Molecular Epidemiology of *Klebsiella pneumoniae* Strains Causing Bloodstream Infections in Adults

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Molecular epidemiology of *Klebsiella pneumoniae* bacteremic strains allows for a better understanding of preventive and therapeutic strategies. Clinical and microbiological characteristics of 348 *K. pneumoniae* bacteremia cases (2007–2009) were retrospectively characterized by multilocus sequence typing and extended-spectrum beta-lactamases (ESBL) production. Overall, 223 (64.08%) cases were nosocomial (NA), 58 (16.67%) healthcare associated, and 67 (19.25%) community acquired. The main infection origins were urinary tract (16.6%, 50.0%, and 43.3%), biliary tract (10.8%, 24.2%, and 31.3%), and catheter-related infection (39.9%, 5.2%, and 0%). The 30-day mortality rate was around 20%. The rates of resistance were around 45% the highest being among NA cases, and ESBL production was detected in 7.2% of cases. A total of 161 different sequence types were grouped into 13 clonal sets by e-burst analysis. No relationship could be established between clonal sets and the origin of infection or the healthcare-related settings. The high genetic variability among the isolates suggests their intrapatient endogenous origin.

Keywords: *Klebsiella pneumoniae*, bloodstream infections, genetic diversity, sequence typing

Introduction

*K*LEBSIELLA PNEUMONIAE IS an important human pathogen in the community and in the hospital setting. *K. pneumoniae* is the second most common cause of Gram-negative bloodstream infections after *Escherichia coli*. This microorganism is also well known because of the increasing rates of antimicrobial resistance mainly associated with the hospital setting and with extended-spectrum beta-lactamase (ESBL) or carbapenemase production.[1,2] In recent years, the number of infections (pneumonia or urinary tract infections) caused by drug-resistant *K. pneumoniae* in hospitalized patients is increasing. Moreover, *K. pneumoniae* bloodstream infections are associated with patients with comorbidities such as diabetes mellitus, cancer, chronic liver disease, and biliary disease.[3]

The molecular epidemiology of multidrug-resistant (MDR) *K. pneumoniae* strains and those associated with nosocomial (NA) outbreaks has been largely studied.[4,5] However, the molecular epidemiology of *K. pneumoniae* causing bacteremia outside the hospital is less known.[6] Recent studies have defined the major lineages of *K. pneumoniae*, including both NA and community-acquired isolates.[7]

Bloodstream infections were detected in patients with comorbid conditions and complications, such as diabetes mellitus (related to community-acquired [CA] bacteremia), malignancy (mainly related to NA bacteremia or healthcare-associated [HCA] bacteremia) or cardiovascular diseases (mainly related to NA).[8,9]

In our study, as in previous reports, urinary tract, biliary tract, catheter, and respiratory tract were the most common point of entry of bacteremia.[3,4,10] Nowadays this is a public health problem according to the clinical and epidemiological impact as well as to the economic and social costs.[11,12]

In this study, we analyze the clinical and molecular epidemiology of *K. pneumoniae* strains isolated from blood cultures of adult patients attended over a 3-year period in a teaching hospital, in Barcelona, Spain. Moreover, NA, HCA, and CA bacteremia episodes caused by *K. pneumoniae* were analyzed.

Materials and Methods

Clinical setting

The study was carried out at the Hospital de Bellvitge, which serves an area of ∼600,000 inhabitants with an average of

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30,000 admissions per year. It is a 900-bed university teaching hospital for adult patients located in the Barcelona metropolitan area, Spain, providing both medical and surgical care without pediatrics, obstetrics, and burn units. As hospital routine, blood cultures are performed on patients with symptoms of infection following standardized protocols.

K. pneumoniae isolates causing bloodstream infection from January 2007 to December 2009 were included in this study. Clinical data of patients, including patient age, sex, comorbidities, and infection source were prospectively collected and included in a computerized prospective.

Definitions

NA *K. pneumoniae* bacteremia was considered when the infection occurred after 48 hours of patient being admitted. HCA *K. pneumoniae* bacteremia was considered when the bloodstream infection sample was detected within 48 hours of the patient's admission, and it met any of the following criteria: a patient being with health system contact (≥2 hospitalization days in the 3 previous months to the recent admission, receiving any nursing care, undergoing hemodialysis in the previous month, or nursing home residents). Finally, CA *K. pneumoniae* bacteremia was considered when a positive bloodstream infection sample was detected in the first 48 hours of admission in a patient without any of the previous criteria.[13]

Bacteremia was defined by the isolation of *K. pneumoniae* in blood cultures together with clinical symptoms of infection. Two episodes were considered different when they occurred at least 1 month apart.

Antimicrobial susceptibility testing

The minimum inhibitory concentration of antibiotics was tested by microdilution using the commercial method MicroScan (Beckman Coulter). The antibiotic susceptibility was interpreted according to the Clinical Laboratory Standards Institute (CLSI) criteria.[14] The multidrug resistance pattern and the presence of ESBL were assessed by the Kirby-Bauer disk diffusion method.

