration sample is not >20% of the β-LA peak area response at the LLOQ, and 5% of the peak area response of the IS.

2.5.3. Lower limit of quantification

The EMA guideline defines the LLOQ as the lowest concentration at which the *S/N* ratio is 5 or more and that could be estimated with an acceptable inter-day imprecision (coefficient of variation ≤ 20%) and bias (≤20%).

To estimate the LLOQ, the plasma calibrator level 2 (1.00 mg/L) was not diluted, diluted 2-fold and diluted 5-fold with the blank plasma calibrator (0.00 mg/L). Each sample was processed repeatedly 10 times in one day, and in a single series per day for 20 nonconsecutive days. The calibration samples used were different from those calibration samples used to obtain the calibration curves.

2.5.4. Calibration curves

Nine-level calibration samples containing the nine β-LA were processed in duplicate once a day. Integration of smoothed peak areas and calculation of β-LA concentrations were performed with TargetLynx™ v 4.1 software (Waters, Milford, MA, USA). According to the EMA guideline, calculated concentrations of the calibration standards should all be within ± 15% of the nominal value, except for the LLOQ for which a ± 20% interval could be allowed.

The calibration curves were generated by linear or quadratic fit of the β-LA/IS standard area response ratio multiplied by IS concentration vs. β-lactam antibiotic concentration (1/X or $1/X^2$ weighting; excluding the option to force through the point of origin). According to Carlier et al. [19,23], to find the appropriate weighting factor and calibration model, the sum of the relative errors for different weighting factors and regression models were calculated. The procedure that gave the

Table 1
Mass spectrometry parameters for the β-lactam antibiotics and their internal standards.

Antibiotic	ESI mode	Retention time (min)	Quantification transition (*m/z*)	Confirmation transition (*m/z*)	Cone voltage (V)	Collision energies (eV)	MRM acquisition number
Amoxicillin	+	1.11	366.0 > 113.9	366.0 > 207.9	20	20 (13[a])	1 and 2[a] (0.50 to 1.20 min)
[D_4]-amoxicillin	+	1.11	370.0 > 113.9	–	20	20	1 (0.5 to 1.20 min)
Ampicillin	+	1.15	350.0 > 106.0	350.0 > 159.9	21	20 (13[a])	3 and 4[a] (1.05 to 2.00 min)
[D_5]-ampicillin	+	1.15	355.0 > 111.0	–	21	20	3 (1.05 to 2.00 min)
Cloxacillin	+	1.91	435.8 > 159.9	435.8 > 355.9	20	15 (10[a])	3 and 4[a] (1.05 to 2.00 min)
[$^{13}C_4$]-cloxacillin	+	1.91	439.9 > 159.9	–	20	15	3 (1.05 to 2.00 min)
Piperacillin	+	1.38	517.9 > 143.0	517.9 > 359.0	25	20 (15[a])	3 and 4[a] (1.05 to 2.00 min)
[D_5]-piperacillin	+	1.38	522.9 > 148.0	–	25	20	3 (1.05 to 2.00 min)
Cefepime	+	1.08	480.9 > 166.9	480.9 > 395.8	22	25 (15[a])	1 and 2[a] (0.50 to 1.20 min)
[D_3]-cefepime	+	1.08	483.9 > 166.9	–	22	25	1 (0.50 to 1.20 min)
Ceftazidime	+	1.11	546.9 > 467.9	546.9 > 166.9	20	12 (10[a])	1 and 2[a] (0.50 to 1.20 min)
[D_5]-ceftazidime	+	1.11	551.9 > 467.9	–	20	12	1 (0.50 to 1.20 min)
Cefuroxime	–	1.26	422.9 > 206.9	422.9 > 317.9	20	13 (15[a])	5 and 6[a] (0.50 to 2.00 min)
[D_3]-cefuroxime	–	1.26	425.9 > 210.0	–	20	13	5 (0.50 to 2.00 min)
Aztreonam	–	1.90	433.9 > 292.8	433.9 > 121.9	25	11 (15[a])	5 and 6[a] (0.50 to 2.00 min)
Carumonam	–	1.18	464.9 > 231.8	–	21	10	5 (0.50 to 2.00 min)
Meropenem	+	1.12	384.0 > 141.0	384.0 > 254.0	25	15 (15[a])	3 and 4[a] (1.05 to 2.00 min)
[D_6]-meropenem	+	1.12	390.0 > 147.0	–	25	15	3 (1.05 to 2.00 min)

ESI, electrospray ionization; *m/z*, mass-to-charge ratio; MRM, multiple reaction monitoring.
[a] Qualifier.

smallest sum of the relative errors was chosen as the most appropriate calibration model.

2.5.5. Imprecision and relative bias

Quality control samples were used to estimate intra- and inter-day imprecision and bias according to the following equations:

$$CV\% = \frac{s}{\bar{x}} \cdot 100.$$

$$\delta_r\% = \left(\frac{\bar{x}-\mu}{\mu}\right) \cdot 100$$

where CV, s, $\bar{x}$, δ_r and μ are the coefficient of variation, standard deviation, mean, relative bias and the conventional value, respectively. The reference value (conventional value) of the QC samples was assigned by weighing.

For intra- and inter-day imprecision and bias, 10 aliquots of each concentration were tested repeatedly in one day and in a single series per day, for 20 nonconsecutive days. Coefficient of variation and δ_r results were analyzed following the EMA acceptance criteria (15% for QC materials and 20% for LLOQ).

2.5.6. Dilution integrity

The dilution integrity experiments were performed to validate the dilution test to be carried out on drug concentrations beyond the calibration interval, which may be encountered during real subject sample analysis. For each β-LA, human drug-free plasma was spiked with the highest working standard solution (1750 mg/L) up to about two times the upper limit of quantification (highest plasma calibrator) and it was further diluted five- and ten-fold with drug-free plasma. The dilution integrity experiment was carried out analyzing six replicates of these samples after processing them following the extraction procedure described above. According to the EMA guideline, imprecision and bias should be within ±15%.

2.5.7. Recovery

For the recovery study, several β-LA-spiked samples were prepared (3.00, 30.0 and 120 mg/L). Recovery was calculated as the mean ratio between the peak area response of six replicates of these samples and the corresponding peak area response of equivalent neat samples. The recoveries of IS were similarly studied at the concentration of 2.50 mg/L. According to the CLSI-IFCC C50-A guideline [31], the variation in recovery among all concentrations should be <15%.

2.5.8. Matrix effect

According to the EMA guideline and Viswanathan et al. [32] the quantitative measure of the matrix effect can be termed as the matrix factor and defined as the ratio of the peak area response in the presence of the matrix (measured by analyzing a blank matrix spiked after extraction with analyte) to the peak area response in the absence of the matrix (pure solution of analyte):

$$\text{Matrix factor} = \frac{\text{Peak area response in presence of matrix components}}{\text{Peak area response in absence of matrix components}}$$

A matrix factor > 1 (or 100%) may be due to ion enhancement, and when it is <1 (or 100%) it may be due to ion suppression. Similarly, the IS can also experience ion enhancement or ion suppression.

Considering the matrix effects of the IS, an IS-normalized matrix factor was calculated by dividing the matrix factor of the β-LA by the matrix factor of the IS. To determine the variability of the matrix effect in samples from different individuals, the IS-normalized matrix factor was calculated in six different batches of plasma matrix at 3.00 mg/L, 30.0 mg/L and 120 mg/L. The matrix of IS was similarly studied but only one concentration was measured (2.50 mg/L).

According to the EMA, the variability in matrix effect as measured by the *CV* should be <15% and the variation in matrix effect among all concentrations should be <15%.

2.5.9. Stability

Stability studies included stock solution stabilities of β-LA and IS, extracted samples in-autosampler stability and short- and long-term stabilities for concentration of β-LA.

To evaluate the stability of stock solutions, the peak area response of the stock solutions refrigerated at (5 ± 3) °C for 1, 3 and 7 days and those kept at (−75 ± 3) °C for 6 months were compared with fresh stock at room temperature. The stability of extracted samples in the autosampler was tested by reinjecting them after 6 h, 12 h and 24 h storage at (4 ± 1) °C. To evaluate short-term stability, the aliquots for QC (3.00, 30.0 and 120 mg/L) were first stored at (5 ± 3) °C for 1, 3 and 7 days and then equilibrated to room temperature and extracted and tested against their fresh counterparts. For long-term stability evaluation, the aliquots for QC samples were first frozen at (−75 ± 3) °C for 6 months and then thawed before extraction and tested against fresh calibration and spiked samples.

All stability experiments were carried out using ten replicates of spiked samples against fresh calibration samples and the results were compared with the freshly spiked samples. The EMA guideline defines stable samples as those having a mean concentration at each level within ±15% of the nominal concentration.

2.6. Application to biological samples

Our UHPLC-MS/MS procedure was developed to be introduced into an institutional antimicrobial stewardship program (ASP) [33,34]. This ASP was approved by local Committee of our hospital and it included the administration of β-LA in CI or EI against difficult-to-treat infections as a routine clinical practice.

2.6.1. Patients and sample collection

We evaluated applicability of the UHPLC-MS/MS procedure by processing plasma samples from patients treated with β-LA therapy admitted in Infectious Diseases or Intensive Care Departments. All these patients suffered serious bacterial infections and were treated with some of the antibiotics included in the present study.

Blood samples were obtained during the period of 24–48 h after the beginning of β-LA in CI in order to assure that they represented concentrations at the steady-state condition. Approximately 3 mL of blood were collected in a lithium-heparin tube (Vacuette, Kremsmünster, Austria) and immediately refrigerated at 2–8 °C for a maximum of 30 min. Samples were then centrifuged at 2000*g* for 10 min at (4 ± 1) °C, aliquoted, and stored at (−75 ± 3) °C until analysis.

2.6.2. Microbiological studies

Microorganisms were identified using the MALDI-TOF Biotyper® measurement system (Bruker, Billerica, MA, USA).

Susceptibility studies were performed using the MicroScan automated microdilution measurement system (Dade International, West Sacramento, CA, USA). In addition, exact MIC values for each antibiotic administrated was measured by E-test® diffusion procedure (bioMérieux, Marcy-l'Étoile, France) on agar plate, according to the current CLSI guideline [35].

3. Results

3.1. Chromatography

Under the chromatographic conditions described above for the UHPLC-MS/MS procedure, β-LA eluted at retention times ranging

between 1.08 and 1.91 min (see Table 1). A typical MRM chromatogram for the lowest QC sample (3.00 mg/L) is shown in Fig. 1. The UHPLC-MS/MS run time was 3.5 min, including the time needed for the solvent gradient to return to baseline conditions before the next injection.

3.2. Validation data

3.2.1. Selectivity

The peak area responses observed in all plasma batches at AMX, AMP, CLX, PIP, FEP, CAZ, CXM, ATM and MEM retention times were ≤7.3%, ≤1.7%, ≤4.4%, ≤6.6%, ≤3.1%, ≤7.3%, ≤5.1% and ≤0.8% and ≤3.3% of the LOQ of AMX, AMP, CLX, PIP, FEP, CAZ, CXM, ATM and MEM, being <1.9% for AMX, 0.9% for AMP, 3.5% for CLX, 0.5% for PIP, 2.3% for FEP, 2.5% for CAZ, 3.0% for CXM, 1.2% for ATM and 1.1% for MEM at their respective IS retention time.

3.2.2. Carry-over

Peak area responses observed in the blank calibration sample after measurement of the highest calibration sample were ≤2.3%, ≤1.8%, ≤3.4%, ≤4.2%, ≤0.9%, ≤1.9%, ≤2.2%, ≤1.7% and ≤2.0% of the LLOQ of AMX, AMP, CLX, PIP, FEP, CAZ, CXM, ATM and MEM peak area response at the LLOQ, respectively. On the other hand, peak area responses were ≤0.9%, ≤0.7%, ≤1.0%, ≤0.4%, ≤1.3%, ≤1.0%, ≤1.3%, ≤2.1% and ≤1.1% of the peak area response of their respective IS.

3.2.3. Lower limits of quantification

Inter-day LLOQ were 0.56 mg/L (*S/N* ratio of 5.2) for AMX, 0.59 mg/L (*S/N* ratio of 6.0) for AMP, 0.52 mg/L (*S/N* ratio of 5.7) for CLX, 0.54 mg/L (*S/N* ratio of 5.6) for PIP, 0.58 mg/L (*S/N* ratio of 5.1) for FEP, 0.51 mg/L (*S/N* ratio of 5.2) for CAZ, 0.96 mg/L (*S/N* ratio of 6.6) for CMX, 0.55 mg/L (*S/N* ratio of 5.5) for ATM and 0.50 mg/L (*S/N* ratio of 5.5) for MEM. Data for intra-day and inter-day imprecision and relative bias at LLOQ are summarized in Tables 3 and 4.

3.2.4. Calibration curves

The calibration curves generated showed that quadratic regression with a weighting scheme of $1/X^2$ best described the data set generated for ATM, CXM, FEP and MEM. On the other hand, the best calibration model for AMX, AMP, CAZ, CLX and PIP was found to be linear regression using a weighting factor of 1/X.

The deviations of the calculated concentrations from their nominal values ranged from 2.2 to 14.2% for AMX, 5.7 to 14.9% for AMP, 1.1 to 10.7% for CLX, 0.6 to 9.8% for PIP, 2.2 to 12.9% for FEP, 3.3 to 11.2% for CAZ, 5.9 to 13.3% for CXM, 7.1 to 13.7% for ATM and 1.7 to 10.1% for MEM.

3.2.5. Imprecision and bias

Data for intra-day and inter-day imprecision and relative bias data are showed in Tables 3 and 4. The imprecision values ranged from 15.4% to 19.7% at LLOQ and from 7.5% to 13.3% at 3.00 mg/L, 5.1% to 9.1% at 30.0 mg/L and 2.0% to 6.2% at 120 mg/L. Relative bias absolute values ranged between 0.3% to 14.7% at 3.00 mg/L, 0.3% to 13.7% at 30.0 mg/L and 0.8% to 13.3% at 120 mg/L.

3.2.6. Dilution integrity

Imprecision values for dilution integrity, at five- and ten-fold dilution, were found to be, respectively, 3.3 and 5.4% for AMX, 5.7 and 6.6% for AMP, 3.5 to 7.7% for CLX, 5.3 and 6.6% for PIP, 5.1 and 5.9% for FEP, 4.4 to 8.8% for CAZ, 4.9 and 5.9% for CXM, 4.5 and 5.2% for ATM, and 4.7 and 5.6% for MEM. Relative bias values were −4.9 and −6.2% for AMX, −3.3 and −4.2% for AMP, −4.1 and −7.5% for CLX, −4.5 and −5.5% for PIP, −3.7 and −4.7% for FEP, −6.3 and −8.9% for CAZ, −5.3 and −6.1% for CXM, −5.1 and −6.2% for ATM, and −2.9 and −3.8% for MEM.

3.2.7. Recovery and matrix effect

Values for recovery, matrix factor, variability of matrix effect and IS-normalized matrix factor of β-LA at different concentrations are showed in Table 5. Evaluation of the matrix effect showed ion enhancement for all β-LA and their internal standards. The variation in recovery and matrix effect among all concentrations was <15%.

