Fosfomycin plus Beta-lactams: Synergistic Bactericidal Combinations in Methicillin-resistant (MRSA) and Glycopeptide-Intermediate Resistant (GISA) *Staphylococcus aureus* Experimental Endocarditis.

Authors: del Río A[1*], García-de-la-Mària C[1]*, Entenza J.M[2], Gasch O[3], Armero Y[1], Soy D[5], Mestres C.A[6], Pericás J.M[1], Falces C[6], Ninot S[6], Almela M[4], Cervera C[1], Gatell J. M[1], Moreno A[1], Moreillon P[2], F. Marco[4], Miró J. M[1] and the Hospital Clinic Experimental Endocarditis Study Group[7]

[1]Infectious Diseases Service, Microbiology Service[4], Pharmacy Service[5] and Cardiovascular Institute[6]. Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona School of Medicine, Barcelona, Catalunya, Spain;

[2] Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland;

[3] Corporació Sanitària Parc Taulí. Universitat Autònoma de Barcelona. Sabadell, Spain.

[*]Equivalent contribution

[7] Members are listed in the Appendix.

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Corresponding author: Dr. Jose M. Miró, Infectious Diseases Service, Hospital Clínic, Villarroel 170, 08036-Barcelona, Spain. (jmmiro@ub.edu).

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Abstract

The urgent need of effective therapies for MRSA infective endocarditis (IE) is cause of concern. We aimed to ascertain the *in vitro* and *in vivo* activity of the old antibiotic fosfomycin combined with different beta-lactams against methicillin-resistant (MRSA) and glycopeptide-intermediate resistant (GISA) *S. aureus* strains. Time-kill tests with ten isolates showed that fosfomycin plus imipenem (FOF+IPM) was the most active evaluated combination. In an aortic valve IE model with two strains (MRSA-277H and GISA-ATCC700788), the following intravenous regimens were compared: fosfomycin (2g q8h) plus imipenem (1g q6h) or ceftriaxone (2g q12h) (FOF+CRO), vancomycin at standard dose (VAN-SD) (1g q12h) and high-dose (VAN-HD) (1g q6h). Whereas a significant reduction of MRSA-227H load in the vegetations was observed with FOF+IPM compared with VAN-SD (0 [0-1] *vs.* 2 [0-5.1] log CFU/g veg; P= 0.01), no statistical differences were found with VAN-HD. In addition, FOF+IPM sterilized more vegetations than VAN-SD (11/15 [73%] vs. 5/16 [31%]; P=0.02). GISA-ATCC700788 load in the vegetations was significantly lower after FOF+IPM or FOF+CRO compared with VAN-SD (2 [0-2] and 0 [0-2] *vs.* 6.5 [2-6.9] logCFU/g veg; P<0.01). The number of sterilized vegetations after FOF+CRO was higher than VAN-SD or VAN-HD (8/15 [53%] vs. 4/20 [20%] or 4/20 [20%]; P=0.03). To assess the effect of FOF+IPM on penicillin binding protein (PBP) synthesis, molecular studies were performed, showing that FOF+IPM significantly decreased PBP1 and PBP3 synthesis. These results allow clinicians considering the use of FOF+IPM or FOF+CRO to treat MRSA or GISA IE.

Introduction.

Staphylococcus aureus is the most frequent causative pathogen in all types of infective endocarditis (IE) (1-3). About 30% of all *S. aureus* isolates causing IE are methicillin-resistant (MRSA) (1). Vancomycin (VAN) is the antibiotic of choice for MRSA IE (4-6) despite its well-known limitations, including low penetration into vegetations (7) and a slow bactericidal effect (8, 9). In addition, *S. aureus* clinical isolates with intermediate resistance to glycopeptides (GISA) and heterorresistance (hGISA) have been observed in the last decades (10-15) and VAN minimum inhibitory concentration (MIC) creep has been matter of concern in the health-care systems around the world (16-19). As a result, clinical failures in the treatment of MRSA invasive infections are frequently observed (8, 20). In order to increase its efficacy, VAN high-dose regimens have been considered in clinical practice, being the area under the curve/MIC (AUC/MIC)>400 a pharmacodynamic predictor of favourable clinical outcomes in MRSA invasive infections (21, 22). However, these regimens are limited by VAN dose-dependent toxicity (23). The addition of other antibiotics has not improved VAN efficacy in most studies (8, 24, 25).

Daptomycin is also considered a first-line therapy for MRSA native valve IE (4). However, clinical failures and emergence of resistance to daptomycin have been reported (20, 26, 27). Other active antibiotics against MRSA such as linezolid (28-32) or telavancin (33) have failed to show clear superiority to VAN in the *in vivo* models. Ceftaroline has been tested in *in vivo* experimental studies (34) and a case-series (35) with promising results. However, further studies are needed to assess its efficacy in MRSA IE. As a result, alternative therapeutic options against MRSA IE are urgently needed (36).

Fosfomycin (FOF), an old antibiotic described in 1969 (37) has shown great bactericidal activity against most Gram-positive and Gram-negative bacteria (38). Despite its potent activity against *S. aureus*, it is only approved by the American Food and Drug Administration as an oral single-dose treatment for acute uncomplicated cystitis (38). FOF has a unique mechanism of action inhibiting the enzyme phosphoenolpyruvate synthetase, involved in the initial step of bacterial cell wall synthesis. This feature makes cross-resistance with other antibiotics highly unusual (39). However, emergence of resistance is often observed when it is administered in monotherapy, thus being mandatory the association of a second antibiotic for the treatment of invasive infections (39, 40).

A large number of available reports of *in vitro* and *in vivo* studies showed synergistic activity of FOF and different beta-lactam antibiotics against MRSA infections (41-46). In addition, clinical studies published in the earlier eighties demonstrated the efficacy of FOF plus cefotaxime in the treatment of MRSA invasive infections, including bacteremia, meningitis and acute osteoarticular infections (47-49). This well-established clinical experience is the basis of the combination use in our experimental IE model in rabbits.