All multidrug-resistant strains were retested by disk diffusion method following the CLSI recommendations. The antibiotics tested were penicillins, third-generation cephalosporins, aztreonam, carbapenems, aminoglycosides, quinolones, and cotrimoxazole. To assess the ESBL phenotype, a double-disk diffusion test was performed to show the synergy of cefuroxime, cefotaxime, ceftazidime, and cefepime with amoxicillin/clavulanic acid.

Molecular typing

K. pneumoniae isolates were typed by pulsed-field gel electrophoresis (PFGE) after restriction with *Xba*I. Band patterns were visually compared using the criteria described by Tenover *et al.*[15] At least one isolate for each PFGE cluster was subjected to Multilocus Sequence Typing (MLST) following protocol 2, described at the MLST web site of the Institute Pasteur (Paris, France; http://bigsdb.web.pasteur.fr/Klebsiella/Klebsiella.html). The allele number and Sequence Type (ST) were assigned using this MLST website.

Bacteremia data were analyzed using the goeBURST algorithm and an extension of the goeBURST rules to draw a full Minimum Spanning Tree (MST). The analysis of MLSTs was performed using *Phyloviz* software (https://online.phyloviz.net/index).[16]

The goeBURST Full MST algorithm represents STs in a combination of circles and lines separating the STs according to their allelic differences.

To differentiate between the recently described *K. pneumoniae*-related species (*Klebsiella variicola*, *Klebsiella quasipneumoniae*, and *K. pneumoniae sensu* stricto), we performed a MLSA (multilocus sequence alignment) as described previously and using the reference ST described for these three species.[17]

Putative ESBL strains as deduced for the results of the double-disk diffusion assay were subjected to a multiplex polymerase chain reaction (PCR) assay confirming the detection of bla_{SHV}, bla_{TEM}, bla_{CTX-M}, and bla_{OXA} family genes.[18] For OXA-positive strains, the PCR amplicons were sequenced. Among CTX-M-positive strains, a PCR plus sequencing to differentiate families CTX-M-1 and CTX-M-9 groups was performed. Finally, because this PCR scheme did not differentiate ESBL or non-ESBL enzymes of TEM and SHV families, further PCR plus sequencing analysis was performed[19–21] (www.lahey.org/Studies). The PCR products were sequenced by Sanger methodology at Macrogen, Inc. (Seoul, Korea)

Statistical analyses

Statistical analyses were carried out using PASW Statistics 18. The odds ratios (ORs) and 95% confidence intervals were calculated, and Fisher's exact test or chi-square test were used for statistical analysis as appropriate. All probabilities were two-tailed, and a *p*-value <0.05 being considered statistically significant.

Results

Microbiological and clinical features of patients with NA, HCA, or CA bacteremia due to K. pneumoniae

During the study period (January 2007 to December 2009) 348 episodes of *Klebsiella spp.* bacteremia were detected in 335 patients. Eleven patients had two episodes and one patient had three episodes (defined as those occurred at least 1 month apart).

Table 1 shows the characteristics of the 348 bacteremia episodes in the three groups analyzed. The overall mean age (±standard deviation) was 63.73 ± 15.81 years and 210/348 (60.35%) were males.

Fifty-three (15.2%) episodes were considered primary bacteremia: 41 NA isolates versus 6 HCA isolates versus 6 CA isolates. There were differences in the foci of infection comparing the three groups (NA vs. HCA vs. CA): catheter-related bacteremia (39.9%, 5.2%, 0%) was more frequent among NA episodes, whereas the urinary tract (16.6%, 50%, 43.3%) and biliary tract origin (12.6%, 29.3%, and 37.3%) were more frequently found among HCA and CA infections.

The presence of underlying diseases was similar for the three groups of patients. Diabetes mellitus, malignancy, and cardiovascular disease were the most frequent comorbidities among NA patients (22.9%, 39%, and 30.5%), HCA patients (27.6%, 60.3%, and 30.5%), and CA patients (32.8%, 14.9%, and 28.3%).

TABLE 1. ANALYSIS OF DEMOGRAPHIC DATA AND SOURCE OF BACTEREMIA AMONG NOSOCOMIAL,
HEALTHCARE-ASSOCIATED, AND COMMUNITY-ACQUIRED *KLEBSIELLA PNEUMONIAE* BACTEREMIA CASES