3.2.8. Stability

β-Lactam antibiotics concentrations in plasma were stable during storage at (5 ± 3) °C for a period of 3 days with absolute percent deviations (%D) from the nominal concentrations lower than 13.9%. On the other hand, β-LA concentrations in extracted samples were stable in

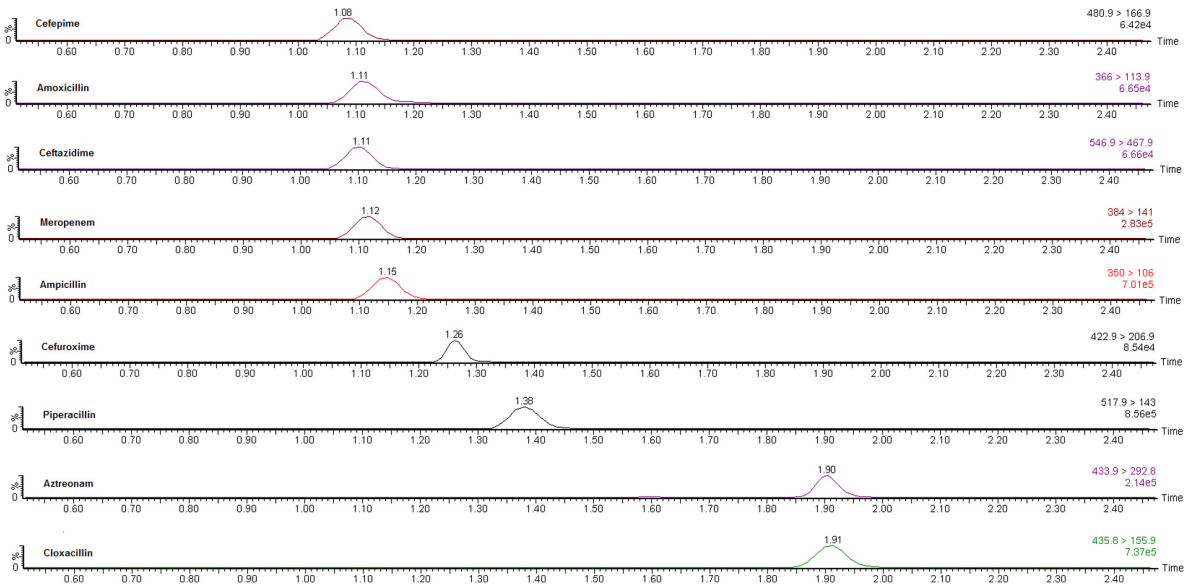

Fig. 1. Multiple reaction monitoring chromatograms of different antibiotics for a quality control sample at 3.00 mg/L.

the autosampler at (4 ± 1) °C for 12 h (absolute %D values ≤ 14.9%). Also, β-LA concentrations in plasma were stable at (−75 ± 3) °C for at least 6 months (absolute %D ≤ 9.9%). Stock solutions of β-LA and IS stored at (5 ± 3) °C were stable for 3 days (absolute %D values ≤ 14.4% and ≤13.9%, respectively) and at (−75 ± 3) °C for 6 months (absolute %D values ≤ 8.7% and 9.9%, respectively). Percent deviations were in all cases negative, indicating a loss of β-LA concentrations with regard to the nominal value, i.e., a decomposition or degradation of β-LA occurred.

3.3. Clinical application

β-Lactam antibiotics concentrations in plasma and MIC's values obtained in selected patients are shown in Table 2. As expected, these results were consistent with the patients' clinical situation (e.g., lack of fever, remission of infection, etc.) but β-LA concentrations were much higher than those recommended in most of the revised literature [1–9, 12] (T > MIC or 3–4 times the MIC values). These data could emphasize the need for TDM of the β-LA.

4. Discussion

The optimization of PK/PD parameters for β-LA when they are used in CI or EI may be essential in particularly difficult-to-treat scenarios. The CI or EI administration maintains the antibiotic concentration above the MIC for longer, thus leading to assure a T > MIC ~ 100% against susceptible microorganisms and to protect from the potential emergence of resistant strains. Also, this mode of administration allows to recover antimicrobial efficacy of β-LA against drug-resistant bacteria, which exhibit higher MIC values. However, the optimal dosage for β-LA in CI or EI has not been established, and also there is a great variability in β-LA plasma concentrations for an identical dose of a β-LA in particular situations. Taking all these considerations into account, TDM appears to be essential for guiding the CI or EI therapy with β-LA.

In the present study, we developed and validated an UHPLC-MS/MS procedure for simultaneous measurement of nine β-LA (AMX, AMP, CLX, PIP, FEP, CAZ, CXM, ATM, and MEM) concentration in plasma that can be used in CI or EI. This procedure could improve dose adjustment of β-LA in our hospital, especially in critically ill patients with unpredictable PK and those with bone and joint infections. Currently, it has been included within the ASP of our hospital.

4.1. Procedure development

Various combinations of mobile phase and reverse-phase UHPLC columns were tested to achieve a good resolution and symmetric peaks, a high response, a short retention time and better peak shape. Different mobile phases were evaluated to improve UHPLC separation and to enhance MS sensitivity. Several experiments were performed testing different mobile phases consisting of water, on one hand, and acetonitrile and methanol as organic phases on the other hand. All these mobile phases were combined with ammonium acetate, with formic acid at 0.1% (*v*/*v*) or with both additives. From all the possible combinations, that composed of water and acetonitrile and both with formic acid at 0.1% (*v*/*v*) offered the highest MS response. Two kinds of Bridget Ethyl Hybrid UPLC columns (Acquity® UPLC® BEH™ C_{18} reverse-phase columns) with the same particle size (1.7 μm) and internal diameter (2.1 mm) but with different length (50 mm vs. 100 mm) were evaluated. With the 50 mm-length BEH column, shorter retention times were obtained but β-LA presented wider peaks and worst peak shapes, probably, because they were near to the elution front. It was found that the use of an Acquity® UPLC® BEH™ C_{18} reverse-phase column, 2.1 × 100 mm; 1.7 μm, in combination with gradient mode of mobile phase, let us achieve the chromatographic conditions mentioned above. Other parameters such as column temperature, flow rate and injection volume were studied in order to get a fast and reliable separation, and the best results were obtained when 30 °C was used as column temperature (versus 40 °C or 50 °C), 0.4 mL/min as flow rate (better than 0.3 mL/min or 0.5 mL/min) and 10 μL were injected (versus 5 μL or 20 μL). Under all these conditions, retention times of all β-LA were constant and reproducible.

All MS parameters were optimized by direct injection of 10 mg/L of each β-LA and IS in an acetonitrile/water solution containing 0.1% formic acid (50/50 *v*/*v*) into the mass spectrometer at a flow rate of 10 μL/min. In our case, the most abundant ions obtained were the $[M + H]^+$ adducts in ESI + for AMX, AMP, CLX, PIP, FEP, CAZ, MEM

Table 2
Details of patients with any infection and their β-lactam antibiotic mass concentration in plasma and minimum inhibitory concentration values obtained.

Patient	Unit	Pathogen causing the infection	Antibiotic administrated	MIC (mg/L)	Dosage/frequency (g/h)	Administration route	Steady-state antibiotic mass concentration (mg/L)
1	IDD	*Enterococcus faecalis*	Ampicillin	0.750	2/24	CI	7.5
2	IDD	*Pseudomonas aeruginosa*	Aztreonam	6.00	4/24	CI	31.3
2	IDD	*Enterobacter cloacae*	Aztreonam	0.190	4/24	CI	31.3
3	IDD	*Pseudomonas aeruginosa*	Aztreonam	1.00	2/24	CI	21.7
3	IDD	*Enterobacter cloacae*	Aztreonam	2.00	2/24	CI	21.7
4	ICD	*Pseudomonas aeruginosa*	Aztreonam	2.00	2/24	CI	45.9
5	ICD	*Pseudomonas aeruginosa*	Aztreonam	2.00	2/24	CI	10.1
6	IDD	*Enterobacter cloacae*	Cefepime	0.094	3/24	CI	21.3
7	IDD	*Enterobacter cloacae*	Cefepime	0.120	2/24	CI	16.0
8	ICD	*Enterobacter cloacae*	Cefepime	0.380	4/24	CI	35.2
9	ICD	*Klebsiella pneumoniae*	Cefepime	0.250	4/24	CI	59.5
10	IDD	*Pseudomonas aeruginosa*	Ceftazidime	2.00	4/24	CI	20.0
10	IDD	*Proteus mirabilis*	Ceftazidime	0.064	4/24	CI	20.0
11	IDD	*Staphylococcus hominis*	Cloxacillin	0.250	8/24	CI	21.6
12	IDD	*Staphylococcus aureus*	Cloxacillin	0.250	8/24	CI	24.9
13	IDD	*Staphylococcus aureus*	Cloxacillin	0.250	6/24	CI	14.6
14	IDD	*Klebsiella pneumoniae*	Meropenem	0.030	2/8	EI	48.8
15	IDD	*Klebsiella pneumoniae*	Meropenem	0.030	2/8	EI	25.8
16	ICD	*Pseudomonas aeruginosa*	Meropenem	0.500	6/24	CI	29.4
17	ICD	*Klebsiella pneumoniae*	Meropenem	0.060	2/24	CI	34.1
18	IDD	*Enterobacter cloacae*	Piperacillin	2.00	10/24	CI	52.4
18	IDD	*Acinetobacter baumannii*	Piperacillin	1.00	10/24	CI	52.4
18	IDD	*Enterococcus faecalis*	Piperacillin	2.00	10/24	CI	52.4
19	ICD	*Klebsiella pneumoniae*	Piperacillin	2.00	12/24	CI	81.8
20	ICD	*Klebsiella pneumoniae*	Piperacillin	8.00	12/24	CI	36.5
20	ICD	*Pseudomonas aeruginosa*	Piperacillin	6.00	12/24	CI	36.5

CI, continuous infusion; EI, extended infusion; ICD, Intensive Care Department; IDD, Infectious Diseases Department; MIC, minimum inhibitory concentration.

Table 3
Intra-day imprecision and bias values obtained in the UHPLC-MS/MS measurement system for different β-lactam antibiotics mass concentration in plasma.

Quantity	LLOQ			QC1			QC2			QC3		
	$\bar{x}$ (mg/L)	*CV* (%)	δ_r (%)	$\bar{x}$ (mg/L)	*CV* (%)	δ_r (%)	$\bar{x}$ (mg/L)	*CV* (%)	δ_r (%)	$\bar{x}$ (mg/L)	*CV* (%)	δ_r (%)
P—amoxicillin; mass c.	0.53	16.3	6.2	3.44	9.7	14.7	33.4	6.6	11.4	130	4.2	8.5
P—ampicillin; mass c.	0.60	17.2	20.0	3.38	10.1	12.7	32.9	5.9	9.7	133	3.9	10.8
P—cloxacillin; mass c.	0.59	17.0	18.0	3.19	10.4	6.3	34.1	7.2	13.7	132	4.8	10.2
P—piperacillin; mass c.	0.58	15.4	16.0	3.33	7.5	11.0	31.5	5.3	5.1	130	2.0	8.5
P—cefepime; mass c.	0.54	16.6	8.8	3.41	9.2	13.7	33.7	6.2	12.3	136	2.8	13.3
P—ceftazidime; mass c.	0.55	15.6	10.2	3.22	8.5	7.3	31.1	5.5	3.7	128	2.3	6.7
P—cefuroxime; mass c.	1.04	16.9	4.4	3.06	7.9	2.0	30.9	6.4	3.0	121	3.4	0.8
P—aztreonam; mass c.	0.52	15.9	4.2	3.42	7.8	14.2	33.3	5.1	11.0	129	3.0	7.5
P—meropenem; mass c.	0.54	16.1	8.6	3.06	8.1	2.0	30.5	5.8	1.7	122	3.4	1.7

LLOQ, lower limit of quantification; QC1, internal quality control 1; QC2, internal quality control 2; QC3, internal quality control 3; $\bar{x}$, mean; *CV*, coefficient of variation; δ_r, relative bias. Quantities are described according to the IFCC and IUPAC recommendations [41]. P, plasma; mass c., mass concentration.

and their IS, and the $[M-H]^-$ adducts in ESI− for CXM, ATM and their respective IS. The choice of the monitored ions was based on β-LA MS/MS fragmentation pattern. All β-LA were quantified using the MRM mode due to its high-sensitivity data acquisition when the precursor and the product ions were monitored. To prevent analytes misidentification, and specifically to confirm the presence of the drugs and the absence of false contributions from similar components coeluted in the samples, two MRM transitions were followed for β-LA. One transition was used for quantification (the quantifier), and the other transition was monitored for identification (the qualifier) (see Table 1). The quantifier to qualifier ratio was used for peak identification based on criteria set forth by the CLSI C50-A [31] and C62-A [36] guidelines. Results report peak ratios of the two peaks which did not deviate from the average ratio in the standards by more than the 20%, indicating that there is no analyte misidentification. On the other hand, because there are several β-LA detected in ESI+ that co-eluted with β-LA with ESI−, polarity switching was not an adequate option and therefore, two injections were carried out, one to monitor the analytes detected in ESI+ and the second one for analytes in ESI− (see Table 1).

Although protein precipitation is not the best procedure to prevent the matrix effects, in our evaluation, the protein precipitation with an organic solvent as acetonitrile followed by a subsequent dilution with water containing 0.1% formic acid in a 1:3 proportion, simplified the extraction procedures published by others [22–24], and provided acceptable normalized matrix factors results.

Besides the simplicity of the sample preparation, the major advantage of our procedure is the possibility of measuring nine β-LA (the most used in our hospital in CI or EI) concentration in plasma in just two runs. Another advantage is a chromatographic run time of only 7.0 min per sample, which is equal or shorter than that of other procedures previously reported with a similar number of antibiotic analyzed [20,21,24,25]. Other studies [19,26] reported better chromatographic run times, what is not surprising if one takes into account that they detect antibiotics in only one ESI mode (ESI+). Although the combination of sample preparation and global chromatographic run time can offer just a moderate throughput, measurement of β-LA concentration could be combined with TDM of other drugs—which in our case will be immunosuppressant, antiepileptic, antitumor or antiviral drugs, on the same instrument and day.

4.2. Procedure validation

4.2.1. Selectivity

According to the EMA, a measurement procedure should be able to differentiate the analytes of interest and their IS from other possible components in the sample (e.g. concomitant drugs). So, unlike other reported procedures [20–22,24–26], a selectivity study should be performed using patient samples receiving other drugs. In our case, no interfering peaks were present in any plasma sample studied indicating that the proposed UHPLC-MS/MS procedure provides acceptable selectivity.

4.2.2. Carry-over

According to the EMA, carry-over should be addressed and minimized (if it exists) during a measurement procedure development. In contrast with other published procedures [20–25], a carry-over study was conducted. In our case, no carry-over was observed.

4.2.3. Lower limits of quantification

The LLOQ of the measurement procedure for each β-LA plasma concentration was near to 0.50 mg/L, except for concentration of CMX for which it was near to 1.00 mg/L. Taking into account that MIC for many bacteria are higher than 1.00 mg/L [37] and that patients included in our hospital protocol receiving a CI or EI administration rarely have low concentrations of β-LA in plasma, we considered that the LLOQ obtained were acceptable.

Furthermore, we obtained LLOQ results similar to others [19,21,23,25]. In other studies [20,22,24], their results were better, what is not

Table 4
Inter-day imprecision and bias values obtained in the UPLC-MS/MS measurement system for different β-lactam antibiotics mass concentration in plasma.