The aim of the current study was to evaluate: (**i**) FOF plus three selected beta-lactam antibiotics (imipenem-IPM, ceftriaxone –CRO- and amoxicillin/clavulanate-AMC) *in vitro* activity against ten MRSA strains (one of them with intermediate resistance to glycopeptides). (**ii**) the efficacy of these combinations in an experimental IE model in rabbits, using a human-like pharmacokinetic model and two selected strains from the *in*

vitro study; and (**iii**) the effect of FOF alone or in combination with imipenem on penicillin binding proteins (PBP) synthesis in MRSA and GISA strains.

Material and Methods

Microorganisms.

In vitro studies. Ten MRSA strains isolated from patients with bacteremia were selected; one of them showing decreased susceptibility to glycopeptides (a GISA strain included in the American Type Culture Collection; ATCC700788).

In vivo studies**.** Two MRSA isolates from the *in vitro* studies were selected for the *in vivo* and molecular studies: MRSA-277H, an isolate from a patient with bacteremia at our institution, and ATCC700788, the GISA strain used in the *in vitro* study.

Antibiotics. IPM powder was supplied by Merck (West Point, PA. USA), FOF by CEPA (Spain) and CRO by Roche (Basel, Switzerland). AMC and VAN were purchased to Sigma (St Louis. MO.USA). For all experiments, the purified powder of each antibiotic was diluted following the CLSI recommendations (50).

Susceptibility testing. Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) were determined using the microdilution method, according to standard recommendations (50). FOF susceptibility testing was done in Mueller-Hinton broth supplemented with D-glucose 6-phosphate (Sigma, St Louis. MO.USA) with a final concentration of 25 mg/L. *S. aureus* ATCC 29213 was used as the test control strain in all the experiments. All the results were double-checked.

Time-kill curves with FOF and three different beta-lactams (IPM, CRO and AMC) at concentrations of ¼xMIC and 1xMIC were performed, being used two different initial inocula of 10^5 (initial standard inoculum ISI) and 10^7 colony-forming units (cfu)/ml

(initial high inoculum, IHI). Before the inoculation, each tube of fresh CAMHB (cation-adjusted Mueller-Hinton broth) was supplemented with D-glucose 6-phosphate. All the experiments were performed in duplicate as recommended (51). Bactericidal activity was defined as a ≥3-log10 decrease in cfu/ml of the initial inoculum at 48 h. The results at 24 h of the combination were compared with those of the most active single drug; synergy, indifference, and antagonism were then defined as a ≥2-log increase in killing, a <2-log change (increase or decrease) in killing, and a ≥2-log decrease in killing, respectively.

PBP studies: Bacteria were incubated at 37° C in TSB alone, in TSB supplemented with either FOF (0.25 x MIC) alone or IPM (0.25 x MIC) alone, or in TSB with FOF (0.25 x MIC) plus IPM (0.25 x MIC) until achieving an optical density of 0.7 at 600 nm. PBPs were determined in membrane fractions of bacterial lysates as published elsewhere (52). Briefly, membrane suspensions (20 µl) containing ca. 4 mg/liter of protein were labelled with 1 mg/liter of the fluorescent penicillin Bocillin FL (Invitrogen, Carlsbad, CA) (53) for 1 h at 37º C. After incubation, the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a NuPAGE 7% Tris-acetate precast gel (Invitrogen). The Bocillin FL-labeled PBPs were then visualized by direct scanning of the gels with a Typhoon Trio+ imager (Amersham Biosciences)."

***In vivo* studies.**

Animals. New Zealand White rabbits (body weight, 2.2 Kg) obtained from San Bernardo farm (Pamplona, Spain) were housed in the animal facilities of the Faculty of Medicine (University of Barcelona), which is equipped with automatic air exchange with a HEPA filter and a circadian light cycle. They were nourished *ad libitum*. This research project

fulfils the requirements stipulated in the Spanish Royal Decree 223/1988 on the protection of animals used in experiments. The Ethical Committee on Animal Research of the University of Barcelona approved the animal studies.

Pharmacokinetics studies. The antibiotics were administered using a computer-controlled infusion pump system designed to reproduce human serum pharmacokinetics in rabbits to mimic the following intravenous antibiotic regimens: FOF 2 g q8h, IPM 1g q6h, CRO 2g q12h and VAN 1g q12h or 1g q6h. The procedure had three steps: (i) estimation of antibiotic parameters in the rabbit; (ii) application of a mathematical model to determine the infusion rate required for reproducing human pharmacokinetics in the animals; and (iii) *in vivo* experimental pharmacokinetics studies performed to simulate in rabbits the antibiotic pharmacokinetic profiles in humans (54).

(i) The antibiotic pharmacokinetic parameters in rabbits were estimated after the administration *in bolus* (50 mg/Kg IPM, 100 mg/Kg FOF and 30 mg/Kg CRO) in five healthy rabbits for each antibiotic. To determine the antibiotic concentrations in serum samples, blood was removed from a carotid catheter at different times (minimum of 10 points for each antibiotic). Blood samples were placed into tubes and centrifuged at 13,000 rpm for 20 min. The serum was removed and stored at -80º C. IPM, CRO and FOF concentrations in serum were determined by the disk-plate bioassay method (55), being *Micrococcus luteus* ATCC 9341 the bioassay microorganism and Mueller-Hinton agar (Difco Laboratories) the growth medium. Standard curves were determined with solutions of the different antibiotics in pooled rabbit serum and the concentrations in serum samples were inferred. Serum samples from rabbits were diluted with pooled rabbit serum (Sigma [St. Louis. MO.USA]), to assure that the antibiotic concentrations

were within the range of the standard curve. Standard and serum samples were assayed in triplicate. The results were expressed in μg/mL of blood. The linearity of the standard curves was 0.99 in all the assays. The sensitivity for FOF, IPM and CRO was 4 μg/mL, 0.5 μg/mL and 1 μg/mL, respectively. The day variation for replicates coefficients were less than 5% in all cases. (ii) The mathematical models were applied to obtain the required infusion doses to simulate human kinetics of FOF and IPM as an open one-compartment model (56), while CRO as a drug with an open two-compartment model (57). (iii) Simulation of the human profiles was done in healthy rabbits as reported previously (58). The study of human-like pharmacokinetics profile for VAN was already done and published previously (58).