Characteristics	NA N = 223 (64.1%)	HCA N = 58 (16.7%)	CA N = 67 (19.2%)	HCA versus NA	CA versus NA p value	CA versus HCA
Mean age	61.5 (±15.815)	65.4 (±13.270)	69.8 (±16.266)	0.088	0	0.102
Sex (male)	62.8%	60.3%	52.2%	0.733	0.122	0.363
Source of bacteremia						
Primary bacteremia	41 (18.4)	6 (10.3)	6 (8.9)	0.144	0.066	0.793
Urinary tract	37 (16.6)	29 (50)	29 (43.3)	**<0.001**	**<0.001**	0.025
With urinary catheter	34 (15.2)	15 (25.9)	0	0.058	**<0.001**	**<0.001**
Without urinary catheter	3 (1.4)	14 (24.2)	29 (43.3)	0	0	0.025
Intra-abdominal						
Abdominal	12 (5.4)	2 (3.5)	4 (6)	0.547	0.854	0.510
Biliary tract	24 (10.8)	14 (24.2)	21 (31.3)	0.008	**<0.001**	0.370
SBP	3 (1.3)	1 (1.7)	2 (3)	0.828	0.366	0.646
Liver abscess	1 (0.5%)	2 (3.5)	2 (3)	0.048	0.072	0.882
Soft tissues and skin	3 (1.3)	0	1 (1.5)	0.374	0.929	0.350
Pneumonia	10 (4.5)	1 (1.7)	2 (3)	0.334	0.589	0.646
Catheter-related bacteremia	89 (39.9)	3 (5.2)	0	**<0.001**	0	0.059
Other focus	3 (1.3)	0	0	0.374	0.340	0
Main underlying diseases						
Diabetes mellitus	51 (22.9)	16 (27.6)	22 (32.8)	0.453	0.110	0.524
Malignancy	87 (39)	35 (60.3)	10 (14.9)	**0.003**	**<0.001**	**<0.001**
Cardiovascular disease	68 (30.5)	7 (12.1)	19 (28.3)	0.005	0.879	0.025
Chronic lung disease	27 (12.1)	8 (13.8)	11 (16.4)	0.729	0.409	0.684
Cerebrovascular disease	22 (9.9)	5 (8.6)	5 (7.5)	0.775	0.810	0.811
Chronic liver disease	13 (5.8)	6 (10.3)	9 (13.4)	0.223	0.061	0.596
End-stage chronic renal failure	4 (1.8)	5 (8.6)	0	0.008	0.577	0.014
Solid organ transplant	13 (5.8)	4 (6.9)	4 (6)	0.762	1	0.832
30-Day mortality	53 (23.8)	12 (20.7)	11 (16.4)	0.621	0.241	0.539

Significant differences are indicated in boldface.
CA, community-acquired; HCA, healthcare-associated; NA, nosocomial; SBP, spontaneous bacterial peritonitis in hepatic cirrhosis patients.

The median length of hospitalization was 23.6 (0–99) days in patients with NA bacteremia. The overall 30 days mortality was also higher among NA episodes (23.8%, 20.7%, and 16.4%, NA, HCA, and CA, respectively); however, these differences did not reach statistical significance (Table 1). Most of NA bacteremia episodes occurred in patients admitted to intensive care 99 (44.4%), hematology 23 (10.3%), or surgery 18 (8.1%) wards.

Differences of antimicrobial resistance rates for *K. pneumoniae* bacteremic isolates were detected among NA, HCA, and CA. In general, NA isolates had higher resistance rates than those of HCA and CA: (NA vs. HCA vs. CA) for amoxicillin/clavulanic acid (25.1%, 13.8%, and 10.5%), gentamicin (5.8%, 1.8%, 0%), tobramycin (13.4%, 3.5%, 0%), amikacin (8.5%, 1.7%, 0%), cotrimoxazole (24.7%, 17.3%, 10.4%), and ciprofloxacin (32.3%, 24.1%, 17.9%). The ESBL

TABLE 2. RATES OF ANTIMICROBIAL RESISTANCE AMONG NOSOCOMIAL, HEALTHCARE-ASSOCIATED,
AND COMMUNITY-ACQUIRED *K. PNEUMONIAE* BACTEREMIA

Episodes[a]	NA N = 223 (64.1%)	HCA N = 58 (16.7%)	CA N = 67 (19.2%)	HCA versus NA	CA versus NA, p values	CA versus HCA
Amox/clav	56 (25.1)	8 (13.8)	7 (10.5)	0.067	0.011	0.566
Piper/taz	44 (19.7)	5 (8.6)	4 (6)	0.047	0.008	0.568
Cefotaxime	24 (10.8)	4 (6.9)	2 (3)	0.381	0.051	0.308
Ceftazidime	33 (14.8)	4 (6.9)	2 (3)	0.113	0.009	0.308
Cefepime	24 (10.8)	4 (6.9)	2 (3)	0.381	0.051	0.308
Gentamicin	13 (5.8)	1 (1.7)	0	0.200	0.044	0.281
Tobramycin	30 (13.4)	2 (3.5)	0	0.033	**<0.001**[c]	0.125
Cotrimoxazole	55 (24.7)	10 (17.3)	7 (10.4)	0.232	0.011	0.269
Ciprofloxacin	72 (32.3)	14 (24.1)	12 (17.9)	0.230	0.031	0.392
Amikacin	19 (8.5)	1 (1.7)	0	0.073	0.009	0.281
Antimicrobial susceptibility[b]	115 (51.6)	34 (58.6)	43 (64.2)	0.338	0.093	0.524

[a]After excluding outbreak isolates, these antimicrobial resistance rates were close similar to those of CA and HCA isolates (data not shown).
[b]Susceptible isolates to all antimicrobial tested, with the exception of ampicillin, piperacillin, and ticarcillin.
[c]Significant differences are indicated in boldface.