Quantity	LLOQ			QC1			QC2			QC3		
	$\bar{x}$ (mg/L)	*CV* (%)	δ_r (%)	$\bar{x}$ (mg/L)	*CV* (%)	δ_r (%)	$\bar{x}$ (mg/L)	*CV* (%)	δ_r (%)	$\bar{x}$ (mg/L)	*CV* (%)	δ_r (%)
P—amoxicillin; mass c.	0.56	18.1	12.2	3.31	11.9	10.3	32.1	7.8	7.0	125	4.8	4.2
P—ampicillin; mass c.	0.59	19.7	18.0	3.24	13.3	8.0	30.7	8.4	2.3	122	5.3	1.7
P—cloxacillin; mass c.	0.52	19.2	4.8	3.09	12.8	3.0	34.1	9.1	13.7	131	6.2	9.2
P—piperacillin; mass c.	0.54	16.9	8.4	3.13	8.9	4.3	31.5	6.7	5.0	124	3.5	3.3
P—cefepime; mass c.	0.58	18.5	16.2	3.15	12.2	5.0	31.7	8.6	5.7	131	5.7	9.2
P—ceftazidime; mass c.	0.51	17.3	2.4	3.36	10.8	12.0	33.4	7.7	11.3	129	5.0	7.3
P—cefuroxime; mass c.	0.96	17.7	−4.0	3.01	11.0	0.3	32.2	8.0	7.3	130	4.4	8.7
P—aztreonam; mass c.	0.55	16.4	10.4	3.05	9.9	1.7	30.1	7.2	0.3	122	4.0	2.0
P—meropenem; mass c.	0.50	18.1	0.8	3.22	10.1	7.3	30.9	7.4	3.0	126	4.9	4.6

LLOQ, lower limit of quantification; QC1, internal quality control 1; QC2, internal quality control 2; QC3, internal quality control 3; $\bar{x}$, mean; *CV*, coefficient of variation; δ_r, relative bias. Quantities are described according to the IFCC and IUPAC recommendations [41]. P, plasma; mass c., mass concentration.

Table 5
Recoveries, matrix factors and internal standard-normalized matrix factors obtained in the UHPLC-MS/MS measurement system for different β-lactam antibiotics mass concentration in plasma.

Quantity	Recovery (%)				Matrix factor (%)				IS-normalized matrix factor (%)		
	2.5 mg/L	3.00 mg/L	30.0 mg/L	120 mg/L	2.5 mg/L	3.00 mg/L	30.0 mg/L	120 mg/L	3.00 mg/L	30.0 mg/L	120 mg/L
P—amoxicillin; mass c.	–	62.2 (6.2)	65.5 (9.9)	70.8 (3.9)	–	124.6 (8.1)	127.6 (6.5)	129.9 (5.0)	100.5 (11.9)	102.8 (9.9)	104.6 (8.5)
P—[D_5]-amoxicillin; mass c.	59.6 (6.4)	–	–	–	124.7 (7.8)	–	–	–	–	–	–
P—ampicillin; mass c.	–	61.2 (8.0)	65.8 (8.2)	71.8 (4.1)	–	117.7 (7.8)	118.4 (7.4)	121.5 (4.1)	100.9 (10.7)	101.4 (9.3)	104.1 (10.8)
P—[D_4]-ampicillin; mass c.	60.6 (11.3)	–	–	–	117.4 (10.2)	–	–	–	–	–	–
P—cloxacillin; mass c.	–	60.6 (12.7)	64.0 (12.6)	68.6 (11.9)	–	119.7 (9.1)	122.5 (5.0)	123.1 (2.4)	100.8 (5.7)	103.4 (5.1)	104.0 (6.1)
P—[$^{13}C_4$]-cloxacillin; mass c.	59.3 (11.4)	–	–	–	118.7 (6.5)	–	–	–	–	–	–
P—piperacillin; mass c.	–	67.2 (14.0)	69.8 (5.9)	72.0 (6.0)	–	121.5 (11.8)	122.8 (6.7)	124.1 (5.4)	100.2 (5.8)	102.2 (11.9)	103.3 (11.8)
P—[D_5]-piperacillin; mass c.	67.6 (4.5)	–	–	–	121.2 (10.0)	–	–	–	–	–	–
P—cefepime; mass c.	–	77.5 (9.4)	80.8 (10.4)	84.8 (13.5)	–	122.2 (7.2)	126.9 (9.2)	129.6 (8.3)	102.9 (11.1)	106.8 (12.8)	109.5 (14.7)
P—[D_3]-cefepime; mass c.	77.0 (14.0)	–	–	–	119.4 (7.6)	–	–	–	–	–	–
P—ceftazidime; mass c.	–	68.0 (13.2)	72.0 (14.9)	77.4 (13.1)	–	132.3 (8.6)	133.3 (9.9)	138.4 (5.1)	100.9 (8.9)	101.9 (13.0)	105.5 (4.2)
P—[D_5]-ceftazidime; mass c.	69.3 (13.7)	–	–	–	131.3 (5.1)	–	–	–	–	–	–
P—cefuroxime; mass c.	–	67.7 (11.8)	71.7 (10.8)	72.7 (11.9)	–	105.7 (10.3)	109.0 (7.6)	111.3 (8.8)	104.2 (11.9)	105.9 (6.8)	108.4 (11.7)
P—[D_3]-cefuroxime; mass c.	67.2 (10.4)	–	–	–	102.1 (10.8)	–	–	–	–	–	–
P—aztreonam; mass c.	–	72.4 (11.7)	75.3 (7.1)	78.4 (5.4)	–	103.4 (6.2)	111.1 (6.7)	117.7 (8.2)	99.3 (10.8)	106.7 (12.1)	112.8 (10.3)
P—Carumonam; mass c.	74.6 (13.6)	–	–	–	104.8 (7.9)	–	–	–	–	–	–
P—meropenem; mass c.	–	55.7 (10.1)	56.2 (9.3)	59.2 (6.7)	–	133.6 (9.5)	137.5 (6.8)	140.5 (10.1)	102.0 (11.6)	105.1 (11.1)	107.1 (11.2)
P—[D_6]-meropenem; mass c.	53.4 (12.0)	–	–	–	131.6 (7.5)	–	–	–	–	–	–

IS, internal standard.
Coefficients of variation (%) between patients are indicated in brackets.
Quantities are described according to the IFCC and IUPAC recommendations [41]. P, plasma; mass c., mass concentration.

surprising if one takes into account that they used a solid-phase extraction or protein precipitation combined with liquid-liquid extraction which are cleaner and allow lower LLOQ.

4.2.4. Imprecision and bias

The imprecision and bias values, for each concentration, were found to neither exceed the 15% for QC samples nor the 20% for LLOQ, thus conforming to the EMA criteria, and were similar or better to those of previous publications [19–26]. These results indicate that the proposed UHPLC-MS/MS procedure provides acceptable precision and trueness.

4.2.5. Dilution integrity

According to the EMA, a dilution integrity study should be performed when a patient sample result is higher than the upper limit of quantification. This consideration may arise when we process samples from critically ill patients with, for example, acute kidney injury. In contrast with other published procedures [19–26], we conducted a dilution integrity study. In our case, the imprecision and bias values obtained, for each dilution, were found to neither exceed the 15%, thus conforming to EMA criteria.

4.2.6. Recovery and matrix effect

According to the results obtained, recoveries could be considered constant and reproducible and, consequently, acceptable.

The evaluation and the variability of the matrix effect in samples from different individuals are crucial aspects. These two issues are often not properly studied and could compromise the analysis of the experimental data. An ideal IS should be a structural analogue or a stable labeled compound, should track the analyte during the extraction and compensate for any analyte on the column and any inconsistent response. We used IS-stable labeled compounds for all β-LA, except for ATM, for which we used a chemical structural analogue with similar physico-chemical properties. Due to problems of availability at the moment of purchase and the high price of stable labeled compound, we used carumonam for ATM as IS. Although they did not elute simultaneously — with a risk for lack of compensation of matrix effect — we observed that the concentrations of the three samples assayed showed a steady value, given that the use of carumonam as IS did compensate for the ion enhancement observed in the AZT. On the other hand, carumonam is unlikely to be co-administered with AZT. For all these reasons, we considered carumonam as adequate IS. With regard to the other β-LA, we observed that the concentration of the three samples assayed showed a steady value in our evaluation of the matrix effect, given that the use of these IS compensates for the matrix effect observed in the measurement of β-LA concentrations.

4.2.7. Stability

In our stability studies, β-LA concentrations in plasma were stable at (5 ± 3) °C for a period of 3 days and in extracted samples in the autosampler at (4 ± 1) °C for 12 h. The poor stability of concentration of β-LA in biological fluids, at room temperature or refrigerated, is well known [38–40]. For this reason, precautions should be taken to prevent β-LA decomposition in the processed samples (i.e., reconstituted extracts in HPLC vials) left at room temperature in the

autosampler rack. The storage time of HPLC vials in the autosampler rack at room temperature should therefore be minimized and the samples placed in the temperature-controlled autosampler just prior to the analysis.

5. Conclusions

In conclusion, we developed a single UHPLC-MS/MS procedure for simultaneous measurement of nine β-LA concentrations in plasma, and validated it following international recommendations. The specificity of tandem spectrometry allows the measurement of different β-LA plasma concentrations with minimal preparation, and the sensitivity of the detector allows the use of small sample volumes. Additionally, considering time of analysis, versatility, flexibility and analytical performance characteristics of selectivity, capability of detection, precision, trueness, recovery and matrix effect, the mentioned procedure is well suited to routine hospital practice for TDM of β-LA in different patients, as are critically ill patients and patients with bone and joint infections.

Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cca.2017.03.009.

References

[1] M. Grupper, J.L. Kuti, D.P. Nicolau, Continuous and prolonged intravenous β-lactam dosing: implications for the clinical laboratory, Clin. Microbiol. Rev. 29 (2016) 759–772.
[2] W.A. Craig, Introduction to pharmacodynamics, in: A.A. Vinks, H. Derendorf, J.W. Mouton (Eds.), Fundamentals of Antimicrobial Pharmacokinetics and Pharmacodinamics, Part I, Springer, New York 2014, pp. 3–22.
[3] J.A. Roberts, R. Norris, D.L. Paterson, J.H. Martin, Therapeutic drug monitoring of antimicrobials, Br. J. Clin. Pharmacol. 73 (2012) 27–36.
[4] M.A. Kohanski, D.J. Dwyer, J.J. Collins, How antibiotics kill bacteria: from targets to networks, Nat. Rev. Microbiol. 8 (2010) 423–435.
[5] T.P. Lodise, B.M. Lomaestro, G.L. Drusano, Application of antimicrobial pharmacodynamic concepts into clinical practice: focus on beta-lactam antibiotics: insights from the Society of Infectious Diseases Pharmacists, Pharmacotherapy 26 (2006) 1320–1332.
[6] S.H. MacVane, J.L. Kuti, D.P. Nicolau, Prolonging β-lactam infusion: a review of the rationale and evidence, and guidance for implementation, Int. J. Antimicrob. Agents 43 (2014) 105–113.
[7] I.P. Korbila, G.S. Tansarli, D.E. Karageorgopoulos, K.Z. Vardakas, M.E. Falagas, Extended or continuous versus short-term intravenous infusion of cephalosporins: a meta-analysis, Expert Rev. Anti-Infect. Ther. 11 (2013) 585–595.
[8] A.E. Muller, J.W. Mouton, Continuous infusion of beta-lactam antibiotics, in: A.A. Vinks, H. Derendorf, J.W. Mouton (Eds.), Fundamentals of Antimicrobial Pharmacokinetics and Pharmacodinamics, Part II, Springer, New York 2014, pp. 223–256.
[9] V.H. Tam, Suppressing resistance development, in: A.A. Vinks, H. Derendorf, J.W. Mouton (Eds.), Fundamentals of Antimicrobial Pharmacokinetics and Pharmacodinamics, Part I, Springer, New York 2014, pp. 135–152.
[10] European Centre for Disease Prevention and Control, Antimicrobial resistance surveillance in Europe 2014, Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2015 Available at http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1400 (accessed 01.06.16).
[11] World Health Organization, Antimicrobial Resistance: Global Report on Surveillance 2014Available at http://www.who.int/drugresistance/documents/surveillancereport/en/ 2014 (accessed 01.06.16).
[12] A. Huttner, S. Harbarth, W.W. Hope, J. Lipman, J.A. Roberts, Therapeutic drug monitoring of the β-lactam antibiotics: what is the evidence and which patients should we be using it for? J. Antimicrob. Chemother. 70 (2015) 3178–3183.
[13] T. Legrand, D. Vodovar, N. Tournier, N. Khoudour, A. Hulin, Simultaneous determination of eight β-lactam antibiotics amoxicillin, cefazolin, cefepime, cefotaxime, ceftazidime, cloxacillin, oxacillin, piperacillin in human plasma using ultra-high performance liquid chromatography with ultraviolet detection, Antimicrob. Agents Chemother. 60 (2016) 4734–4742.
[14] F. Wolff, G. Deprez, L. Seyler, F. Taccone, M. Hites, B. Gulbis, J.-L. Vincent, F. Jacobs, F. Cotton, Rapid quantification of six β-lactams to optimize dosage regimens in severely septic patients, Talanta 103 (2013) 153–160.
[15] M.-C. Verdier, O. Tribut, P. Tattevin, Y. Le Tulzo, C. Michelet, D. Bentué-Ferrer, Simultaneous determination of 12 beta-lactam antibiotics in human plasma by high-performance liquid chromatography with UV detection: application to therapeutic drug monitoring, Antimicrob. Agents Chemother. 55 (2011) 4873–4879.
[16] B.C. McWhinney, S.C. Wallis, T. Hillister, J.A. Roberts, J. Lipman, J.P.J. Ungerer, Analysis of 12 beta-lactam antibiotics in human plasma by HPLC with ultraviolet detection, J. Chromatogr. B 878 (2010) 2039–2043.
[17] E. Nemutlu, S. Kir, D. Katlan, M.S. Beksaç, Simultaneous multiresponse optimization of an HPLC method to separate seven cephalosporins in plasma and amniotic fluid: application to validation and quantification of cefepime, cefixime and cefoperazone, Talanta 80 (2009) 117–126.
[18] R. Denooz, C. Charlier, Simultaneous determination of five beta-lactam antibiotics (cefepime, ceftazidime, cefuroxime, meropenem and piperacillin) in human plasma by high-performance liquid chromatography with ultraviolet detection, J. Chromatogr. B 864 (2008) 161–167.
[19] M. Carlier, V. Stove, J.J. De Waele, A.G. Verstraete, Ultrafast quantification of β-lactam antibiotics in human plasma using UPLC-MS/MS, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 978–979 (2015) 89–94.
[20] F.B. Sime, M.S. Roberts, J.A. Roberts, T.A. Robertson, Simultaneous determination of seven β-lactam antibiotics in human plasma for therapeutic drug monitoring and pharmacokinetic studies, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 960 (2014) 134–144.
[21] R. Cazorla-Reyes, R. Romero-González, A.G. Frenich, M.A. Rodríguez Maresca, J.L. Martínez Vidal, Simultaneous analysis of antibiotics in biological samples by ultra high performance liquid chromatography-tandem mass spectrometry, J. Pharm. Biomed. Anal. 89 (2014) 203–212.
[22] P. Colin, L. De Bock, H. T'jollyn, K. Boussery, J. Van Bocxlaer, Development and validation of a fast and uniform approach to quantify β-lactam antibiotics in human plasma by solid phase extraction-liquid chromatography-electrospray-tandem mass spectrometry, Talanta 103 (2013) 285–293.
[23] M. Carlier, V. Stove, J.A. Roberts, E. Van de Velde, J.J. De Waele, A.G. Verstraete, Quantification of seven β-lactam antibiotics and two β-lactamase inhibitors in human plasma using a validated UPLC-MS/MS method, Int. J. Antimicrob. Agents 40 (2012) 416–422.
[24] T. Ohmori, A. Suzuki, T. Niwa, H. Ushikoshi, K. Shirai, S. Yoshida, S. Ogura, Y. Itoh, Simultaneous determination of eight β-lactam antibiotics in human serum by liquid chromatography-tandem mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 879 (2011) 1038–1042.
[25] M. Cohen-Wolkowiez, N.R. White, A. Bridges, D.K. Benjamin Jr., A.D. Kashuba, Development of a liquid chromatography-tandem mass spectrometry assay of six antimicrobials in plasma for pharmacokinetic studies in premature infants, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 879 (2011) 3497–3506.
[26] M.J. Ashman, E.D. Wildschut, D. Tibboel, R.A. Mathot, Microanalysis of β-lactam antibiotics and vancomycin in plasma for pharmacokinetic studies in neonates, Antimicrob. Agents Chemother. 53 (2009) 75–80.
[27] M. Gumustas, S. Kurbanoglu, B. Uslu, S.A. Ozcan, UPLC versus HPLC on drug analysis: advantageous, applications and their validation parameters, Chromatographia 76 (2013) 1365–1427.
[28] L. Novakova, L. Matysova, P. Solich, Advantages of application of UPLC in pharmaceutical analysis, Talanta 68 (2006) 908–918.
[29] M.I. Churchwell, N.C. Twaddle, L.R. Meeker, D.R. Doerge, Improving LC-MS sensitivity through increases in chromatographic performance: comparisons of UPLC-ES/MS/MS to HPLC-ES/MS/MS, J. Chromatogr. B 825 (2005) 134–143.
[30] European Medicines Agency, EMA, Guideline on Bioanalytical Method Validation (EMEA/CHMP/EWP/192217/2009) Available at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf 2011 (accessed 01.06.16).
[31] Clinical and Laboratory Standards Institute, International Federation of Clinical Chemistry and Laboratory Medicine, Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance (CLSI C50-P), CLSI, Wayne, Pennsylvania, 2007.
[32] C.T. Viswanathan, S. Bansal, B. Booth, A.J. De Stefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays, Pharm. Res. 24 (2007) 1962–1973.
[33] Society for Healthcare Epidemiology of America, Infectious Diseases Society of America, Pediatric Infectious Diseases Society, Policy statement on antimicrobial stewardship by the Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA), and the Pediatric Infectious Diseases Society (PIDS), Infect. Control Hosp. Epidemiol. 33 (2013) 322–327.
[34] T.F. Barlam, S.E. Cosgrove, L.M. Abbo, C. MacDougall, A.N. Schuetz, E.J. Septimus, A. Srinivasan, T.H. Dellit, Y.T. Falck-Ytter, N.O. Fishman, C.W. Hamilton, T.C. Jenkins, P.A. Lipsett, P.N. Malani, L.S. May, G.J. Moran, N.M. Neuhauser, J.G. Newland, C.A. Ohl, M.H. Samore, S.K. Seo, K.K. Trivedi, Implementing an Antibiotic Stewardship Program: guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America, Clin. Infect. Dis. 62 (2016) e51–e77.
[35] Clinical and Laboratory Standard Institute, Liquid Chromatography-mass Spectrometry Methods; Approved Guideline (CLSI C62-A), CLSI, Wayne, Pennsylvania, 2014.
[36] Clinical and Laboratory Standard Institute, Performance Standards for Antimicrobial Susceptibility Testing, Twenty-sixth Edition, (CLSI M100-S26), CLSI, Wayne, Pennsylvania, 2016.
[37] European Committee on Antimicrobial Susceptibility Testing, European Society of Clinical Microbiology and Infectious Diseases, Antimicrobial Wild Type Distributions of Microorganisms, ESCMIDAvailable at http://mic.eucast.org/Eucast2/SearchController/search.jsp?action=init 2016 (accessed 01.06.16).