In vivo efficacy **studies.** Experimental aortic valve IE was induced according to the method described by Garrison and Freedman (59). A catheter was inserted through the right carotid artery into the left ventricle while antibiotics were administered into the inferior vena cava through a jugular vein catheter, as previously described (58).

Rabbit aortic valve endocarditis model. Twenty-four hours after placement of the intracardiac catheter, all animals were infected via the marginal ear vein with 1 mL of saline solution containing 7-8 $\times$ 10^5 cfu/ml of microorganisms. Eighteen hours after the inoculation, antibiotic treatments were initiated. The infected rabbits were randomised to receive intravenously: no therapy (control); VAN-SD (simulating 1g q12h), VAN-HD (simulating 1g q6h), FOF (simulating 2g q6h), IPM (simulating 1g q6h); CRO (simulating 2g q24h) and FOF plus IPM (FOF+IPM) or CRO (FOF+CRO) at the same dosage regimens mentioned above. At that time, control animals were sacrificed and vegetations quantified for bacterial cfu. Antibiotics were administered via the computer-

controlled infusion pump system for 48 hours. After finishing the 48-hour treatment, animals were sacrificed after an additional 6 half-lives had elapsed, thus allowing residual viable bacteria in the endocardial vegetations growth.

Analysis of endocardial vegetations. After antibiotic treatment, rabbits were killed and aortic valve vegetations were removed and processed as previously described (58). Only those animals with proper placement of the catheter, macroscopic evidence of vegetations at the time of death, and *S. aureus* in cultures of blood obtained before the start of antimicrobial therapy were included in the study. The results were expressed as the number of $\log_{10}$ cfu/gr. of vegetation. If no growth was observed on the quantitative plates but contrary it was observed in the qualitative culture (the rest of the homogenate in tryptic soy broth), a value of 2 $\log_{10}$ cfu/gr. of vegetation was assigned. If no growth was observed from the initial quantitative culture and from the homogenates cultured for a week, a value of zero $\log_{10}$ cfu/gr. of vegetation was assigned, thus being considered the vegetation sterile. The recovered bacteria antibiotic susceptibility was retested and compared to that from the pretreatment isolates.

Statistical analysis. The results were expressed as the median and the interquartile range (IQR) of $\log_{10}$ cfu per gram of vegetation. The Mann-Whitney rank-sum test was used to compare the $\log_{10}$ cfu/g values between the different treatment groups. The Fisher exact test was used to compare the vegetations sterilization rate and to assess differences between treatment groups.

Results

In vitro studies:

Susceptibility testing. The ten strains MICs and MBCs to the antibiotics used in the study are shown in **Table 1**. Of note, only one strain was resistant to FOF (4E, MIC=64 μg/mL), while the other nine strains were susceptible, being the MBC of two susceptible strains 32 μg/mL. Four isolates had IPM MIC≥32μg/mL and all ten isolates were resistant to CRO. Regarding the two strains selected for the *in vivo* study, MRSA-277H was susceptible to VAN and FOF (MIC/MBC, 2/2 μg/ml and 4/4 μg/ml, respectively) and resistant to IPM and CRO (MIC/MBC, 64/64 μg/ml and >512/>512 μg/ml). GISA-ATCC700788 was susceptible to FOF and IPM (MIC/MBC 16/16 μg/ml and 1/2μg/ml, respectively), had intermediate resistance to VAN (MIC/MBC 8/128 μg/ml) and was resistant to ceftriaxone (MIC/MBC 128/256 μg/ml).

***In vitro* time-kill studies.** In **Table 2** are shown the results of the time-kill studies against initial standard inocula (ISI, 10^5 cfu/mL, **Table 2a**) and initial high inocula (IHI, 10^7 cfu/mL, **Table 2b**). After 24h of incubation with FOF+IPM, synergistic and bactericidal effect was observed in nine of the ten experiments with ISI (**Table 2a**), and in eight of ten experiments with IHI (**Table 2b**). The combination FOF+CRO showed synergistic effect against ISI of all the studied strains but bactericidal effect was detected in seven of ten. Against IHI, synergy was observed in seven of ten strains and bactericidal effect in five. Meanwhile, FOF+AMC was the less active combination, being synergy observed against six of ten strains at both ISI and IHI, while bactericidal effect was observed in five strains at ISI and four at IHI. And the comparison among the combinations of FOF+IPM or

FOF+CRO vs. FOF+AMC was statistically significant (P=0.05). The experiments with MRSA-277 and GISA-ATCC700788 are represented in **Figures 1a** and **1b**.

PBPs studies: After incubation of both MRSA-277 and GISA-ATCC 700788 with FOF, the production of PBP1 and PBP2 (but not PBP2a) was drastically reduced. The addition of IPM enhanced the effect of FOF, particularly on PBP2 production. In addition, the generation of PBP3 also decreased with the addition of IPM (**Figure 2**). No apparent changes were observed on PBP4 production (not shown in the Figure).

Pharmacokinetic studies

The pharmacokinetic data in rabbit used in the mathematical model are summarized in **Table 3** and **Figure 3.**

Treatment of established endocarditis (**Table 4**)**.** Comparisons between treatment groups revealed that FOF+IPM and FOF+CRO were more effective than VAN-SD sterilizing the vegetations caused by MRSA 277 (P=0.02 and P=0.08, respectively) and reducing the density of bacteria in vegetations (P=0.01 and P=0.06, respectively). VAN-HD sterilized a higher percentage of vegetations than VAN-SD (50% [8/16] vs. 31% [5/16]), but a lower percentage compared with FOF+IPM and FOF+CRO (73% [11/15], 62% [10/16]). However, differences did not reach statistical significance.