TABLE 3. EXTENDED-SPECTRUM BETA-LACTAMASES
PRODUCTION PATTERNS AMONG NOSOCOMIAL,
HEALTHCARE-ASSOCIATED, AND COMMUNITY-ACQUIRED
K. PNEUMONIAE BACTEREMIA

Type of beta-lactamase No.	NA N = 223 (64.1%)	HCA N = 58 (16.7%)	CA N = 67 (19.2%)
Total number of ESBL producers	21 (9.4)	4 (6.9)	0
CTX-M-1 and SHV-1	4	2	0
CTX-M-1, SHV-1, and TEM-1	2	0	0
CTX-M-1, SHV-1, and OXA-1	2	1	0
CTX-M-1 and SHV-11	1	0	0
CTX-M-1 and SHV-2	1	0	0
CTX-M-1 and SHV-100	0	1	0
CTX-M-1, SHV-26, and TEM-1	1	0	0
CTX-M-9 and SHV-1	1	0	0
CTX-M-9 and SHV-11	1	0	0
CTX-M-9 and SHV-2	1	0	0
CTX-M-9 and SHV-2A	1	0	0
CTX-M-9 and SHV-12	1	0	0
CTX-M-9 and SHV-26	1	0	0
CTX-M-9, SHV-11, and TEM-1	1	0	0
CTX-M-9, SHV-1, and OXA-1	1	0	0
SHV-2 and TEM-1	2	0	0

Non-ESBLs are underlined (SHV-1, SHV-11, TEM-1, and OXA-1). ESBL, extended-spectrum beta-lactamases.

production according to the positivity of double-disk diffusion test was detected in 24 (10.8%) episodes of NA, in 4 (6.9%) of HCA, and in 2 (3%) of CA. All of the ESBL producers belonged to Kp I-*K. pneumoniae*.

After sequencing, 5 of 30 isolates with positive double-disk synergy were not classified as ESBL. These five strains had one or two beta-lactamases: SHV-1 plus TEM-1 ($n = 2$), SHV-1 plus TEM-1 plus OXA-1 ($n = 1$), SHV-11 plus TEM-1 ($n = 1$), and SHV-83 ($n = 1$). The remaining 25 strains showed different beta-lactamase production patterns as shown in Table 3. The bla_{CTX-M} was the most common ESBL. Of note, the presence of more than one ESBL in eight strains (Table 3).

Comparative of molecular typing in NA, HCA, and CA bacteremia

Molecular typing of bacteremias was performed by PFGE and MLST. Although we identified minor clusters among NA isolates, a high genetic diversity was observed in all three groups. In fact, the Simpson's diversity index was 0.983 in NA, 0.988 in HCA, and 0.996 in CA.

Overall, by PFGE/MLST analysis we obtained 161 different STs. By groups using *Phyloviz* software, we found 127 STs among 191 NA isolates, 42 STs among 54 HCA isolates, and 55 STs among 59 CA isolates. Fourteen STs were found in NA and HCA isolates, 14 STs in NA and CA, and 3 STs in HCA and CA. Finally, seven STs were found in all three groups: ST13, ST15, ST17, ST35, ST37, ST42, and ST45 (Supplementary Table S1 [Supplementary Data are

available online at www.liebertpub.com/mdr] and Fig. 1). Seventeen STs (shared by three or more isolates) accounted for 38.1% of the isolates.

After e-burst analysis, there were 13 clonal groups (GC1–CG13; defined as an ST and single locus variant [SLV]), and 125 singletons. The 13 clonal sets accounted for 123 isolates; however, there were differences among them (Supplementary Table S1). There was 28 singletons with more than one isolate ($n = 87$ isolates) and 97 singletons, which only presented one isolate. For instance, clonal complex 14 accounted for 28 isolates with 5 STs. Most of them had caused NA bacteremia. In fact, this CC14 was related to an outbreak of *K. pneumoniae* producing OXA-1 beta-lactamase in our hospital.[22]

Other clonal groups accounting for more than 10 isolates were associated either with CA, NA, and HCA episodes. The same occurred among the 28 singletons accounting for more than one isolate. When the origin of bacteremia was analyzed, no relationship between ST and focus of infection could be established (Supplementary Table S2).

By MLSA analysis (Fig. 2), the species distribution was as follows: Kp III-*K. variicola* $n = 25$ (8.1%); Kp II-*K. quasipneumoniae* $n = 16$ (5.2%), Kp I-*K. pneumoniae sensu stricto* $n = 266$ (86.6%), being similar in all three groups by CA, NA, and HCA.