[38] J. Zander, B. Maier, M. Zoller, G. Döbbeler, L. Frey, D. Teupser, M. Vogeser, Effects of biobanking conditions on six antibiotic substances in human serum assessed by a novel evaluation protocol, Clin. Chem. Lab. Med. 54 (2016) 265–274.
[39] M. Carlier, J.J. De Waele, A.G. Verstraete, V. Stove, Exploration of the preanalytical stability of β-lactam antibiotics in plasma and blood—implications for therapeutic drug monitoring and pharmacokinetic studies, Clin. Chem. Lab. Med. 53 (2015) e227–e230.
[40] P.C. Van Krimpen, W.P. Van Bennekom, A. Bult, Penicillins and cephalosporins, physicochemical properties and analysis in pharmaceutical and biological matrices, Pharm. Weekbl. Sci. S9 (1987) 1–23.
[41] U. Magdal, R. Dybkær, R.H. Olesen, Properties and units in the clinical laboratory sciences, part XXIII, the NPU terminology, principles, and implementation: a user's guide (IUPAC technical report), Pure Appl. Chem. 84 (2012) 137–165.

Beta-lactams in continuous infusion for difficult-to-treat osteoarticular infections caused by Gram-negative bacilli: validation of an easy method for clinical use.

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Running title: Beta-lactams in continuous infusion for bone infections.

Keywords: Beta-lactams, Continuous infusion, Biofilm-related infections, Osteoarticular infections, Gram-negative bacilli, Antibiotic plasma levels

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ABSTRACT

We used ceftazidime, aztreonam and piperacillin-tazobactam in continuous infusion (CI) in a prospectively collected cohort of patients (n=24) for difficult-to-treat Gram-negative bacilli osteoarticular infections, and aimed to validate an easy-to-use method to guide its dosage (Daily dose=24h-Total Body Clearance X target “steady-state” concentration). The plasma observed concentration (UPLC-MS/MS) was higher overall than predicted concentration by formula (*Spearman* correlation: rho=0.6, *P*=0.005). The simple method applied may be useful for planning the dosage of beta-lactams in CI.

Beta-lactams (BL) have traditionally been treated with standard intermittent bolus (IB) administration, to achieve a time above the pathogen's MIC (T>MIC) of 40-60% (1, 2) and a peak concentration that often exceeds the established maximum killing rate cutoff (3-4 timesxMIC) (1, 3, 4). However, a longer T>MIC may be needed in difficult-to-treat scenarios (5, 6), and that could be assured by using BL in continuous infusion (BL-CI). BL-CI administration may achieve T>MIC≈100% and also recover the antimicrobial efficacy against multidrug-resistant (MDR) bacteria, which exhibit high MIC values (7-10). The ideal dosage for CI is not well defined, but using the same BL total dose than in IB may pose a risk of overdosing (11). In biofilm-related osteoarticular infections (OAI), CI might improve the questioned effectiveness of BL (12); however, little previous experiences exist (13). In this study we analysed cases of Gram-negative bacilli (GNB) OIA treated with BL-CI during our clinical practice, and aimed to validate an easy-to-use method to guide its doses.

The research was conducted in accordance with the Declaration of Helsinki and approved by Hospital Universitari de Bellvitge Ethics Committee (Barcelona). It is a retrospective analysis of a prospectively collected cohort (April 2012- December 2015). All patients received BL-CI (ceftazidime, aztreonam and piperacillin-tazobactam). BL were used in combination according to our protocol (ciprofloxacin, in cases of susceptible *P. aeruginosa*, and colistin in quinolone-resistant GNB).

To calculate the dosage of BL-CI, we considered that daily dose is directly related to the BL-Total Body Clearance (TBC) and the desired target concentration, as defined from the following Equation (3, 4):

Daily dose (mg)= 24(h) X TBC$^{(1)}$ *(L/h) X target "steady-state" concentration*$^{(2)}$ *(*C_{ss}*,mg/L)*.

62 [(1)]For ceftazidime TBC, which is basically cleared by glomerular filtration, we used
63 patient's creatinine clearance (CrCL, calculated using the *Cockcroft-Gault* formula) (14).
64 For piperacillin and aztreonam, which have renal (glomerular filtration and active
65 tubular secretion) and non-renal clearance, we used piperacillin- or aztreonam-TBC
66 values previously reported 15, 16).

67 [(2)]C_{ss} changes for each strain (3-4 times x MIC).

68 The Daily dose was calculated to reach this C_{ss}, avoiding concentrations above 100mg/L
69 for safety reasons (17). When this "Theoretical daily dose" represented a significant
70 reduction in comparison with the usual daily dosage by IB we administered a dose
71 considered more appropriate (named "Real Dose"); this especially happened at the
72 beginning of the study and due to our inexperience. Using the above *Equation*, we
73 calculated the predicted concentration (C_{pred}) for a specific administered Real Dose, as
74 follows:

75 - C_{pred} (mg/L) = Daily dose (mg/24h)/ TBC (L/h)
76 where, Daily dose refers to the Real Dose administered to the patient.

77 We correlated our predicted concentration (C_{pred}) with the patient's observed
78 concentration (C_{obs}), using the *Spearman's* rank correlation coefficient.
79 In order to determine serum BL C_{ss}, blood samples were taken at least 24h after the
80 start of therapy (26), they were immediately centrifuged and frozen at -80ºC until
81 analysis. Plasma concentrations of all patients were measured together (and double-
82 checked in different days) afterwards by UPLC-MS/MS following our methodology
83 previously described (18)

Out of 27 patients, 3 were excluded due to methodological inconveniences in the measurement of BL concentration. Finally, we included 24 patients: 11 osteomyelitis, 10 prosthetic joint or arthrodesis infections and three septic arthritis [median age: 66 years (IQR 54-75), 14 (58.3%) women, and 15 (66.6%) with renal impairment (CrCL<90mL/min)]. Ceftazidime (14 cases), aztreonam (seven), and piperacillin-tazobactam (three) were used to treat mainly *P. aeruginosa* OAI (21 cases, 87.5%; 9 MDR), with a median treatment duration of 34.5 days (IQR 20.3-42). BL was combined with ciprofloxacin (five cases), and with colistin (twelve cases, nine were BL-resistant). Resistant strains required higher doses than susceptible ones: ceftazidime (median dose-grams-/24h, IQR) 6 (4-6) versus 4.5, and aztreonam 5.5 (4.3-6) versus 3, respectively. This therapy was well tolerated and only one case, which was treated with ceftazidime (6 Grams/24hours– $C_{ss:}$ 50.9 mg/L), presented a *Clostridium difficile* colitis that was cured with metronidazole and a reduction in ceftazidime dosage. Twenty-four patients underwent concomitant surgery (debridement or implant removal). Finally, all patients except one (who required a supracondylar amputation), were clinically cured after a median follow-up of 18.4 months (IQR 10-32).

In total we had 37 antibiotic plasma determinations: 24 initial (Table 1) and 13 monitoring levels. The C_{obs} were higher than the C_{pred} in cases with normal renal function [ΔC_{obs}-C_{pred} –percentage- (**$\%\Delta_{Conc}$**): from 19% to 54%], and it was more variable with renal impairment (from -33% to +31%). *Spearman* correlation between C_{pred} and C_{obs} was: rho=0.6 (*P*=0.005), for all BL; and rho=0.8 (*P*<0.001), for ceftazidime exclusively (Figure 1A,B). This correlation was better for patients with weigh<75kg (rho 0.6) than for those weighting ≥75kg (rho 0.3) (Figure 1A).

The optimized use of BL-CI may be essential in difficult-to-treat scenarios (3, 8), such as GNB-OAIs. In our experience, this therapy was safe, achieved drug concentrations above the MIC for longer, and allowed the treatment of BL resistant strains. Although we can't conclude about antimicrobial efficacy, mainly due to the lack of a comparative treatment and the use of concomitant antibiotics or surgery, these results encourage further studies to confirm the potential benefits of BL-CI based on their pharmacodynamic properties and synergisms with other therapies.

The dosages of BL-CI need to be defined (3, 4). We show a simple way to estimate those dosages and the BL plasma levels in the early hours of treatment; thus, it could be useful for clinicians since therapeutic drug monitoring (TDM) for BL is usually not applied in routine practice. Nevertheless, C_{obs} was higher overall than the C_{pred}, probably because the established BL clearance values were not perfectly adjusted to our population cohort. Newer sophisticated pharmacokinetic models, if developed in the field of OAI, might better represent the nonlinear pharmacokinetic of some BL (19, 20). Overall, clinicians should be very cautious when using these formulas for different BLs or patient's features (weight or renal function).

To conclude, the use of BL-CI was safe, and its efficacy should be further evaluated in OAIs. A simple equation may be useful for planning BL-CI dosage and estimating BL concentration in the early hours of treatment. However, TDM is advisable and population pharmacokinetic models could improve the clinical management.

ACKNOWLEDGEMENTS

We thank Michael Maudsley for helping with the English in this manuscript.

All authors have no conflicts of interest to disclose.

FUNDING

This work was supported by Ministerio de Economía y Competitividad, Instituto de Salud Carlos III—co-financed by European Development Regional Fund '*A way to achieve Europe'* ERDF, Spanish Network for Research in Infectious Diseases (REIPI RD12/0015). A. R. was supported by a research grant from the Bellvitge Biomedical Research Institute (IDIBELL).

REFERENCES

1. **Craig WA**. 1998. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis **26**:1-10.

2. **Drusano GL**. 2004. Antimicrobial pharmacodynamics: critical interactions of "bug and drug". Nat Rev Microbiol **2**:289–300.

3. **Mouton JW, Vinks AA**. 2007. Continuous infusion of beta-lactams. Curr Opin Crit Care **13**:598–606.

4. **Mouton JW, Vinks AA**. 1996. Is continuous infusion of beta-lactam antibiotics worthwhile? Efficacy and pharmacokinetic considerations. J Antimicrob Chemother **38**:5–15.

5. **McKinnon PS, Paladino JA, Schentag JJ**. 2008. Evaluation of area under the inhibitory curve (AUIC) and time above the minimum inhibitory concentration (T>MIC) as predictors of outcome for cefepime and ceftazidime in serious bacterial infections. Int J Antimicrob Agents **31**:345–351.

6. **Van Herendael B, Jeurissen A, Tulkens PM, Vlieghe E, Verbrugghe W, Jorens PG, Ieven M**. 2012. Continuous infusion of antibiotics in the critically ill: The new holy grail for beta-lactams and vancomycin? Ann Intensive Care **2**:22.

7. **Mohd Hafiz A-A, Staatz CE, Kirkpatrick CMJ, Lipman J, Roberts JA**. 2012. Continuous infusion vs. bolus dosing: implications for beta-lactam antibiotics. Minerva Anestesiol **78**:94–104.

8. **Roberts JA, Paratz J, Paratz E, Krueger WA, Lipman J**. 2007. Continuous infusion

of beta-lactam antibiotics in severe infections: a review of its role. Int J Antimicrob Agents **30**:11–18.

9. **Dulhunty JM, Roberts JA, Davis JS, Webb SAR, Bellomo R, Gomersall C, Shirwadkar C, Eastwood GM, Myburgh J, Paterson DL, Lipman J**. 2013. Continuous infusion of beta-lactam antibiotics in severe sepsis: a multicenter double-blind, randomized controlled trial. Clin Infect Dis **56**:236–244.

10. **Roberts JA, Abdul-Aziz M-H, Davis JS, Dulhunty JM, Cotta MO, Myburgh J, Bellomo R, Lipman J**. 2016. Continuous versus Intermittent β-Lactam Infusion in Severe Sepsis. A Meta-analysis of Individual Patient Data from Randomized Trials. Am J Respir Crit Care Med **194**:681–691.