Both VAN–SD and VAN-HD sterilized 20% vegetations caused by GISA-ATCC700788, while FOF+IPM and FOF+CRO sterilized a higher percentage (35%, P=0.24 and 53%, P=0.03, respectively). FOF+IPM and FOF+CRO reduced the median number of GISA cfu in the vegetations to a greater extent than VAN-HD (P=0.04 and P<0.01 respectively).

The rate of isolates recovered in the vegetations showing emergence of resistance to FOF when it was administered in monotherapy was 42% (5/12 isolates) of MRSA-277 and 61% (8/13 isolates) of GISA-ATCC700788. No emergence of resistance was observed in the combination arms.

Discussion

MRSA IE is a difficult-to-treat infection even when using the currently recommended antibiotics (2, 5). In addition, most patients have associated comorbidities that preclude valvular surgical treatment, which otherwise would be advisable in many cases. Therefore, it is necessary to optimize antibiotic therapy to rapidly achieve the control of bacteremia and the vegetations sterilization.

Recent studies encouraged the use of combined therapy for the treatment of serious MRSA infections (24, 25). In this regards, fosfomycin combined with beta-lactam antibiotics had encouraging results (38, 40).

This study shows the efficacy of some beta-lactams (CRO and IPM) plus FOF combinations in the treatment of MRSA and GISA experimental IE animal model, in agreement with previous studies (41, 42, 44, 45). Ours and previous results showed the significant synergism between these antibiotics, especially with carbapenems and cephalosporins. As we observed, it correlates well with our *in vivo* results. Against the MRSA strain, FOF+IPM was more effective than VAN at standard guideline-recommended doses of 1g/12h i.v., while significant differences with VAN at higher doses were not found. However, from a clinical perspective, the risk of renal toxicity at higher doses of VAN, is a limitation that in most cases precludes these regimens (23). In the setting of IE caused by a GISA strain, our results showed an increased efficacy of FOF+CRO to sterilize vegetations. The combination achieved similar percentages of GISA sterile vegetations to those published previously by our group with daptomycin (54). Notably, the combined regimens against both MRSA and GISA IE avoided the

development of resistance to FOF in contrast to the 42% of resistant isolates recovered from the FOF monotherapy arm.

The *in vivo* synergism between FOF and beta-lactams was also supported by the few available old studies in experimental models of meningitis (43), mouse infection (44) or foreign body infection (60). A recent study involving a foreign-body infection model obtained better activity with FOF when combined with daptomycin than with IPM (61), stressing the significance of the type of infection on antibiotic activity. These results are in accordance with the predominance of growths of planktonic or stationary, non-growing bacteria in each setting (62).

FOF plus beta-lactams efficacy against MRSA is also supported by some clinical studies; the combination with CRO was used in 22 cases with invasive staphylococcal infections with excellent results (47), while FOF plus cefotaxime was used in 16 cases of methicillin-resistant and aminoglycoside-resistant staphylococcal infections obtaining 100% of cure with no relapses (48). As secondary effects, three cases of neutropenia, one of serum ALAT levels increase due to the high doses of cefotaxime used and one case of *Candida spp*. infection was observed. The same group also reported excellent results with this combination in the treatment of staphylococcal and enterobacteriaceae meningitis (49). More recently, our group reported (based on the experimental data presented herein) the experience with FOF+IPM as rescue therapy for 16 patients with IE and complicated bacteremia. In all cases, blood cultures were negative 72 hours after the first dose of the combination and the success rate was 69%, not being observed breakthrough bacteremia or relapse (63).

The mechanism of synergy between FOF and beta-lactams is not well known. However the modification in the different PBP production, leading to re-sensitization of MRSA against beta-lactams, has been proposed (44). Apparently, the mechanism of action might not be related to the strain susceptibility to VAN, being therefore expected similar results in MRSA and GISA strains. Our findings in this study suggest that the synergy FOM and IPM against MRSA and GISA is not due to decreased synthesis of PBP2a, but is due to the reduction of PBP1, PBP2 and PBP3 synthesis, which may impair PBP2a function, thus increasing IPM effect on MRSA and GISA strains. These results highlight the need for further studies addressing the mechanism of action of the combination.

In summary, according to our experimental results and the available clinical data, the combination of FOF+IPM and FOF+CRO significantly overcame the efficacy of VAN in the treatment of MRSA or GISA experimental IE. These combined regimens might be a good alternative to cure these difficult-to-treat infections, allowing the rapid control of bacteremia and achieving better outcomes. There is enough evidence to justify further clinical studies with this combined therapy for MRSA or GISA IE, such as the randomized clinical trial leaded by our group comparing FOF+IPM with VAN for IE caused by MRSA isolates with VAN MIC <2 μg/mL (ClinicalTrials.gov Identifier: NCT00871104).

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Appendix: Members of the Hospital Clínic Endocarditis Study Group, Hospital Clínic-IDIBAPS, University of Barcelona School of Medicine, Barcelona, Spain: José M. Miró, Asunción Moreno, Ana del Río, Juan M. Pericás, Ximena Castañeda, Cristina García de

la Mària, Yolanda Armero, Carlos Cervera, Jose M. Gatell (Infectious Diseases Service); Francesc Marco, Manel Almela, Jordi Vila (Microbiology Service); Carlos A. Mestres, Juan C. Paré, Carlos Falces, Ramón Cartañá, Salvador Ninot, Manel Azqueta, Marta Sitges, Magda Heras, José L. Pomar (Cardiovascular Institute); Jose Ramírez, Teresa Ribalta (Pathology Department); Merce Brunet (Toxicology Service); Dolors Soy (Pharmacy Service); Jaume Llopis (Statisticians).

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Figures' legends:

Figure 1a. Results of time-kill experiments for MRSA strain (MRSA-277) incubated with fosfomycin and imipenem or ceftriaxone at the MIC, alone or in combination. fosfomycin (4 µg/mL),imipenem (16 µg/mL) and ceftriaxone (64 µg/mL).