Discussion

Klebsiella pneumoniae is an important cause of Gram-negative bacteremia all over the world and has been largely analyzed in the hospital setting causing infections due to multiresistant strains.[9,23–26] In addition, *K. pneumoniae* is a frequent cause of other endogenous NA infections without bacteremia (urinary tract infection, surgical infection, etc.), although some geographical differences have been detected.[8,10] In the community, *K. pneumoniae* is the second cause of both urinary tract infections and Gram-negative CA bacteremia.[27–29] In this study, we analyzed clinical and molecular characteristics associated with *K. pneumoniae* bacteremia, including NA, HCA, and CA episodes.[13]

Our study, in agreement with others, shows that *K. pneumoniae* bacteremia has different clinical features in the community or in the hospital.[9] Moreover, healthcare-related episodes were similar to CA ones. There were differences regarding the foci of infection, the urinary and the bile tract being the most frequent origin in the CA and HCA, whereas catheter-related bacteremia was the most frequent among NA cases. In our series, as in others from Europe and North America, the bile tract is a main origin of *K. pneumoniae* bacteremia in outpatients, whereas in Asian or Oceania countries the origin related to liver abscess is more frequent.[3,9,29,30]

In any case, *K. pneumoniae* bacteremia occurred mainly in elderly patients with underlying conditions. As described for other infections, diabetes mellitus was an important underlying disease for *K. pneumoniae* bacteremia in all the three groups.[6,26] Malignancy was mainly present among HCA and NA bacteremia episodes; in fact, this microorganism is a frequent cause of Gram-negative bacteremia in these patients.[31] Although the highest mortality rate was found among patients with NA bacteremia, these differences were not statistically significant and were similar to those found by other authors.[9,32–34]

In our study only 7.2% of the isolates produced an ESBL and these were mainly associated with CTX-M family.[2,35,36]

FIG. 1. The Minimum Spanning Tree was computed using the goeBURST algorithm and ST nodes shown in three colors representing ST nodes (https://online.phyloviz.net/ index). STs, sequence types. CA, community acquired; HCA, healthcare associated; NA, nosocomial.

FIG. 2. Phylogenetic tree of MLSA based on six concatenated housekeeping genes of the genus *Klebsiella*. MLSA, multilocus sequence alignment.

Longer hospitalization, invasive procedures, and inappropriate antibiotic therapy or antibiotic use could raise the prevalence of resistant strains or ESBL-producing *K. pneumoniae*.[9,25,33] In the early 1990s and 2000s the ESBL-producing *K. pneumoniae* were associated with the hospital setting. However, the number of HCA and CA ESBL infections is currently increasing.[2,4,35,36] Nevertheless, community-acquired ESBL–*K.pneumoniae* infections were infrequent in our series, reflecting that hospital environment is a predominant site to disseminate these strains.[3,4,32,36] In this way, the higher antibiotic resistance rates were associated with NA cases. However, these rates were similar to CA or HCA isolates when the clonal outbreak-related isolates were excluded. On the other hand, the rates of quinolone and cotrimoxazole resistance were up to 10% and 15% for CA and HCA isolates, which preclude their use as empirical therapy in this setting. Moreover, the presence of mobile elements with resistance determinants to fluoroquinolones and to cotrimoxazole has been described for other pathogens, such as *E. coli*.[2,37,38]

The genetic diversity of *K. pneumoniae* isolates has not been extensively studied outside hospital outbreaks. In the present study, we analyzed this diversity and we found a high heterogeneity among the isolates. However, some clonal groups were found and were present in both the hospital and the community. Moreover, we found some antibiotic-susceptible strains with STs previously associated with ESBL or carbapenemase-producing strains.[39–42] For instance, all ST20 isolates in our series were antibiotic susceptible, whereas this ST has been associated with KPC- or NDM-producing isolates. However, all four isolates of this ST20 caused NA or HCA bacteremia, and this is probably a hospital-adapted clone with the ability to acquire resistance determinants.

Nevertheless, the high genetic variability among NA *K. pneumoniae* bacteremia isolates suggests that these infections had an endogenous origin. Despite the heterogeneity, the major cluster (CG1) included 16 patients with NA bacteremia episodes caused by ST14 strain harboring bla_{OXA-1} gene and resistant to quinolones and aminoglycosides.[22]

We could not link the origin of infection with specific STs or clonal complex (CC), including the hypervirulent clones. For instance, ST23 commonly associated with patients with pyogenic liver abscess was also found causing catheter-related bacteremia as described previously.[43] Similar results were observed in other studies, where no relationship was detected between pathogenicity of strains and specific MLST.[26] It seems that *K. pneumoniae* infections varied regarding clinical characteristics of patients, geographic distribution of serotypes, and the presence of epidemic strains.[29,44]

The present study has two main limitations: first, it was performed at a single hospital; and second, the molecular typing was performed retrospectively, and some cases were missing. However, it is an important series which improves the knowledge of molecular features of *K. pneumoniae* causing bacteremia and its relationship with the origin of infection and the contact with the healthcare system.

Conclusions

This study shows a high genetic variability among *K. pneumoniae* isolates causing bacteremia and suggests they may be considered endogenous infections. The higher resistance rates observed in the hospital setting could be linked to an NA outbreak. Finally, high-risk clones associated to MDR could be found as susceptible in the healthcare-related settings.

Disclosure Statement

The authors declare that they have no conflicts of interest.