11. **Huttner A, Harbarth S, Hope WW, Lipman J, Roberts JA**. 2015. Therapeutic drug monitoring of the β-lactam antibiotics: what is the evidence and which patients should we be using it for?. J Antimicrob Chemother **70**:3178-3183.

12. **Gilbert P, Brown MR**. 1998. Biofilms and beta-lactam activity. J Antimicrob Chemother **41**:571–572.

13. **Ribera A, Benavent E, Lora-Tamayo J, Tubau F, Pedrero S, Cabo X, Ariza J, Murillo O**. 2015. Osteoarticular infection caused by MDR Pseudomonas aeruginosa: the benefits of combination therapy with colistin plus β-lactams. J Antimicrob Chemother **70**:3357–3365.

14. **Cockcroft DW, Gault MH**. 1976. Prediction of creatinine clearance from serum creatinine. Nephron **16**:31–41.

15. **Xu H, Zhou W, Zhou D, Li J, Al-Huniti N.** 2017. Evaluation of aztreonam dosing

regimens in patients with normal and impaired renal function: a population pharmacokinetic modeling and Monte Carlo simulation analysis. J Clin Pharmacol **57**:336-344.

16. **Hayashi Y, Roberts JA, Paterson DL, Lipman J.** 2010. Pharmacokinetic evaluation of piperacillin-tazobactam. Expert Opin Drug Metab Toxicol **6**:1017-1031.

17. **Moriyama B, Henning SA, Neuhauser MM, Danner RL, Walsh TJ**. 2009. Continuous-infusion beta-lactam antibiotics during continuous venovenous hemofiltration for the treatment of resistant gram-negative bacteria. Ann Pharmacother **43**:1324–1337.

18. **Rigo-Bonnin R, Ribera A, Arbiol-Roca A, Cobo-Sacristan S, Padulles A, Murillo O, Shaw E, Granada R, Perez-Fernandez XL, Tubau F, Alia P.** 2017. Development and validation of a measurement procedure based on ultra-high performance liquid chromatography-tandem mass spectrometry for simultaneous measurement of beta-lactam antibiotic concentration in human plasma. Clin Chim Acta **10**:215-224.

19. **Georges B, Conil J-M, Seguin T, Ruiz S, Minville V, Cougot P, Decun J-F, Gonzalez H, Houin G, Fourcade O, Saivin S**. 2009. Population pharmacokinetics of ceftazidime in intensive care unit patients: influence of glomerular filtration rate, mechanical ventilation, and reason for admission. Antimicrob Agents Chemother **53**:4483–4489.

20. **Roberts JA, Abdul-Aziz MH, Lipman J, Mouton JW, Vinks AA, Felton TW, Hope WW, Farkas A, Neely MN, Schentag JJ, Drusano G, Frey OR, Theuretzbacher U,**

Kuti JL, International Society of Anti-Infective Pharmacology and the Pharmacokinetics and Pharmacodynamics Study Group of the European Society of Clinical Microbiology and Infectious Diseases. 2014. Individualised antibiotic dosing for patients who are critically ill: challenges and potential solutions. Lancet Infect Dis **14**:498–509.

TABLE 1. Patient characteristics, microorganisms details, antibiotic dose and plasma concentration of patients treated with ceftazidime (n=17)

Case/ ATB	Patient characteristics		Microorganisms details			Antibiotic dose (mg/24h)		Antibiotic plasma concentration (mg/L)		
	CrCl	Weight (kg)	Microorganism	MIC (mg/L)	Susceptibility	TimesxMIC, Theoretical dose	Real dose	C_{pred}	C_{obs}	$\%\Delta_{Conc}$
1/CAZ	118.08	80	PA	2	S	4×2, 1360	7000	41.17	70.40	41
2/ CAZ	65.39	72	PA	12	R	4×12, 4520	8000	84.96	103.00	17
3/CAZ	56.17	85	PA	8	S	4×8, 2588	9000	111.27	90.00	-23
4/CAZ	88.37	70	PA	4	S	4×4, 2036	5000	39.29	65.20	40
5/CAZ	18.95	75	PA	8	S	4×8, 873	3500	128.29	94.20	-36
6/ CAZ	77.75	80	PA	8	S	4×8, 3583	5000	44.66	25.40	-75
7/ CAZ	87.29	75	AX	4	S	4×4, 2011	4000	31.82	46.80	32
8/CAZ	73.21	74	PA	2	S	4×2, 843	2000	18.97	15.50	-22
9/CAZ	115.32	56	PA	8	S	4×8, 5314	6000	36.13	50.90	29
10/CAZ	148.62	72	PA	4	S	4×4, 3424	4000	18.69	23.80	21
11/CAZ	108.59	90	PA	4	S	4×4, 2502	4000	25.58	28.90	11
12/ CAZ	22.30	80	PA	32	R	2×32, 2055	4000	124.57	104.60	-19
13/ CAZ	71.99	68	PA	16	R	3×16, 4976	6000	57.88	82.40	30
14/ CAZ	61.12	92	PA	16	R	3×16, 4225	6000	68.17	37.20	-83
15/ATM	238,21	64	PA	16	R	3x16, 5679	6000	50.71	62.20	18
16/ATM	74.23	75	PA	16	R	3x16, 5679	5000	42.26	42.60	1
17/ATM	69.92	50	PA	8	R	4x8, 3786	4000	33.81	82.20	59
18/ATM	204.19	110	PA	4	S	4x4, 1893	3000	25.35	77.90	67
19/ATM	155.03	65	PA	8	R	4x8, 3786	6000	50.71	115.50	56
20/ATM	58.05	73	PA	4	S	4x4, 1893	3000	25.35	39.20	35
21/ATM	109.55	75	PA	4	S	4x4, 1893	3000	25.35	49.30	48
22/TZP	86.70	100	ABAU	8	S	4x8, 7327	10000	43.68	52.40	16
23/TZP	96.04	100	ECLO	8	S	4x8, 10368	12000	37.04	45.20	18
24/TZP	34.22	40	PA	16	S	3x16, 7880	12000	73.10	33.00	-121

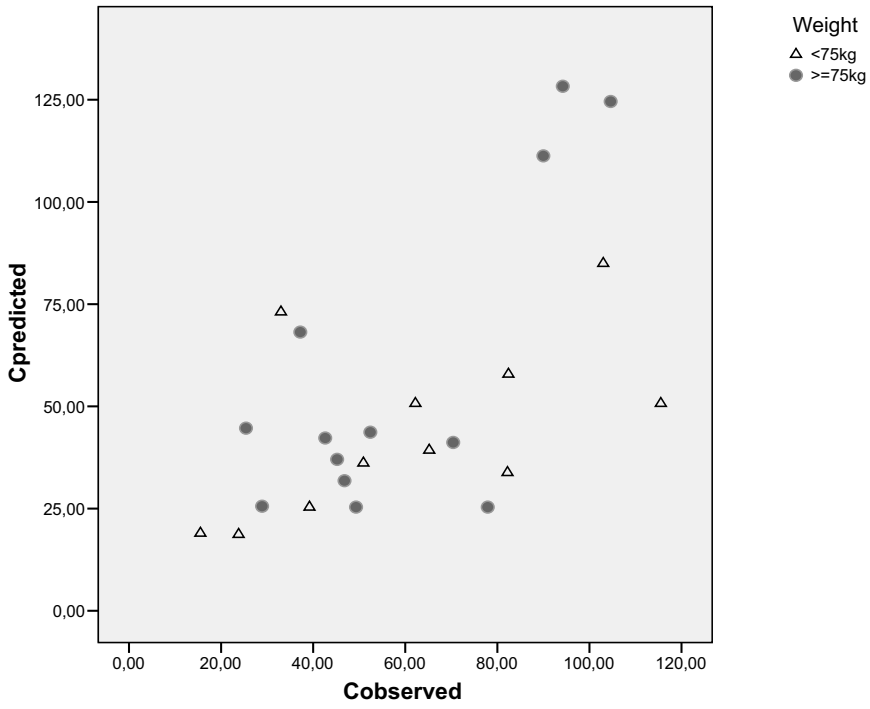
FOOTNOTE Table 1:

CrCl= Creatinine clearance (calculated using the *Cockcroft-Gault* formula, Ref. 14); PA= *Pseudomonas aeruginosa*, AX= *Achromobacter xylosidans*; ECLO: *Enterobacter cloacae*; S= susceptible, R=resistant, mg: milligrams. MIC= each value refers to the BL used in CI for the respective microorganism. TIMES×MIC= the expected number of times over the MIC, used to achieve the desired C_{ss} concentrations to calculate a daily Theoretical Dose of BL in CI (see methodology). Theoretical dose: maintenance dose predicted by equation (see methodology). Real dose: maintenance dose finally administered to patients (see methodology; discrepancies between "Theoretical dose" and "Real dose" was due to our interpretation that the theoretical dose represented a significant reduction in comparison with the usual total daily dosage by IB. This fact especially happened at the beginning of the study).

C_{pred}=predicted concentration by using equation C_{pred} (mg/L) = Real dose (mg/24h)/ TBC (L/h) (see manuscript text); C_{obs}= observed concentration determined by UPLC-MS/MS; $\%\Delta_{Conc}$ = difference C_{obs} - C_{pred} expressed in percentage.

FIGURE 1

A.


Weight
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>=75kg
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100,00
75,00
50,00
25,00
0,00
Cpredicted
0,00
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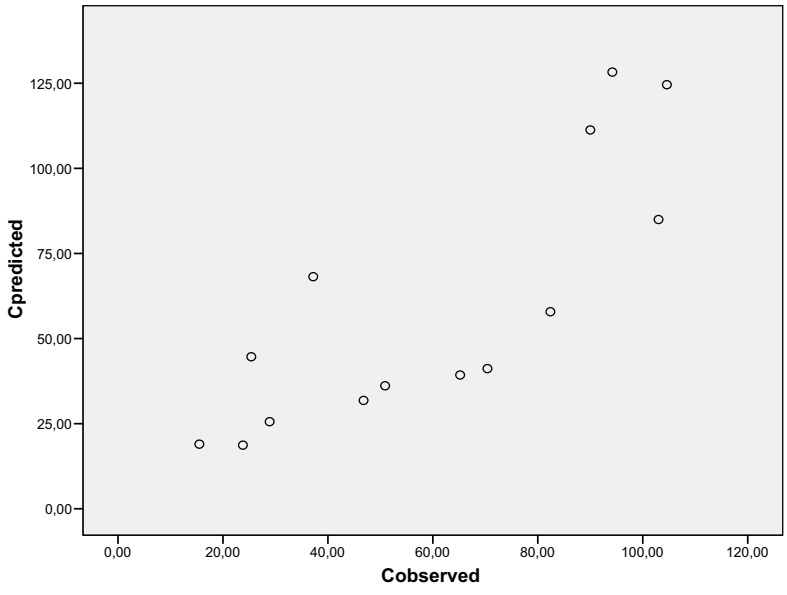
60,00
80,00
100,00
120,00
Cobserved

B.


125,00
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Cpredicted
0,00
20,00
40,00
60,00
80,00
100,00
120,00
Cobserved

FIGURE LEGEND

Figure 1:

A: Correlation between C_{obs} and C_{pred} in all patients (Spearman rho=0.6). Results are presented according to the patient's weight: less than 75kg (white triangle; Spearman rho=0.6) and equal or greater than 75kg (grey circle; Spearman rho=0.3).

B: Correlation between C_{obs} and C_{pred} in patients treated with ceftazidime (Spearman rho=0.8).

Journal of Antimicrobial Chemotherapy Advance Access published September 28, 2015

J Antimicrob Chemother
doi:10.1093/jac/dkv281

Journal of Antimicrobial Chemotherapy

Osteoarticular infection caused by MDR *Pseudomonas aeruginosa*: the benefits of combination therapy with colistin plus β-lactams

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Received 17 June 2015; returned 16 July 2015; revised 7 August 2015; accepted 11 August 2015

Objectives: In the era of emergence of MDR *Pseudomonas aeruginosa*, osteoarticular infections (OIs) add more difficulties to its treatment. The role of β-lactams (BLs) is questioned and older drugs need to be reconsidered. The objective of this study was to describe our experience in the management of OIs caused by MDR *P. aeruginosa* and evaluate different therapeutic options.

Methods: This was a retrospective analysis of a prospectively collected cohort (2004–13) of patients with OI caused by MDR *P. aeruginosa*. We created two groups: (i) Group A (more difficult to treat), prosthetic joint infections (PJIs) and osteoarthritis (OA) managed with device retention; and (ii) Group B (less difficult to treat), OA managed without device retention. Antibiotic treatment was administered according to clinician criteria: monotherapy/combined therapy; and BL used by intermittent bolus (IB)/continuous infusion.

Results: Of 34 patients, 15 (44.1%) had PJI and 19 (55.9%) had OA (8 related to an orthopaedic device). Twenty-three cases (68%) were caused by XDR *P. aeruginosa*. The initial management included removal of an orthopaedic device in 14 cases, together with antibiotic [alone, 19 (55.9%; 4 colistin, 14 BL-IB and 1 BL continuous infusion); and in combination, 15 (44.1%; 5 BL-IB and 10 BL continuous infusion)]. The overall cure rate was 50% (39% and 63% in Groups A and B, respectively), ranging from 31.6% with monotherapy to 73.3% with combined therapy ($P=0.016$), with special interest within Group A (cure rate with combined therapy 71.4%, $P=0.049$). After rescue therapy, which included removal of remaining devices, the cure rate reached 85.3%.

Conclusions: We suggest that the BL/colistin combination is an optimized therapy for OI caused by MDR *P. aeruginosa*, together with an appropriate surgical treatment.

Introduction

Gram-positive bacteria are the most frequent infective agents in osteoarticular infection (OI), whereas Gram-negative bacteria (GNB) may be responsible of 10%–23% of cases.[1–3] In particular settings, such as prosthetic joint infections (PJIs),[4–7] *Pseudomonas aeruginosa* may cause up to 20% of these GNB infections.[7] While current antibiotic recommendations for the treatment of OIs caused by GNB are β-lactams and ciprofloxacin,[8,9] there is no standard of treatment for MDR GNB infection.

The progressive emergence of MDR GNB represents a new challenge in the treatment of nosocomial infection. In the field of PJI, a recent study showed that the percentage of MDR GNB almost tripled from 3.3% in 2003 to 9.4% in 2012.[10] Among these pathogens, *P. aeruginosa* is particularly problematic, with few therapeutic options.[11] Some strains are resistant or not fully susceptible to β-lactams, and the only active antimicrobials are polymyxins and aminoglycosides.[12]

Moreover, the pathology of OIs, especially when an orthopaedic device is present, adds further complexity to the clinical and surgical management of these infections.[3,13–16]

It is well known that bacteria involved in OIs can live in a stationary phase or non-growing condition, either intracellularly or within biofilms around the orthopaedic device.[17] Inside the complex glycoproteic matrix of the biofilm, the low concentration of oxygen and nutrients leads to heterogeneous phenotypic changes in the bacteria. In turn, this results in different antimicrobial tolerances to different families of antibiotics.[17,18] Indeed, the use of β-lactams to treat PJIs caused by quinolone-resistant GNB was associated with a poor cure rate,[7] since the role of antibiotics was even more complicated by the reduced susceptibility or resistance to β-lactams.