Figure 1b. Results of time-kill experiments for GISA-ATCC700788) incubated with fosfomycin and imipenem or ceftriaxone at the MIC, alone or in combination. fosfomycin (16 µg/mL),imipenem (1 µg/mL) and ceftriaxone (128 µg/mL).

Figure 2. PBP profiles by polyacrylamide gel electrophoresis (SDS-PAGE) of MRSA and GISA strains incubated with FOM and IPM alone or in combination **.**

Figure 3. Results of the antibiotic pharmacokinetic profiles on rabbits using a human-like pharmacokinetic model for fosfomycin (A), imipenem (B) and ceftriaxone (C).

Table 1. Antibiotic activity (MIC/MBC) of the antimicrobial agents (µg/mL) tested against 10 MRSA clinical isolates and one GISA collection strain.

Strains	Fosfomycin	Imipenem	Ceftriaxone	Amoxicillin/Clavulanate
4E	64/64	32/64	512/>512	16/8-64/32
7E	4/8	0.03/0.03	16/16	0.5/0.25-0.5/0.25
10E	2/4	0.25/0.5	16/64	8/4-16/8
9M	4/8	2/8	256/512	8/4-16/8
14H	8/16	0.12/0.5	32/32	32/16-32/16
45H	4/8	64/128	512/>512	32/16-32/16
3T	16/32	0.25/1	16/64	8/4-16/8
107V	16/32	64/64	>512/>512	32/16-64/32
277H	4/4	64/64	>512/>512	16/8-32/16
GISA sTCC700	16/16	1/2	128/256	32/16-64/32

Table 2a. Results of time-kill curves with fosfomycin plus imipenem, ceftriaxone or amoxicillin/clavulanate against 10 MRSA strains (ISI, inoculum equal to 10^5 CFU/mL).

Strains	***4E***		***7E***		***10 E***		***9M***		***14H***		***45H***		***3T***		***107V***		***277H***		***GISA ATCC***	
Time-kill	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**
Curves																				
FOF+IPM																				
Control	+1.5	+3.3	+1.4	+3	+1.9	+2.7	+1.3	+3.4	+1.9	+3.2	+1.9	+3.1	+2.1	+2.8	+1.4	+3.5	+1.8	+3	+0.8	+2.7
FOF	-1.2	+3.4	-0.3	+1.5	+1.2	0	0	+1	-0.5	+2	-0.9	+1.5	-0.2	-1	-0.2	+0.3	-1.5	+3.2	+0.1	+1.3
IPM	-1.3	+2.6	-1.7	+1.4	-1.5	-1	-1.3	+2.3	-1	+0.5	-1.1	+1.3	-0.8	+2.3	0	+2.1	-0.4	+2.6	-0.8	+1.5
FOF+IPM	-1.6	-3.5	-1.7	-3.9	-1.9	-3.5	-1.3	-3.5	-1.2	-1.5	-0.8	-3.7	-0.6	-3.1	-0.3	-3.7	-1.4	-3.4	-1.2	-3
FOF+CRO																				
Control	+1.6	+3.4	+1.7	+2.9	+1.4	+3.2	+1.6	+3.1	+1.8	+3	+1.9	+3.6	+1.7	+2.9	+1.7	+3.2	+1.6	+2.9	+1.3	+3.4
FOF	-0.7	+2.5	-0.5	-1.4	+0.1	+1.1	-0.9	+1.6	-0.3	+2.4	-0.2	+2.3	-0.1	+1.7	-0.1	+2.4	-0.9	+0.9	+0.8	+1.4
CRO	+0.5	+3.2	-1.2	-1.4	-0.5	+1.2	-1	+2.8	-0.5	-0.3	+0.5	+3.4	-0.3	+0.7	+1	+3	-0.8	+1.2	-0.1	+2.3
FOF+CRO	-1.3	-3.4	-2	-4	-0.9	-2.2	-1.2	-1.1	-0.9	-3	-0.1	-3.2	-0.5	-3.1	-0.2	-1	-2	-3.9	0	-3.3
FOF+AMC																				

Control	+1.1	+3.4	+1.9	+3.1	+1.7	+3.4	+1.3	+3.5	+1.7	+3.2	+0.5	+3.1	+1.9	+3.2	+1.3	+3.7	+1.5	+3.3	+1.3	+3.1
FOF	-0.3	+1.8	-0.3	+1.1	-0.6	+1.4	0	+1.8	-0.1	+1.1	+0.1	+2.2	-0.4	+1.1	-0.4	+0.2	-0.4	+3	+0.1	+2.7
AMC	-0.3	+3.4	+0.9	+3.1	+0.2	+2.5	0	+3.2	-1.7	-1.3	-0.2	+2.1	-0.8	+2.8	+0.3	+3.2	+0.1	+3.2	+1.1	+1.4
FOF+AMC	-1	-3.3	-1.9	-2.1	-0.4	-3.4	-1	-2.9	-1.8	-3.4	0	-2.5	-1.5	-3.7	-0.4	-1.7	-0.7	+1.4	-0.4	-3.4

1 FOF fosfomycin; CRO ceftriaxone, AMC amoxicillin-clavulanate, IPM imipenem

Table 2b. Results of time-kill curves with fosfomycin plus imipenem, ceftriaxone or amoxicillin/clavulanate against 10 MRSA strains (IHI, inoculum equal to 10^7 CFU/mL).