References

1. Hussein, K., A. Raz-Pasteur, R. Finkelstein, A. Neuberger, Y. Shachor-Meyouhas, I. Oren, and I. Kassis. 2013. Impact of carbapenem resistance on the outcome of patients' hospital-acquired bacteraemia caused by *Klebsiella pneumoniae*. J. Hosp. Infect. 83:307–313.
2. Peralta, G., M. Lamelo, P. Alvarez-García, M. Velasco, A. Delgado, J.P. Horcajada, M. Montero, M.P. Roiz, M.C. Fariñas, J. Alonso, L.M. Martínez, A. Gutiérrez-Macías, J.A. Alava, A. Rodríguez, A. Fleites, V. Navarro, E. Sirvent, and J.A. Capdevila. 2012. Impact of empirical treatment in extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella spp*. bacteremia. A multicentric cohort study. BMC Infect. Dis. 12:245.
3. Meatherall, B.L., D. Gregson, T. Ross, J.D.D. Pitout, and K.B. Laupland. 2009. Incidence, risk factors, and outcomes of *Klebsiella pneumoniae* bacteremia. Am. J. Med. 122:866–873.
4. Marra, A.R., S.B. Wey, A. Castelo, A.C. Gales, R.G.R. Cal, J.R.D.C. Filho, M.B. Edmond, and C.A.P. Pereira. 2006. Nosocomial bloodstream infections caused by *Klebsiella pneumoniae*: impact of extended-spectrum beta-lactamase (ESBL) production on clinical outcome in a hospital with high ESBL prevalence. BMC Infect. Dis. 6:24.
5. Yang, C.-C., S.-H. Li, F.-R. Chuang, C.-H. Chen, C.-H. Lee, J.-B. Chen, C.-H. Wu, and C.-T. Lee. 2012. Discrepancy between effects of carbapenems and flomoxef in treating nosocomial hemodialysis access-related bacteremia secondary to extended spectrum beta-lactamase producing *Klebsiella pneumoniae* in patients on maintenance hemodialysis. BMC Infect. Dis. 12:206.
6. Ito, R., Y. Shindo, D. Kobayashi, M. Ando, W. Jin, J.I. Wachino, K. Yamada, K. Kimura, T. Yagi, Y. Hasegawa, and Y. Arakawa. 2015. Molecular epidemiological characteristics of *Klebsiella pneumoniae* associated with bacteremia among patients with pneumonia. J. Clin. Microbiol. 53:879–886.
7. Lee, J., C.-I. Kang, E.-J. Joo, Y.E. Ha, S.-J. Kang, S.Y. Park, D.R. Chung, K.R. Peck, K.S. Ko, N.Y. Lee, and J.-H. Song. 2011. Epidemiology and clinical features of community-onset bacteremia caused by extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*. Microb. Drug Resist. 17:267–273.
8. Pien, B.C., P. Sundaram, N. Raoof, S.F. Costa, S. Mirrett, C.W. Woods, L.B. Reller, and M.P. Weinstein. 2010. The clinical and prognostic importance of positive blood cultures in adults. Am. J. Med. 123:819–828.

9. Tsay, R.-W., L.K. Siu, C.-P. Fung, and F.-Y. Chang. 2002. Characteristics of bacteremia between community-acquired and nosocomial *Klebsiella pneumoniae* infection. Arch. Intern. Med. 162:1021.

10. Marschall, J., V.J. Fraser, J. Doherty, and D.K. Warren. 2009. Between community and hospital: healthcare-associated gram-negative bacteremia among hospitalized patients. Infect. Control Hosp. Epidemiol. 30:1050–1056.

11. Neidell, M.J., B. Cohen, Y. Furuya, J. Hill, C.Y. Jeon, S. Glied, and E.L. Larson. 2012. Costs of healthcare-and community-associated infections with antimicrobial-resistant versus antimicrobial-susceptible organisms. Clin. Infect. Dis. 55:807–815.

12. Rosenthal, V.D., D.G. Maki, Y. Mehta, H. Leblebicioglu, Z.A. Memish, H.H. Al-Mousa, H. Balkhy, B. Hu, C. Alvarez-Moreno, E.A. Medeiros, A. Apisarnthanarak, L. Raka, L.E. Cuellar, A. Ahmed, J.A. Navoa-Ng, A.A. El-Kholy, S.S. Kanj, I. Bat-Erdene, W. Duszynska, N. Van Truong, L.N. Pazmino, L.C. See-Lum, R. Fernández-Hidalgo, G. Di-Silvestre, F. Zand, S. Hlinkova, V. Belskiy, H. Al-Rahma, M.T. Luque-Torres, N. Bayraktar, Z. Mitrev, V. Gurskis, D. Fisher, I.B. Abu-Khader, K. Berechid, A. Rodríguez-Sánchez, F.G. Horhat, O. Requejo-Pino, N. Hadjieva, N. Ben-Jaballah, E. García-Mayorca, L. Kushner-Dávalos, S. Pasic, L.E. Pedrozo-Ortiz, E. Apostolopoulou, N. Mejía, M.O. Gamar-Elanbya, K. Jayatilleke, M. De Lourdes-Dueñas, and G. Aguirre-Avalos. 2014. International Nosocomial Infection Control Consortium (INICC) report, data summary of 43 countries for 2007–2012. Device-associated module. Am. J. Infect. Control 42:942–956.