In the difficult scenario outlined, the most appropriate antibiotic therapy remains a matter of concern that is poorly defined. Older antibiotics, such as the polymyxins [mostly polymyxin B and polymyxin E (colistin)], have recently gained prominence in the treatment of problematic MDR GNB such as *P. aeruginosa*, and their activity against the associated biofilms has been demonstrated by *in vitro* and *in vivo* experimentation.[19–24] Several publications based on pharmacokinetic, pharmacodynamic and experimental models have suggested the potential clinical benefits of systemic colistin in combination with other antimicrobials (such as β-lactams).[19,25–27]

In this study, we describe our experience with the management of OIs caused by MDR *P. aeruginosa* in the presence and absence of an orthopaedic device, and evaluate the different therapeutic options available. We aimed to identify the prognostic factors for failure, so that we could propose optimized treatment guidance for these difficult-to-treat infections.

Methods

Setting

The study was performed by a multidisciplinary team in a tertiary-care teaching hospital in Barcelona. The team included specialists in infectious diseases, orthopaedics and microbiology, with extensive experience in these fields.

Study design

This was a retrospective analysis of a prospectively collected cohort, with data collection carried out from January 2004 to May 2013. The study cohort included all patients admitted with OI caused by MDR *P. aeruginosa*.

Definitions

The term OI included patients with PJIs and patients with osteoarthritis (OA) that may or may not have been related to an orthopaedic device. Polymicrobial infections with participation of *P. aeruginosa* and cases where *Pseudomonas* was involved after a different primary infection (superinfection) were all included. Patients with a 'diabetic foot' or distal-toe osteomyelitis were excluded because these required particular management.

P. aeruginosa resistance was defined as follows:[11] (i) MDR when *P. aeruginosa* was non-susceptible to one or more agent(s) in three or more antimicrobial categories (aminoglycosides, anti-pseudomonal carbapenems, anti-pseudomonal cephalosporins, anti-pseudomonal fluoroquinolones, anti-pseudomonal penicillins + β-lactamase inhibitors, monobactams, phosphonic acids and polymyxins); or (ii) XDR when *P. aeruginosa* was non-susceptible to one or more agent(s) in all but two or fewer antimicrobial categories.

OI caused by *P. aeruginosa* was defined by positive cultures in two or more surgical samples, or by one positive culture in surgical samples or joint-aspirate or blood cultures, plus the presence of typical clinical symptoms and signs of infection.

Although all patients were assumed to have more difficult-to-treat infections, we considered that prosthesis removal could introduce a new foreign body (i.e. spacer) or a new cavity with liquid retention (i.e. Girdlestone resection), which could actually promote the persistence of infection. Thus, we created two groups according to the type of infection and the initial surgical treatment: Group A comprised those with OIs considered more difficult to treat (including patients with PJIs and OA managed with device retention), while Group B comprised OIs considered less difficult to treat (including patients with OA managed without device retention).

Renal impairment was defined as follows: (i) creatinine increase to >85 μmol/L or a glomerular filtrate rate decrease to <60 mL/min/1.73 m^2 in cases with previous normal renal function; or (ii) creatinine increase to twice the initial value or a glomerular filtration rate decrease of >50% (defined as renal injury by the RIFLE classification)[28] in cases with previous chronic renal dysfunction.

Microbiology processes

All specimens (tissue samples, joint aspirates and blood cultures) were processed in our microbiology laboratory. Cultures of tissue and joint-aspirate samples were produced by prolonged incubation (10 days) at 30–35°C under aerobic and anaerobic conditions. Blood samples were processed using a Bactec 9240 (Becton-Dickinson Microbiology Systems); the inoculated bottles were incubated for 5 days at 35°C before being discharged.

Identification of microorganisms and susceptibility testing were performed using commercial panels from the MicroScan automated system (Siemens Healthcare Diagnostics Ltd, West Sacramento, CA, USA). The antibiotics tested were piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, ciprofloxacin, gentamicin, tobramycin, amikacin, colistin and fosfomycin. Criteria of susceptibility or resistance to the various antibiotics were according to EUCAST guidelines.[29]

Clinical study

The clinical features of patients with OI caused by *P. aeruginosa* in our hospital were prospectively evaluated and added to a database during the study period. We collected the following clinical data: underlying medical conditions; clinical presentation, including symptoms, signs and duration; type of infection, divided into PJI or OA, haematogenous or post-surgical infection (acute/chronic) and those involving an orthopaedic device; microbiological diagnosis, divided into monomicrobial or polymicrobial infection, and infection or super-infection; and C-reactive protein and erythrocyte sedimentation rate results.

Clinical and surgical management

Patients with an acute post-surgical PJI or with OA and devices were managed with debridement, antibiotic and implant retention (DAIR) according to current recommendations (patients with acute infection, implant stability and integrity of surrounding soft tissues).[1,3,14,16] We also recommended DAIR when, in addition to the established criteria, anti-biofilm antimicrobials were not active, which departed from current recommendations. DAIR was not used for patients with an unstable prosthesis/osteosynthesis or with severely damaged soft tissue around the joint. The antimicrobial therapy was chosen from the available agents, which included colistin, aminoglycosides or β-lactams (used in intermittent bolus or continuous infusion) alone or in combination. Of the anti-pseudomonal β-lactams, we choose the one with the lowest MIC value. Continuous β-lactam infusions were administered to achieve target drug concentrations at or above the MIC, using the same intermittent total daily dose over 24 h or by calculating individual dose regimens.[30,31] Patients were treated with the selected intravenous antibiotic plan for 6 weeks; in patients with combined therapy, colistin was used with β-lactams from when susceptibility to *P. aeruginosa* was known until the end of the treatment (when renal function was normal), or earlier when renal injury occurred. The colistin dose was started at 2 million IU (MIU) every 8 h (without a loading dose) when renal function was normal, and adjusted to renal function in patients with chronic renal failure or treatment-induced renal impairment. The attending medical team was responsible for treatment choice and dose regimen.

Outcome and follow-up

After treatment, patients were clinically assessed in the outpatient clinic at months 1, 3, 6 and 12; after 1 year, patients were reviewed at the discretion of each researcher. Failure was defined as: (i) death related to the

infection; (ii) amputation of the affected limb; or (iii) persistence of clinically relevant MDR *P. aeruginosa* (i.e. signs/symptoms of infection and/or positive cultures) despite appropriate initial therapy. Rescue therapy was evaluated as part of the outcome assessment.

Statistical analysis

Continuous variables were expressed as medians with the IQR and were compared using the Mann–Whitney *U*-test. Categorical variables were expressed as number (percentage) and were compared using the χ^2 test or Fisher's exact test, as appropriate. Statistical significance was defined as a two-tailed *P* value <0.05. Predictor parameters of failure were analysed by logistic regression. In addition, Kaplan–Meier curves and the log-rank test were used to compare the cumulative likelihood of failure between patients treated with combined therapy or monotherapy. Data were analysed using IBM SPSS for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA).

Results

We included 34 patients: 15 (44%) with PJI, 11 (32%) with OA not related to an orthopaedic device and 8 (24%) with OA related to an orthopaedic device. The median age was 68.7 years (IQR 59.5–78) and 59% were men, with >70% having at least one comorbidity. Polymicrobial infection was initially present in 16 (47%) patients and 20 (59%) had a super-infection caused by MDR *P. aeruginosa* (Table 1).

Of the 34 patients, 31 (92%) initially underwent surgery. Three patients with OA (without device) were managed conservatively with antibiotics alone: two had post-surgical pubic symphysis osteomyelitis following a prostatic resection, and one had sacroiliitis because of a sacral pressure sore. Among the 23 patients with OI related to an orthopaedic device (8 OA plus 15 PJI), surgery

Table 1. Main characteristics of patients with OI caused by MDR PA; *N*=34

	Median (IQR) or *n* (%)
Age (years)	68.7 (59.5–78)
Male	20 (58.8)
Comorbidities	
diabetes mellitus	6 (17.6)
immunosuppressive therapy	8 (23.5)
autoimmune disease	5 (14.7)
chronic renal failure	6 (17.6)
malignancy	4 (11.8)
others[a]	6 (17.6)
no comorbidity[b]	10 (29.4)
Type of infection	
PJI	15 (44.1)
OA (without related device)	11 (32.4)
OA (related to an orthopaedic device)	8 (23.5)
Polymicrobial infection	16 (47.1)
Super-infection	20 (58.8)
MDR PA/XDR PA	11 (32.4)/23 (67.6)

PA, *P. aeruginosa*.
[a]Includes patients with chronic pulmonary disease, chronic heart disease or advanced dementia.
[b]Includes patients without any of the previously defined comorbidities.

Table 2. Initial management of patients with OI caused by MDR PA; *N*=34

	n (%) or *n*
Antibiotic	
monotherapy	19 (55.9)
colistin	4
BL-IB	14
BL continuous infusion	1
combined therapy	15 (44.1)
colistin+BL-IB	3
colistin+BL continuous infusion	10
amikacin+BL-IB	2
Surgery	
no surgery	3 (8.8)
surgery without device maintenance[a]	22 (64.7)
debridement with device retention	9 (26.5)

BL, β-lactam; IB, intermittent bolus.
Monotherapy: BL-IB, ceftazidime (4), cefepime (1), aztreonam (1), piperacillin/tazobactam (4) and carbapenem (4); and BL continuous infusion, piperacillin/tazobactam (1).
Combined therapy: colistin+BL-IB: ceftazidime (1), aztreonam (1) and carbapenem (1); colistin+BL continuous infusion: ceftazidime (5), aztreonam (2), piperacillin/tazobactam (2) and carbapenem (1); and amikacin+BL-IB: cefepime (1) and piperacillin/tazobactam (1).
[a]Includes patients with OI without a device managed by debridement and patients in which the involved devices were removed.

Table 3. Prognostic factors for persistence of infection after the initial therapy; analysis of risk of failure considering main characteristics and antibiotic treatment; *N*=34

	Cured infection, *n*=17	Non-cured infection, *n*=17	*P*
Main characteristics			
age (years), median (IQR)	71 (59–76)	67 (51–79)	1
male, *n* (%)	12 (70.6)	8 (47.1)	0.163
polymicrobial infection, *n* (%)	6 (35.3)	10 (58.8)	0.169
super-infection, *n* (%)	11 (64.7)	9 (52.9)	0.486
MDR PA, *n* (%)	3 (17.6)	8 (47.1)	0.067
XDR PA, *n* (%)	14 (82.4)	9 (52.9)	
related to an orthopaedic device, *n* (%)	10 (58.8)	13 (76.5)	0.271
Antibiotic			
monotherapy, *n* (%)	6 (35.3)	13 (76.5)	0.016
combined therapy, *n* (%)	11 (64.7)	4 (23.5)	
BL-IB, *n* (%)	8 (53.3)	11 (73.3)	0.256
BL continuous infusion, *n* (%)	7 (46.7)	4 (26.7)	

PA, *P. aeruginosa*; BL, β-lactam; IB, intermittent bolus.

involved debridement and device removal in 14 (60.9%; 9 PJI and 5 OA), while the device was retained in 9 (39.1%; 6 PJI and 3 OA).

Monotherapy was used in 19 (56%) patients, mainly with intermittent boluses of β-lactams (14/19), but 4 patients received colistin alone. When the clinician used combination therapy (15, 44%), it was mostly with continuous infusion of a β-lactam plus colistin (10/15). Overall, 30 patients received β-lactams: in 12 patients, *P. aeruginosa* strains were susceptible (6 to anti-pseudomonal cephalosporins, 2 to piperacillin/tazobactam and 4 to carbapenems), but the other 18 were not susceptible: 2 intermediate (1 to aztreonam and 1 to carbapenem) and 16 resistant (6 to anti-pseudomonal cephalosporins, 6 to piperacillin/tazobactam, 1 to aztreonam and 3 to carbapenems). The median dose of colistin was 5 MIU/day (IQR 2.8–6), for a median of 40.5 days (IQR 26–43). Amikacin was administered only in two patients, where it was combined with intermittent boluses of β-lactams (Table 2).

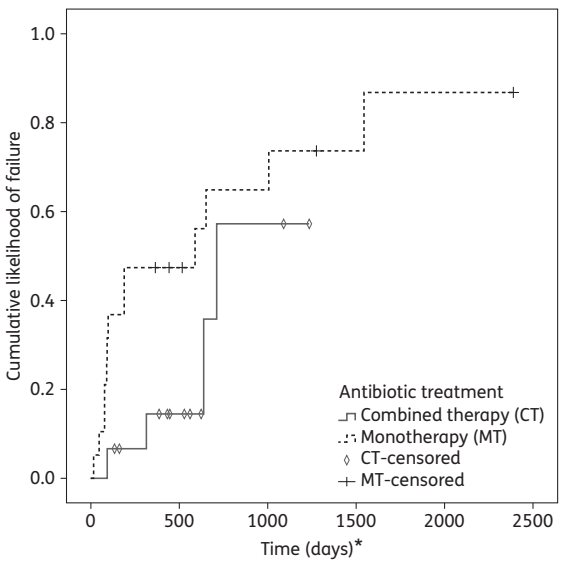

Figure 1. Likelihood of failure according to the antibiotic treatment (combined therapy or monotherapy). *Time from the start of antibiotic therapy to the end of follow-up or to failure (in cases not initially cured). Grey continuous line, combined therapy; black broken line, monotherapy. Log-rank=0.079.

After initial therapy, the cure rate reached 50%. Among the remaining patients, 15 (44%) had persistent infection caused by MDR *P. aeruginosa* and 2 died during the initial treatment. The factors predicting treatment failure were therefore evaluated, focusing on the host, the type of infection and the therapeutic plan. No significant difference was seen in the prognosis when comparing polymicrobial and monomicrobial infections or the presence of *P. aeruginosa* super-infection. XDR *P. aeruginosa* was present in 23 patients and MDR *P. aeruginosa* in 11 patients, with no differences in management (surgical or antibiotic regimen) between the groups (data not shown). Of the 11 patients with OI caused by MDR *P. aeruginosa*, just three (27%) were cured after the first therapeutic plan; but the cure rate more than doubled when the pathogen was an XDR *P. aeruginosa* strain (cure rate 14/23, 61%, $P=0.067$) (Table 3).

Combination therapy (mainly with colistin plus β-lactams) was significantly more effective than monotherapy (with either β-lactams or colistin), with cure rates of 11/15 (73%) and 6/19 (32%), respectively ($P=0.016$) (Table 3). Figure 1 illustrates the likelihood of failure according to the antibiotic treatment and follow-up period (log-rank=0.079). In our case series, colistin was well tolerated, and although 10 patients presented renal impairment during the treatment, creatinine was normalized after reducing the dose. The use of β-lactams in continuous infusion was safe and seemed to offer more benefits than β-lactams in an intermittent bolus (cure rates of 64% and 42%, respectively, $P=0.256$) (Table 3).

The failure rate was also analysed between the two groups by the difficulty of treatment. Patients in Group A had a higher failure rate (61.1%) compared with patients in Group B (37.5%). Focusing on those patients managed with implant retention ($n=9$), three patients were cured after initial debridement (3/9, 33%), but six required further surgery for device removal (Table 4). Combined antibiotic treatment (mainly with colistin plus β-lactams) also appeared to be associated with better outcomes than monotherapy in patients with infections considered more difficult to treat (Group A), despite the added management difficulties, with cure rates of 5/7 (71%) and 2/11 (18%), respectively ($P=0.049$) (Figure 2).