Strains	***4E***		***7E***		***10 E***		***9M***		***14H***		***45H***		***3T***		***107V***		***277H***		***GISA ATCC***	
Time-kill	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**	**4h**	**24h**
Curves																				
FOF+IPM																				
Control	+0.8	+1.5	+0.9	+1.2	+0.7	+1.1	+0.9	+1.8	+1	+1.2	+1	+1.8	+0.6	+1.4	+0.9	+1.8	+0.6	+1.6	-0.8	+1.1
FOF	-0.2	+1	-0.1	-0.7	0	-1.3	-0.1	+0.4	+0.2	-1.2	-0.4	+0.8	0	-0.8	0	+0.1	-0.2	-1.8	-1.5	-0.3
IPM	-0.1	-0.7	-1	-2.2	+0.7	+0.9	0	+1.6	+0.8	+1.1	+0.3	+1.7	+0.2	+0.9	+0.4	+1.8	+0.1	+1.4	-2.4	-0.1
FOF+IPM	-0.7	-2.4	-1.8	-4.4	+0.1	-3.4	-1	-4.5	0	-3.5	-0.4	-3	-0.6	-3.6	-0.2	-1.5	-0.9	-4.6	-2.8	-3.6
FOF+CRO																				
Control	+0.9	+1.8	+0.7	+1.4	+0.6	+1.4	+0.7	+1.6	+0.7	+1.5	+0.6	+1.5	+0.6	+1.2	+0.9	+1.6	+0.4	+1.4	+0.5	+1.4
FOF	-0.4	+1.1	-0.2	+0.4	-0.3	-2	-0.1	0	-0.3	-0.4	-0.3	-0.4	-0.1	-1.3	-0.3	-1.2	-0.6	+0.3	-0.1	-0.3
CRO	+0.6	+1.4	-0.9	+1.2	-0.5	-1	+0.4	+1.4	-0.2	+0.8	+0.3	+1.3	0	+0.4	+0.3	+1.3	-0.9	+1.2	+0.5	+0.7
FOF+CRO	-0.7	-2.4	-1.3	-3.1	-1.1	-4.4	-0.2	-3.9	-0.9	-2.9	0	-1.5	-0.3	-3.3	-0.1	-1.9	-1.2	-4.8	-0.5	-2.5
FOF+AMC																				

Control	+0.5	+1.3	+0.8	+0.4	+0.5	+1.4	+1	+1.7	+0.8	+1.5	+0.5	+1.4	+0.5	+1.3	+1	+1.8	+0.4	+1.7	+1	+2.2
FOF	-0.4	+0.6	+0.6	-1.3	-0.1	-0.7	-0.2	+0.6	-0.3	0	-0.1	+0.6	-0.5	-1.6	-0.3	-1.4	-0.3	+0.3	-0.2	+1.4
AMC	+0.4	+0.8	+0.7	+1.2	0	+0.7	+0.1	+1.4	-0.7	-1.5	-0.1	+0.9	-0.5	-0.1	+0.2	+1.1	+0.2	+1.3	+0.4	-1.5
FOF+AMC	-0.8	-2.6	-0.4	-0.7	-0.7	-4.3	-0.5	-3.5	-0.8	-2.7	0	-3.5	-0.7	-2.3	-0.4	-2.3	-0.2	-2.3	-0.7	-3.4

1 FOF fosfomycin; CRO ceftriaxone, AMC amoxicillin-clavulanate, IPM imipenem

Table 3. Pharmacokinetic and pharmacodynamic parameters

	Fosfomycin	Imipenem	Ceftriaxone
Previously Reported Human Values (Single Dose)			
Dose	2 g i.v.[a]	1 g i.v.[b]	2 g i.v.[c]
C_{max} (µg/ml)	90	52	256
C_{min} (µg/ml)	4.6	0.7	14
K_{el} (h^{-1})	0.34	0.74	0.1
$t_{½}$ β (h)	2	0.93	6.8
AUC (µg·h/ml)	264.7	69.8	1538
Animal Values (n = 5)			
K_{el} (h^{-1} ± SD)	0.82 ± 0.06	2.1 ± 0.08	0.43 ± 0.1
$t_{½}$ β (h ± SD)	0.85 ± 0.08	0.33 ± 0.01	1.6 ± 0.08
AUC (µg·h/ml ± SD)	109.7 ± 15	49.1 ± 9.7	222.9 ± 15
Human-like Values (n = 5)			
C_{max} (µg/ml)	89.7 ± 1.4	51± 1	290 ± 17
C_{min} (µg/ml)	5± 0.2	0.3± 0.01	21 ± 3
K_{el} (h^{-1} ± SD)	0.43 ± 0.05	0.91 ± 0.16	0.096 ± 0.003
$t_{½}$ β (h ± SD)	1.63 ± 0.2	0.8 ± 0.14	7.2 ± 0.19
AUC (µg·h/ml ± SD)	209.3 ± 14	62.7 ± 11	1819 ± 82.7

[a](64); [b](65); [c](66); AUC: Area under the curve; $K_{el:}$ elimination rate constant; NA: not available; $t_{½:}$ half life.

Table 4. Treatment of experimental endocarditis caused by MRSA-277H and GISA-ATCC700788 strains.

Treatment group	Survival, no./ Total, no. (%)	Sterile vegetation, no./ Total, no. (%)	Median (IQR) (log_{10} cfu/g of veg)
MRSA (# 277)			
Control	–/–	0/15 (0)	9.1 (8.3-9.3)
FOF	12/12 (100)	0/12 (0)	8.5 (8-9.1)
CRO	11/11 (100)	0/11 (0)	8.3 (7-8.9)
IPM	14/16 (88)	1/14 (7)	5.1 (4-7.5)
VAN-SD	16/16 (100)	5/16 (31)[a,b]	2 (0-5.1)[c,d]
VAN-HD	16/16 (100)	8/16 (50)	1 (0-2.2)
FOF+IPM	14/16 (88)	11/15 (73)[a]	0 (0-1)[c]
FOF+CRO	16/17 (94)	10/16 (62)[b]	0 (0-2.2)[d]
GISA-ATCC 700788			
Control	–/–	0/15 (0)	9.5 (9.1-9.9)
FOF	13/13 (100)	0/13 (0)	9.4 (9.1-9.9)
CRO	15/16 (94)	0/15 (0)	8.6 (7.7-9.4)
IPM	16/18 (94)	1/16 (6)	4.6 (2-5.9)
VAN-SD	20/23 (87)	4/20 (20)[e,f]	6.5 (2-6.9)[g,h]
VAN-HD	20/21 (95)	4/20 (20) [e,f]	2.4 (2-4) [g,i,j]
FOF+IPM	17/17 (100)	6/17 (35)[e]	2 (0-2)[i]
FOF+CRO	15/15 (100)	8/15 (53)[f]	0 (0-2)[h,j]

[a]$P = 0.02$; [b]$P = 0.08$; [c]$P = 0.01$; [d]$P = 0.06$; [e]$P = 0.24$; [f]$P = 0.03$; [g]$P < 0.01$; [h]$P < 0.01$; [i]$P = 0.04$; [j]$P < 0.01$.

cfu= colony-forming unit; GISA = glycopeptide-intermediate-resistant *S. aureus*; IQR= interquartile range; MRSA= methicillin-resistant *S. aureus*; veg = vegetations. FOF fosfomycin; CRO ceftriaxone, AMC amoxicillin-clavulanate, IPM imipenem, VAN-SD vancomycin, standard dose, VAN-HD vancomycin, high dose.