13. Friedman, N.D., K.S. Kaye, J.E. Stout, S.A. Mcgarry, S.L. Trivette, J.P. Briggs, W. Lamm, C. Clark, J. Macfarquhar, A.L. Walton, L.B. Reller, and D.J. Sexton. 2002. Health care-associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann. Fam. Med. 137:791–798.

14. CLSI. 2015. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement M100-S25Clinical and Laboratory Standards Institute (CLSI). Wayne, PA.

15. Tenover, F.C., R.D. Arbeit, Goering R V., P.A. Mickelsen, B.E. Murray, D.H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33:2233–2239.

16. Ribeiro-Gonçalves, B., A.P. Francisco, C. Vaz, M. Ramirez, and J.A. Carriço. 2016. PHYLOViZ Online: web-based tool for visualization, phylogenetic inference, analysis and sharing of minimum spanning trees. Nucleic Acids Res. 44: W246–W251.

17. Maatallah, M., M. Vading, M.H. Kabir, A. Bakhrouf, M. Kalin, P. Nauclér, S. Brisse, and C.G. Giske. 2014. *Klebsiella variicola* is a frequent cause of bloodstream infection in the Stockholm area, and associated with higher mortality compared to *K. pneumoniae*. PLoS One 9:e113539.

18. Fang, H., F. Ataker, G. Hedin, and K. Dornbusch. 2008. Molecular epidemiology of extended-spectrum β-lactamases among *Escherichia coli* isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. J. Clin. Microbiol. 46:707–712.

19. Coque, T.M., A. Oliver, J.C. Pérez-Díaz, F. Baquero, and R. Cantón. 2002. Genes encoding TEM-4, SHV-2, and CTX-M-10 extended-spectrum β-lactamases are carried by multiple *Klebsiella pneumoniae* clones in a single hospital (Madrid, 1989 to 2000). Antimicrob. Agents Chemother. 46:500–510.

20. Hujer, K.M., A.M. Hujer, E.A. Hulten, S. Bajaksouzian, J.M. Adams, C.J. Donskey, D.J. Ecker, C. Massire, M.W. Eshoo, R. Sampath, J.M. Thomson, P.N. Rather, D.W. Craft, J.T. Fishbain, A.J. Ewell, M.R. Jacobs, D.L. Paterson, and R.A. Bonomo. 2006. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob. Agents Chemother. 50:4114–4123.

21. Bush, K., and G.A. Jacoby. 2010. Updated functional classification of beta-lactamases. Antimicrob. Agents Chemother. 54:969–976.

22. Cubero, M., L. Calatayud, F. Tubau, J. Ayats, C. Peña, R. Martín, J. Liñares, M.Á. Domínguez, and C. Ardanuy. 2013. Clonal spread of *Klebsiella pneumoniae* producing OXA-1 betalactamase in a Spanish hospital. Int. Microbiol. 16:227–233.

23. Bodro, M., N. Sabé, F. Tubau, L. Lladó, C. Baliellas, J. Roca, J.M. Cruzado, J. Carratalà, N. Sabe, F. Tubau, L. Llado, C. Baliellas, J. Roca, J.M. Cruzado, and J. Carratala. 2013. Risk factors and outcomes of bacteremia caused by drug-resistant ESKAPE pathogens in solid-organ transplant recipients. Transplantation 96:843–849.

24. Haddy, R.I., M. Lee, III, S.P. Sangal, G.S. Walbroehl, C.S. Hambrick, and G.M. Sarti. 1989. *Klebsiella pneumoniae* bacteremia in the community hospital. J. Fam. Pract. 28: 686–690.

25. Koupetori, M., T. Retsas, N. Antonakos, G. Vlachogiannis, I. Perdios, C. Nathanail, K. Makaritsis, A. Papadopoulos, D. Sinapidis, E.J. Giamarellos-Bourboulis, I. Pneumatikos, C. Gogos, A. Armaganidis, and E. Paramythiotou. 2014. Bloodstream infections and sepsis in Greece: over-time change of epidemiology and impact of de-escalation on final outcome. BMC Infect. Dis. 14:272.

26. Tseng, C.P., H.S. Wu, T.H. Wu, Y.T. Lin, and C.P. Fung. 2013. Clinical characteristics and outcome of patients with community-onset *Klebsiella pneumoniae* bacteremia requiring intensive care. J. Microbiol. Immunol. Infect. 46:217–223.

27. Decré, D., C. Verdet, A. Emirian, T. Le Gourrierec, J.C. Petit, G. Offenstadt, E. Maury, S. Brisse, and G. Arlet. 2011. Emerging severe and fatal infections due to *Klebsiella pneumoniae* in two university hospitals in France. J. Clin. Microbiol. 49:3012–3014.

28. Isendahl, J., C. Manjuba, A. Rodrigues, W. Xu, B. Henriques-Normark, C.G. Giske, and P. Nauclér. 2014. Prevalence of community-acquired bacteraemia in Guinea-Bissau: an observational study. BMC Infect. Dis. 14:3859.

29. Ko, W.C., D.L. Paterson, A.J. Sagnimeni, D.S. Hansen, A. Von Gottberg, S. Mohapatra, J.M. Casellas, H. Goossens, L. Mulazimoglu, G. Trenholme, K.P. Klugman, J.G. McCormack, and V.L. Yu. 2002. Community-acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. Emerg. Infect. Dis. 8:160–166.