Details of the treatment received by the 17 (50%) patients in whom initial therapy was not curative are summarized in Table 5. Two patients died (Table 5, cases 16 and 17). Among the patients who were not cured by initial therapy, one had a PJI that was retained with a persistent infection [Table 5, case 7, managed conservatively with careful follow-up of a persistent fistula, but without antibiotics (no oral option was possible)]. Another 14 patients required second-line treatment (7 PJIs and

Table 4. Prognostic factors for persistence of infection after the initial therapy; analysis of risk of failure according to the difficulty of treatment; $N=34$

Type of infection	Surgical management		Failure *n*/*N* (%), 17/34 (50%)	*P*
PJI	implant retention	4/6 (66.7%)	more difficult-to-treat OI (Group A), 11/18 (61.1%)	0.169
PJI	implant removal[a]	5/9 (55.6%)		
OA (with device)	implant retention	2/3 (66.7%)		
OA (with device)	implant removal	2/5 (40%)	less difficult-to-treat OI (Group B), 6/16 (37.5%)	
OA (no device)	no surgery or debridement	4/11 (36.4%)		

[a]Management: 3 Girdlestone (2 failures), 5 two-step revision (3 failures) and 1 arthrodesis.

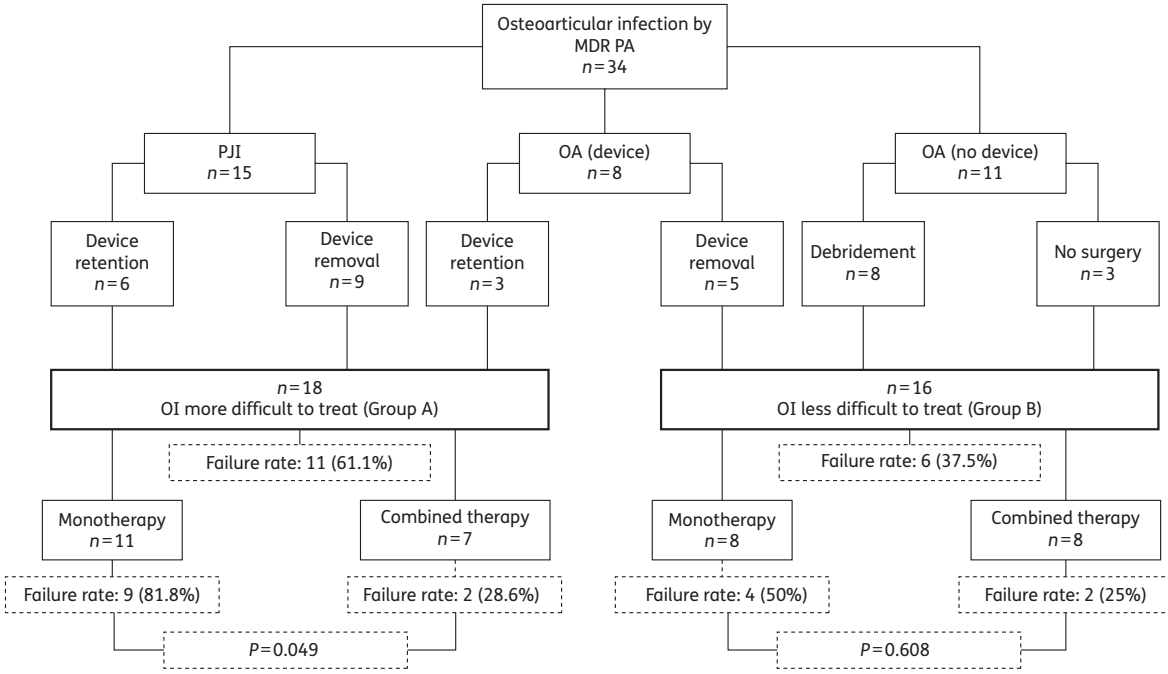

Figure 2. Chart of OI initial management (antibiotic and surgery) according to the difficulties considered (Group A and Group B). Boxes with broken lines show percentages of failure in the various situations. PA, *P. aeruginosa*.

7 OA), which consisted of device removal in 6 patients (always together with an antibiotic plan) or debridement in 8 patients (Table 5). In one patient, prosthetic removal consisted of an infracondylar amputation (Table 5, case 5, total knee prosthesis after resection of an osteosarcoma). The concomitant antibiotic treatment included combination therapy (7 patients), colistin monotherapy (2 patients) or β-lactam monotherapy (4 patients) (Table 5). There was no emergence of colistin-resistant strains in patients with persistent infections.

Overall, three patients died and two had infections that could not be healed, so satisfactory outcomes were achieved in up to 85% of patients (29/34). If we focus on the patients with PJI, 11 of the 15 patients (73.3%) were finally cured; of these, 4 retained a functional prosthesis (2 with the initial prosthesis and 2 with a new prosthesis), 1 with a spacer, 4 with a Girdlestone resection and 2 with an arthrodesis.

Discussion

We have presented a case series of OI caused by MDR *P. aeruginosa* at our hospital. Given the few published reports on this topic,[32,33] our results provide potentially relevant information about the efficacy of β-lactams and colistin when used in combination.

Management of OI caused by MDR GNB represents a new challenge for the clinician, and no specific treatment has been defined. The role of β-lactams in treatment needs to be questioned. Indeed, when treating PJI caused by ciprofloxacin-resistant GNB, β-lactam monotherapy was associated with poorer outcomes than fluoroquinolone monotherapy (treatment response in 40% and 80%, respectively).[7] This scenario is further complicated in infections caused by MDR *P. aeruginosa*, since several strains show reduced susceptibility or resistance to β-lactams. Thus, limited antibiotic availability has led specialists to rediscover old drugs, such as colistin, and to apply them to new therapeutic strategies.

In our case series of 34 patients with OI caused by MDR *P. aeruginosa*, the overall cure rate was 50% after first-line therapy and >85% at the final outcome after rescue therapy. This sample contained more XDR than MDR strains of *P. aeruginosa*, at rates of 68% and 32%, respectively. Curiously, despite the greater degree of resistance in the latter, they seemed to be easier to eradicate. Our findings were not explained by differences in the difficulty of treatment (Group A versus Group B) or in surgical and antimicrobial management. This seems to be consistent with our previous experience regarding the lower virulence and pathogenicity of XDR *P. aeruginosa* in patients with bacteraemia and infections in ICUs,[34] suggesting a trade-off for the acquisition of MDR.

In terms of the antibiotic treatment, combination therapy with β-lactams plus colistin was significantly more effective than monotherapy (with either β-lactams or colistin) overall. We noted that the benefits of combined therapy were particularly shown in patients from Group A (more difficult to treat), with a failure rate of 81.8% with monotherapy and 28.6% with the

Table 5. Details of patients not cured after receiving initial therapy

					Initial therapy			Rescue therapy			
					surgery[a]	antibiotic		surgery[a]	antibiotic		
Case	Age (years)	Type of infection	PA	Orthopaedic device	managed with device retention	MT/CT	BL-IB/BL continuous infusion	managed with device retention	MT/CT	BL-IB/BL continuous infusion	Final outcome
1	63	PJI	XDR	yes	device retention	MT (colistin)	—	no	MT (colistin)	—	cured
2	67	PJI	MDR	yes	device retention	MT	IB	no	CT	IB	cured
3	71	PJI	MDR	yes	no	MT	IB	no	MT	IB	cured
4	70	PJI	MDR	yes	no	MT	IB	no	CT	IB	cured
5	28	PJI	XDR	yes	device retention	CT	continuous infusion	no		—	amputation
6	78	PJI	XDR	yes	device retention	MT	IB	no	MT	IB	cured
7	79	PJI	MDR	yes	no	CT	IB		—		persistence
8	84	PJI	XDR	yes	no	MT	IB	no	CT	continuous infusion	died
9	67	OA	XDR	yes	no	MT (colistin)	—	no	CT	IB	cured
10	41	OA	XDR	yes	device retention	MT	IB	no	CT	IB	cured
11	39	OA	MDR	yes	device retention	MT	IB	no	MT	IB	cured
12	30	OA	MDR	no	no	MT	IB	no	MT (colistin)	—	cured
13	61	OA	MDR	no	no	CT	continuous infusion	no	CT	continuous infusion	cured
14	82	OA	MDR	no	—	MT	IB	no	MT	IB	cured
15	61	OA	XDR	no	no	CT	continuous infusion	no	CT	continuous infusion	cured
16	79	PJI	XDR	yes	no	MT	continuous infusion			died	
17	93	OA	XDR	yes	no	MT	IB			died	

[a]'No' means no device retention (so include cases managed without a device: because there was no previous device or it has been removed). A '—' means that there was no surgery.
PA, *P. aeruginosa*; MT, monotherapy; CT, combined therapy; BL, β-lactam; IB, intermittent bolus.

combination ($P < 0.05$). Although the limited previous information on this topic makes it difficult to compare our results, two clinical studies do exist.[32,33] Valour *et al.*[32] reported a unique case series of bone and joint infection caused by MDR GNB (16 caused by *P. aeruginosa*), with a cure rate of 41% for orthopaedic device-associated infections (despite implant removal), using colistin alone. In our results the outcome was clearly optimized by combination of a β-lactam with colistin (cure rate 71%), and these data supported the potential role of colistin in synergy with β-lactams, especially against biofilm-associated infections. Of course, the individual contribution of each antibiotic in the combination (β-lactams and colistin) is difficult to separate out. Our clinical results are consistent with pharmacokinetic and pharmacodynamic considerations and with the results of experimental studies on this topic. In their *in vitro* model, Bergen *et al.*[35] reported the benefits of combined therapy for the treatment of infection caused by *P. aeruginosa* (adding doripenem to low-dose colistin) even in the presence of high bacterial densities. Moreover, in biofilms caused by GNB, colistin has been shown to be effective against less active bacteria located in the deeper layers of the biofilm structure, which contrasts with the majority of antibiotics that operate at the upper layers only, thereby targeting different subpopulations of the biofilm.[20,21,36] This observation is supported by colistin's particular bactericidal activity, which is independent of hydroxyl radical formation and consumption.[23] Our group also showed colistin to have a higher bacterial killing rate within biofilms than against planktonic bacteria, using an *in vitro* pharmacokinetic/pharmacodynamic biofilm model with several *P. aeruginosa* strains.[27] If further studies confirm our results, the recommendation of combined treatment (colistin plus a β-lactam) could be extended not only to treat OI caused by MDR *P. aeruginosa*, but also to treat OI caused by all ciprofloxacin-resistant GNB.

β-Lactams are known to lose activity inside biofilms.[37,38] This is because their target is on the bacterial wall during the exponential growth phase, even when strains are fully susceptible to them. In addition, little is known about the efficacy of β-lactams (alone or in combination) when strains are resistant or not fully susceptible. Even at lower doses, the synergistic effect of β-lactams in combination with colistin could result from colistin's properties as a cationic peptide, placing β-lactams in a better position against resistant strains by providing better antibiotic penetration.[35,39] Also, it is important to consider the potential benefit of β-lactams administered by continuous infusion (one-third of patients in our case series; cure rate, 64%) to achieve prolonged antibiotic concentrations above the MIC, thereby making several initially resistant strains become susceptible in terms of drug pharmacokinetics and pharmacodynamics.[30,31,40–43] While this needs further exploration, our results are consistent with these benefits. We did not find any differences in outcomes for patients treated with β-lactams (alone or in combination) between those with strains identified as susceptible and those identified as resistant to the particular β-lactam administered.

According to a pharmacokinetic analysis, it is unlikely that intravenous administration of colistimethate sodium (colistin's prodrug) could provide the required colistin concentrations to treat planktonic[44–46] or biofilm-associated infections.[25] Moreover, colistin heteroresistance has been described for several strains of *P. aeruginosa*,[27,47] being a potential problem after exposure to colistin monotherapy. Given these considerations, current recommendations for patients admitted to the ICU suggest using very high doses of colistin (4.5 MIU twice a day) after an initial loading dose of 9 MIU.[45] Nevertheless, it should be balanced with the increased risk of renal toxicity, which is the most common dose-dependent adverse effect of colistin.[48] We believe that, because OIs caused by MDR *P. aeruginosa* (in biofilm-associated infections) require long-term antibiotic therapy, they represent a different scenario from acute life-threatening infection. Moreover, the difference is greater when the role of combination therapy is considered because, due to their synergistic relationship, the addition of β-lactams should allow the clinician to use lower doses of colistin without a loading dose. In our case series, patients with normal renal function were initially given colistin at 6 MIU/day without a loading dose, which was adjusted in patients with renal failure. Tolerance of this regimen was good and, although some patients suffered renal impairment due to colistin, renal function normalized after reducing the dose in all cases. In addition, the clinical results with lower doses of colistin in combination with β-lactams remained acceptable, without colistin resistance. Although older studies have suggested that the diffusion of colistin into bone is poor,[49] recent studies have demonstrated good outcomes when using lower colistin doses without a loading dose.[32]

In conclusion, we have added clinical experience to the pharmacokinetic, pharmacodynamic and experimental models of colistin in combination with β-lactams. There is growing evidence that current recommendations should consider the combination of low-dose colistin with β-lactams as an optimized treatment for OI caused by MDR *P. aeruginosa*. When used as part of a comprehensive treatment plan that includes appropriate surgical treatment (which included implant removal in some situations during initial therapy and in all cases in rescue therapy), this antibiotic combination is essential for achieving good outcomes in these difficult-to-treat infections. Further studies are needed to confirm these results and to consider the role of this therapy for OI caused by ciprofloxacin-resistant GNB.

Acknowledgements

The preliminary results of this study were presented in part at the Fifty-fourth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 2014 (Abstract L-416).

We thank Michael Maudsley for helping with the English in this manuscript.

Funding

This work was supported by Ministerio de Economía y Competitividad, Instituto de Salud Carlos III—co-financed by European Development Regional Fund 'A way to achieve Europe' ERDF, Spanish Network for Research in Infectious Diseases (REIPI RD12/0015). A. R. was supported by a research grant from the Bellvitge Biomedical Research Institute (IDIBELL).

Transparency declarations

None to declare.

References

1 Trampuz A, Zimmerli W. Diagnosis and treatment of implant-associated septic arthritis and osteomyelitis. *Curr Infect Dis Rep* 2008; **10**: 394–403.

2 Murillo O, Grau I, Lora-Tamayo J *et al*. The changing epidemiology of bacteraemic osteoarticular infections in the early 21st century. *Clin Microbiol Infect* 2015; **21**: 254.e1–8.

3 Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med* 2004; **351**: 1645–54.

4 Hsieh P-H, Lee MS, Hsu K-Y *et al*. Gram-negative prosthetic joint infections: risk factors and outcome of treatment. *Clin Infect Dis* 2009; **49**: 1036–43.

5 Tattevin P, Crémieux AC, Pottier P *et al*. Prosthetic joint infection: when can prosthesis salvage be considered? *Clin Infect Dis* 1999; **29**: 292–5.

6 Zimmerli W, Ochsner PE. Management of infection associated with prosthetic joints. *Infection* 2003; **31**: 99–108.

7 Rodríguez-Pardo D, Pigrau C, Lora-Tamayo J *et al*. Gram-negative prosthetic joint infection: outcome of a debridement, antibiotics and implant retention approach. A large multicentre study. *Clin Microbiol Infect* 2014; **20**: O911–9.

8 Osmon DR, Berbari EF, Berendt AR *et al*. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2013; **56**: e1–25.

9 Lew DP, Waldvogel FA. Osteomyelitis. *Lancet* 2004; **364**: 369–79.

10 Benito N, Franco M, Ribera A *et al*. Etiology of prosthetic joint infections (PJI) according to the type of infection in a large multicenter cohort: impact of antimicrobial resistance. In: *Abstracts of the Twenty-fifth European Congress of Clinical Microbiology and Infectious Diseases, Copenhagen, 2015*. Abstract 0301. European Society of Clinical Microbiology and Infectious Diseases, Basel, Switzerland.