Figure 1a.

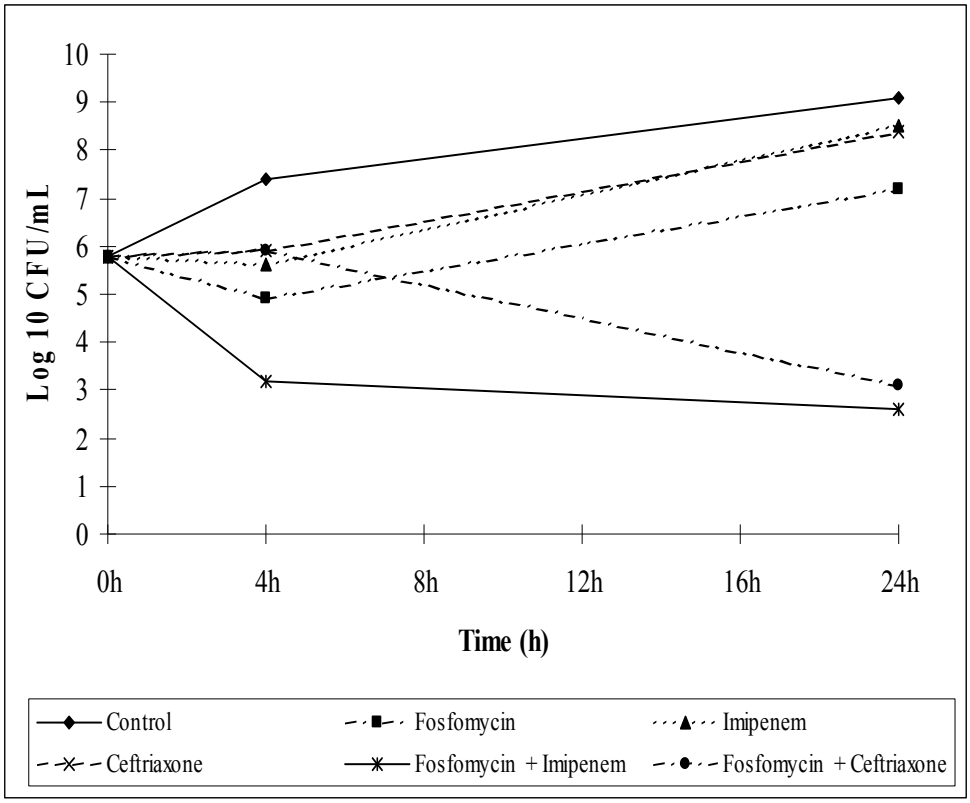

Log 10 CFU/mL
10
9
8
7
6
5
4
3
2
1
0
0h
4h
8h
12h
16h
24h
Time (h)
Control
Fosfomycin
Imipenem
Ceftriaxone
Fosfomycin + Imipenem
Fosfomycin + Ceftriaxone

Figure 1b.

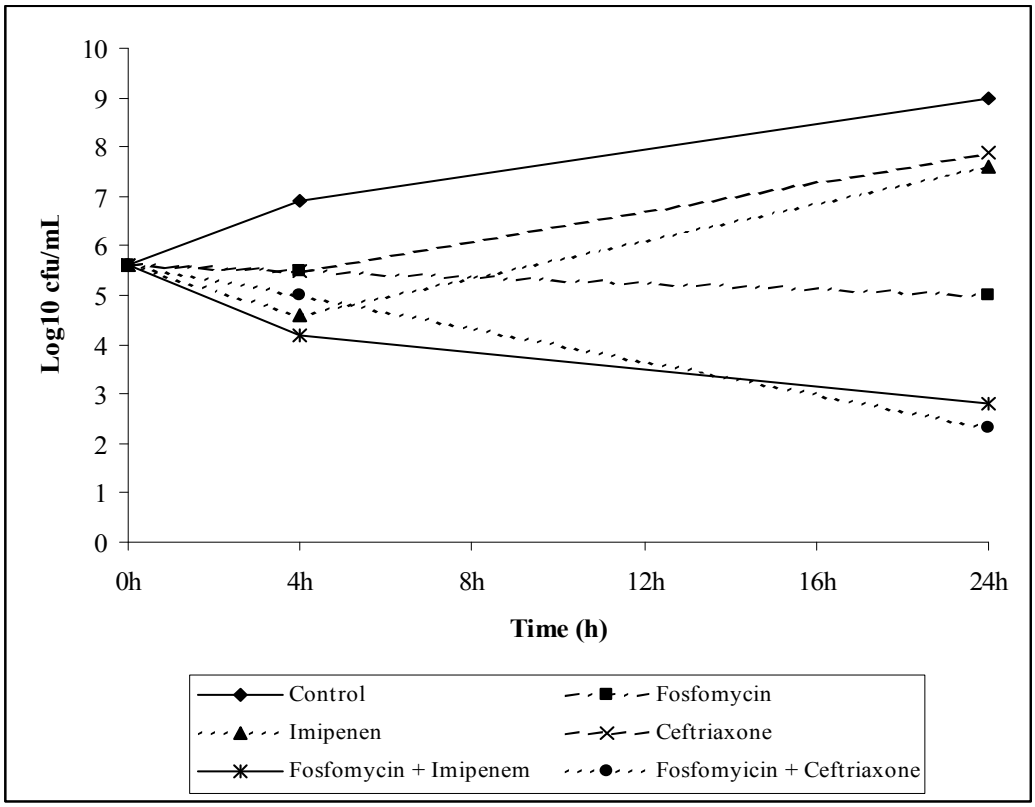

Log10 cfu/mL
10
9
8
7
6
5
4
3
2
1
0
0h
4h
8h
12h
16h
24h
Time (h)
Control
Fosfomycin
Imipenen
Ceftriaxone
Fosfomycin + Imipenem
Fosfomyicin + Ceftriaxone

Figure 2.