30. Melot, B., J. Colot, and G. Guerrier. 2015. Bacteremic community-acquired infections due to *Klebsiella pneumoniae*: clinical and microbiological presentation in New Caledonia, 2008–2013. Int. J. Infect. Dis. 41:29–31.

31. Marín, M., C. Gudiol, C. Garcia-Vidal, C. Ardanuy, and J. Carratalà. 2014. Bloodstream infections in patients with solid tumors: epidemiology, antibiotic therapy, and outcomes in 528 episodes in a single cancer center. Medicine (Baltimore) 93:143–149.

32. Hongsuwan, M., P. Srisamang, M. Kanoksil, N. Luanga-sanatip, A. Jatapai, N.P. Day, S.J. Peacock, B.S. Cooper, and D. Limmathurotsakul. 2014. Increasing incidence of hospital-acquired and healthcare-associated bacteremia in northeast Thailand: a multicenter surveillance study. PLoS One 9:e109324.

33. Wolfe, C.M., B. Cohen, and E. Larson. 2014. Prevalence and risk factors for antibiotic-resistant community-associated bloodstream infections. J. Infect. Public Health 7:224–232.

34. Chetcuti Zammit, S., N. Azzopardi, and J. Sant. 2014. Mortality risk score for *Klebsiella pneumoniae* bacteraemia. Eur. J. Intern. Med. 25:571–576.

35. Calbo, E., and J. Garau. 2015. The changing epidemiology of hospital outbreaks due to ESBL-producing *Klebsiella pneumoniae*: the CTX-M-15 type consolidation. Future Microbiol. 10:1063–1075.

36. Webster, D.P., B.C. Young, R. Morton, D. Collyer, B. Batchelor, J.F. Turton, S. Maharjan, D.M. Livermore, P. Bejon, B.D. Cookson, and I.C.J.W. Bowler. 2011. Impact of a clonal outbreak of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in the development and evolution of bloodstream infections by *K. pneumoniae* and *Escherichia coli*: an 11 year experience in Oxfordshire, UK. J. Antimicrob. Chemother. 66:2126–2135.

37. Talan, D.A., A. Krishnadasan, F.M. Abrahamian, W.E. Stamm, and G.J. Moran; Group for the EmergeIDNETS. 2008. Prevalence and risk factor analysis of trimethoprim-sulfamethoxazole- and fluoroquinolone-resistant *Escherichia coli* infection among emergency department patients with pyelonephritis. Clin. Infect. Dis. 47:1150.

38. Rodríguez-Martínez, J.M., C. Velasco, I. García, M.E. Cano, L. Martínez-Martínez, and A. Pascual. 2007. Characterisation of integrons containing the plasmid-mediated quinolone resistance gene qnrA1 in *Klebsiella pneumoniae*. Int. J. Antimicrob. Agents 29:705–709.

39. Ruiz-Garbajosa, P., T. Curiao, M. Tato, D. Gijón, V. Pintado, A. Valverde, F. Baquero, M.I. Morosini, T.M. Coque, and R. Cantón. 2013. Multiclonal dispersal of KPC genes following the emergence of non-ST258 KPC-producing *Klebsiella pneumoniae* clones in Madrid, Spain. J. Antimicrob. Chemother. 68:2487–2492.

40. Jin, Y., C. Shao, J. Li, H. Fan, Y. Bai, and Y. Wang. 2015. Outbreak of multidrug resistant NDM-1-producing *Klebsiella pneumoniae* from a neonatal unit in Shandong Province, China. PLoS One 10:e0119571.

41. Bialek-Davenet, S., A. Criscuolo, F. Ailloud, V. Passet, L. Jones, A.S. Delannoy-Vieillard, B. Garin, S. Le Hello, G. Arlet, M.H. Nicolas-Chanoine, D. Decré, and S. Brisse. 2014. Genomic definition of hypervirulent and multidrug-resistant *Klebsiella pneumoniae* clonal groups. Emerg. Infect. Dis. 20: 1812–1820.

42. Brisse, S., C. Fevre, V. Passet, S. Issenhuth-Jeanjean, R. Tournebize, L. Diancourt, and P. Grimont. 2009. Virulent clones of *Klebsiella pneumoniae*: identification and evolutionary scenario based on genomic and phenotypic characterization. PLoS One 4:e4982.

43. Cubero, M., I. Grau, F. Tubau, R. Pallarés, M.A. Dominguez, J. Liñares, and C. Ardanuy. 2016. Hypervirulent *Klebsiella pneumoniae* clones causing bacteraemia in adults in a teaching hospital in Barcelona, Spain (2007–2013). Clin. Microbiol. Infect. 22:154–160.

44. Liao, C.H., Y.T. Huang, C.C. Lai, C.Y. Chang, F.Y. Chu, M.S. Hsu, H.S. Hsu, and P.R. Hsueh. 2011. *Klebsiella pneumoniae* bacteremia and capsular serotypes, Taiwan. Emerg. Infect. Dis. 17:1113–1115.

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