11 Magiorakos A-P, Srinivasan A, Carey RB *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; **18**: 268–81.

12 Suarez C, Peña C, Arch O *et al*. A large sustained endemic outbreak of multiresistant *Pseudomonas aeruginosa*: a new epidemiological scenario for nosocomial acquisition. *BMC Infect Dis* 2011; **11**: 272.

13 Darouiche RO. Treatment of infections associated with surgical implants. *N Engl J Med* 2004; **350**: 1422–9.

14 Trampuz A, Zimmerli W. Diagnosis and treatment of infections associated with fracture-fixation devices. *Injury* 2006; **37** Suppl 2: S59–66.

15 Del Pozo JL, Patel R. Infection associated with prosthetic joints. *N Engl J Med* 2009; **361**: 787–94.

16 Cobo J, Del Pozo JL. Prosthetic joint infection: diagnosis and management. *Expert Rev Anti Infect Ther* 2011; **9**: 787–802.

17 Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999; **284**: 1318–22.

18 Høiby N, Bjarnsholt T, Givskov M *et al*. Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents* 2010; **35**: 322–32.

19 Herrmann G, Yang L, Wu H *et al*. Colistin-tobramycin combinations are superior to monotherapy concerning the killing of biofilm *Pseudomonas aeruginosa*. *J Infect Dis* 2010; **202**: 1585–92.

20 Haagensen JAJ, Klausen M, Ernst RK *et al*. Differentiation and distribution of colistin- and sodium dodecyl sulfate-tolerant cells in *Pseudomonas aeruginosa* biofilms. *J Bacteriol* 2007; **189**: 28–37.

21 Pamp SJ, Gjermansen M, Johansen HK *et al*. Tolerance to the antimicrobial peptide colistin in *Pseudomonas aeruginosa* biofilms is linked to metabolically active cells, and depends on the *pmr* and *mexAB-oprM* genes. *Mol Microbiol* 2008; **68**: 223–40.

22 Chiang W-C, Pamp SJ, Nilsson M *et al*. The metabolically active subpopulation in *Pseudomonas aeruginosa* biofilms survives exposure to membrane-targeting antimicrobials via distinct molecular mechanisms. *FEMS Immunol Med Microbiol* 2012; **65**: 245–56.

23 Brochmann RP, Toft A, Ciofu O *et al*. Bactericidal effect of colistin on planktonic *Pseudomonas aeruginosa* is independent of hydroxyl radical formation. *Int J Antimicrob Agents* 2014; **43**: 140–7.

24 Chambers JR, Sauer K. The MerR-like regulator BrlR impairs *Pseudomonas aeruginosa* biofilm tolerance to colistin by repressing PhoPQ. *J Bacteriol* 2013; **195**: 4678–88.

25 Hengzhuang W, Høiby N, Ciofu O. Pharmacokinetics and pharmacodynamics of antibiotics in biofilm infections of *Pseudomonas aeruginosa* in vitro and in vivo. *Methods Mol Biol* 2014; **1147**: 239–54.

26 Hengzhuang W, Wu H, Ciofu O *et al*. In vivo pharmacokinetics/pharmacodynamics of colistin and imipenem in *Pseudomonas aeruginosa* biofilm infection. *Antimicrob Agents Chemother* 2012; **56**: 2683–90.

27 Lora-Tamayo J, Murillo O, Bergen PJ *et al*. Activity of colistin combined with doripenem at clinically relevant concentrations against multidrug-resistant *Pseudomonas aeruginosa* in an in vitro dynamic biofilm model. *J Antimicrob Chemother* 2014; **69**: 2434–42.

28 Bellomo R, Ronco C, Kellum JA *et al*. Acute renal failure—definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004; **8**: R204–12.

29 EUCAST. *Breakpoint Tables For Interpretation of MICs and Zone Diameters, Version 5.0*. 2015. http://www.eucast.org.

30 Moriyama B, Henning SA, Neuhauser MM *et al*. Continuous-infusion β-lactam antibiotics during continuous venovenous hemofiltration for the treatment of resistant gram-negative bacteria. *Ann Pharmacother* 2009; **43**: 1324–37.

31 Moriyama B, Henning SA, Childs R *et al*. High-dose continuous infusion β-lactam antibiotics for the treatment of resistant *Pseudomonas aeruginosa* infections in immunocompromised patients. *Ann Pharmacother* 2010; **44**: 929–35.

32 Valour F, Dutronc H, Dinh A *et al*. Difficult-to-treat Gram-negative bone and joint infections: efficacy and safety of prolonged intravenous colistin. *Int J Antimicrob Agents* 2013; **41**: 197–9.

33 Papagelopoulos PJ, Mavrogenis AF, Giannitsioti E *et al*. Management of a multidrug-resistant *Pseudomonas aeruginosa* infected total knee arthroplasty using colistin. A case report and review of the literature. *J Arthroplasty* 2007; **22**: 457–63.

34 Peña C, Gómez-Zorrilla S, Oriol I *et al*. Impact of multidrug resistance on *Pseudomonas aeruginosa* ventilator-associated pneumonia outcome: predictors of early and crude mortality. *Eur J Clin Microbiol Infect Dis* 2013; **32**: 413–20.

35 Bergen PJ, Tsuji BT, Bulitta JB *et al*. Synergistic killing of multidrug-resistant *Pseudomonas aeruginosa* at multiple inocula by colistin combined with doripenem in an in vitro pharmacokinetic/pharmacodynamic model. *Antimicrob Agents Chemother* 2011; **55**: 5685–95.

36 Klausen M, Aaes-Jørgensen A, Molin S *et al*. Involvement of bacterial migration in the development of complex multicellular structures in *Pseudomonas aeruginosa* biofilms. *Mol Microbiol* 2003; **50**: 61–8.

37 Gilbert P, Collier PJ, Brown MR. Influence of growth rate on susceptibility to antimicrobial agents: biofilms, cell cycle, dormancy, and stringent response. *Antimicrob Agents Chemother* 1990; **34**: 1865–8.

38 Gilbert P, Brown MR. Biofilms and β-lactam activity. *J Antimicrob Chemother* 1998; **41**: 571–2.

39 Zhang L, Dhillon P, Yan H *et al*. Interactions of bacterial cationic peptide antibiotics with outer and cytoplasmic membranes of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2000; **44**: 3317–21.

40 Mouton JW, Vinks AA. Continuous infusion of β-lactams. *Curr Opin Crit Care* 2007; **13**: 598–606.

41 Roberts JA, Paratz J, Paratz E *et al*. Continuous infusion of β-lactam antibiotics in severe infections: a review of its role. *Int J Antimicrob Agents* 2007; **30**: 11–8.

42 Dulhunty JM, Roberts JA, Davis JS *et al*. Continuous infusion of β-lactam antibiotics in severe sepsis: a multicenter double-blind, randomized controlled trial. *Clin Infect Dis* 2013; **56**: 236–44.

43 Van Herendael B, Jeurissen A, Tulkens PM *et al*. Continuous infusion of antibiotics in the critically ill: the new holy grail for β-lactams and vancomycin? *Ann Intensive Care* 2012; **2**: 22.

44 Garonzik SM, Li J, Thamlikitkul V *et al*. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrob Agents Chemother* 2011; **55**: 3284–94.

45 Plachouras D, Karvanen M, Friberg LE *et al*. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by gram-negative bacteria. *Antimicrob Agents Chemother* 2009; **53**: 3430–6.

46 Nation RL, Li J. Colistin in the 21st century. *Curr Opin Infect Dis* 2009; **22**: 535–43.

47 Bergen PJ, Bulitta JB, Forrest A *et al*. Pharmacokinetic/pharmacodynamic investigation of colistin against *Pseudomonas aeruginosa* using an in vitro model. *Antimicrob Agents Chemother* 2010; **54**: 3783–9.

48 Antonucci E, Taccone FS, Regolisti G *et al*. [Colistin: a review]. *G Ital Nefrol* 2014; **31** (http://www.ncbi.nlm.nih.gov/pubmed/25504163).

49 Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clin Infect Dis* 2005; **40**: 1333–41.

ACKNOWLEDGMENTS / AGRAÏMENTS

És ben cert que totes aquestes pàgines no s'escriuen en quatre dies. Són el resultat de tot un procés que ha durat més de cinc anys i que ha estat possible gràcies a l'ajuda i col·laboració d'una llarga llista de companys, amics i familiars. I aquí us ho vull agrair.

Encapçalant la llista vull donar les gràcies al Javier Ariza. Treballar al seu costat ha estat un gran luxe. La seva pausa, rigor i entreteniment m'ha fet aprendre l'ofici de metge, qüestionant-ho tot per després entendre-ho millor; repassant el que diu la literatura mèdica més clàssica, sòlida, per no perdre'ns entre l'allau de *papers* actuals; trobant l'empatia equilibrada amb el pacient perquè aquest se senti segur i confortable entre les paraules incompressibles dels metges i les parets fredes de l'hospital. I aquesta mateixa manera de fer transportada al món de la investigació no ha fet més que enriquir-me cada dia. Les múltiples vegades que he entrat al despatx del Javier, fred, gelat, però a la vegada càlid, amb un cap ennuvolat ple d'informació desordenada, visualitzant a la sortida la imatge d'un arbre amb el seu tronc, i cada una de les branques i branquillons dibuixades perfectament. Gràcies, gràcies per aconseguir tot això i estar sempre darrera de la porta quan hi truco.

I a l'Oscar que m'ha ensenyat, amb paciència, com afrontar el paper en blanc dels meus primers articles, corregint sempre amb detall cada paràgraf i triomfant en la retallada final de paraules. Gràcies per saber ocupar aquest lloc difícil del triangle, incorporant les idees de l'Ariza i respectant amb cura les meves inquietuds; per transportar-me al món de la pipeta i per encarregar-te de procurar i liderar els projectes del *bone team*; per entendre'm i cuidar-me.

I a tots els components del *bone team*: a les noves generacions que arranquen amb entusiasme, però sobretot als que ja fa dies que volten pel món. La Gorane que va fer tan plàcida la meva arribada instruint-me sobre el maneig de la infecció osteoarticular i amb qui vaig compartir les vivències d'unes mares primerenques. El Jaime, per la seva paciència infinita per situar-me dins del món dels protocols i les bases de dades, per ensenyar-me i ajudar-me a qualsevol hora. La Cris que és la principal culpable de la meva actual destresa amb el reactor i la companya de batalla contra totes les adversitats del laboratori (mai oblidaré el dia de la fumigació). I, finalment, el Joan, ajudant incombustible d'aquest final d'etapa i l'Eva per no deixar mai de banda el seu sentit comú.

A l'equip de traumatòlegs. Al Xavier Cabo, un gran professional, que m'ha explicat, des de capçalera del pacient, tot allò que no diuen els llibres sobre la infecció osteoarticular. Al Salva, al José Moranas, al Víctor i als residents de traumatologia per fer que el dia a dia a la 10.2 sigui sempre ben entretingut; i a la Isabel Vila i totes les infermeres que són la base de la unitat.

També el meu agraïment als companys de Medicina Interna, Malalties Infeccioses i a l'equip d'Urgències per acollir-me i per formar-me des del primer dia que vaig arribar en aquest hospital. Amb especial estima per l'Antonio Vidaller, la Maruja Pac, la Xesca Mitjavila, l'Abelardo Montero, el Ramon Pujol, la Carme Cabellos i els meus companys de despatx, la Carme Peña i el Miguel Santín.

A les meves CoR, grans amigues, ja que amb elles aquest camí ha estat molt més fàcil: l'Anto, la Laura, la Silvia. A tots els becaris amb qui he compartit durant tots aquests anys moments fantàstics, encara que també contratemps i *deadlines* que hem acabat tirant endavant: l'Oriol, la Laura, l'Ivan, la Marta, la Isa, la Silvana, el Guille. Als residents de medicina interna. Al *soso pool* pel seu esperit sempre alegre i tan sa, per les nits en blanc i les pujades a la Mola, pel vostre entorn tan familiar.

A la Fe Tubau i al Jordi Càmara, que tremolen cada cop que entro per la porta de microbiologia, gràcies per atendre sempre la meva llista de dubtes; i a la Dolors Garcia per no tenir mai en compte la meva impuntualitat a la sessió de micro, i per tenir sempre preparat el túper del sonicador; a la resta de microbiòlegs de l'hospital. Al Joan M Nolla per les seves lliçons en reumatologia. Al JA Narváez i al Javi per fer-me anar més enllà de les imatges. Al Raül Rigo que ha sabut transmetre'm el seu entusiasme pel que fins ara era per mi ciència ficció. A l'Ariadna Padullés per parlar-me del comportament dels fàrmacs. A la Sara Martí per compartir els seus coneixements sobre el biofilm. A la Susana Arnedo del servei d'Anatomia Patològica per obrir sempre la porta amb una rialla. Al Joan Ribera i al servei cientificotècnic de l'IRB Lleida per deixar-nos veure el biofilm i facilitar-nos aquestes imatges tan espectaculars. Al Cristian Tebé pel seu assessorament en estadística. Al Kevin per fer seus els enormes bidons i a la Carmen per donar-nos tants cops de mà al laboratori.

Als companys del Nuffield Orthopaedic Centre (Oxford) que em van acollir i integrar a la seva manera tan *british*, fent-me sentir una més del grup; tinc tan bons records d'aquells dies...

Als companys del Parc Sanitari de Sant Joan de Déu de St Boi que em transporten cada setmana a la realitat de les urgències del Baix Llobregat.

Als bons amics de Ponent, de Barcelona i de més lluny. Veig molt difícil aconseguir un bon clima per a la ciència sense la vostra companyia, suport i estima. I és que les vostres tertúlies, els dinars que s'allarguen fins a la nit i les jornades improvisades són essencials per carregar piles.

A la meva família.

Al meu pare per fer-me interessar per la investigació; per transmetre'm el seu entusiasme, la seva energia; i per ensenyar-me a no tenir por de res, a ser valenta. A la meva mare pel seu suport en majúscules, per patir per mi i amb mi, per haver-me sabut impregnar del seu pragmatisme, per recordar-me sovint que les coses es fan una darrera l'altra i per escoltar-me sempre. Al Guillem i a l'Eugènia per mostrar-me la calma enmig del meu neguit, per creure en mi. Als meus padrins que em segueixen des de la distància i em transporten als meus orígens, perquè no m'oblidi mai de com va la vida per Ponent. I a la tieta Teresa, per posar el seu granet de sorra en la meves ganes d'aprendre d'infeccions.

Al Norbert per fer que sempre tingui ganes d'arribar a casa, per crear un entorn càlid. Per fer que les coses siguin més fàcils del que jo em pensava, per fer-me centrar en allò que és important. Per la seva visió crítica de les coses, per veure'm venir. Per estimar-me tant i pensar en mi. Pels sopars d'estrella i per acompanyar-me lluny. Per haver collit amb mi totes aquelles pomes i des d'aleshores tantes coses més.

A l'Ona i al Pere que em fan moure cada dia amunt i avall. Per sorprendre'm cada moment i perquè és emocionant veure com creixen. Per la seva innocència i tendresa, per aquesta explosió d'emocions encara incontrolades. Per les seves abraçades espontànies que són com petites descàrregues de vitalitat. Pels concerts de cada tarda, pels contes inventats...

I, per acabar, no em puc oblidar del paper que hi hagi pogut tenir la màgia.