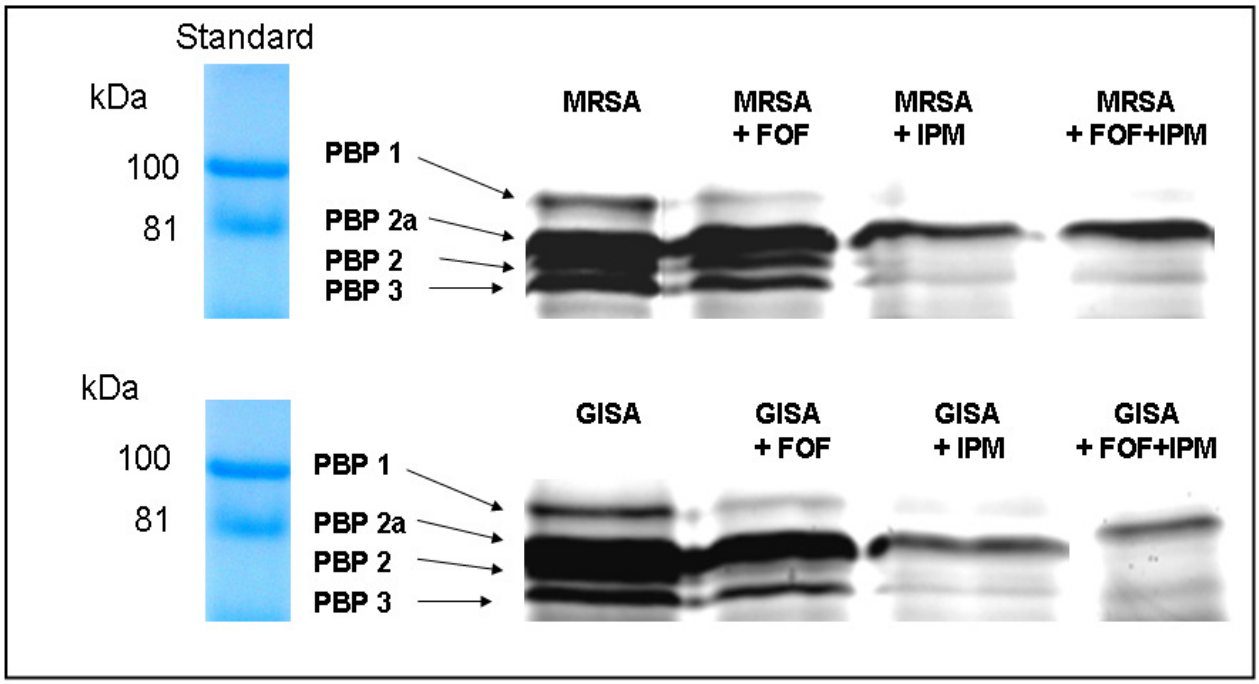
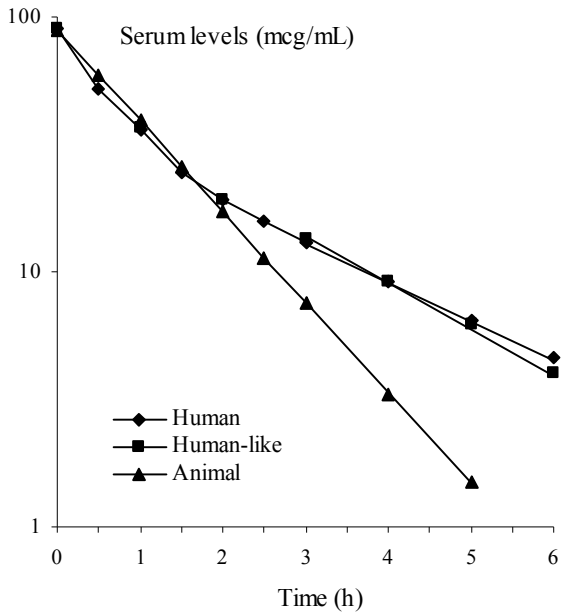
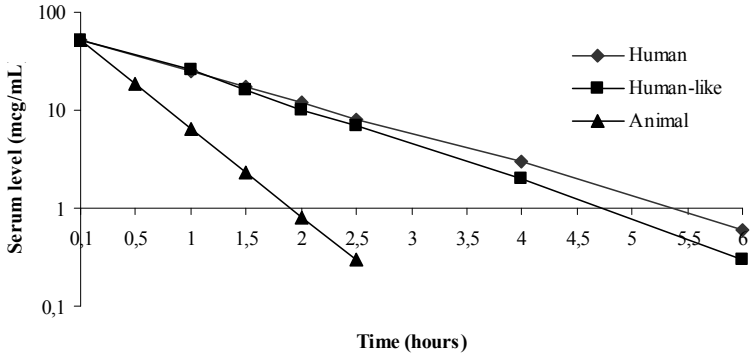

Standard
kDa
100
81
PBP 1
PBP 2a
PBP 2
PBP 3
MRSA
MRSA + FOF
MRSA + IPM
MRSA + FOF+IPM
kDa
100
81
PBP 1
PBP 2a
PBP 2
PBP 3
GISA
GISA + FOF
GISA + IPM
GISA + FOF+IPM

Figure 3.

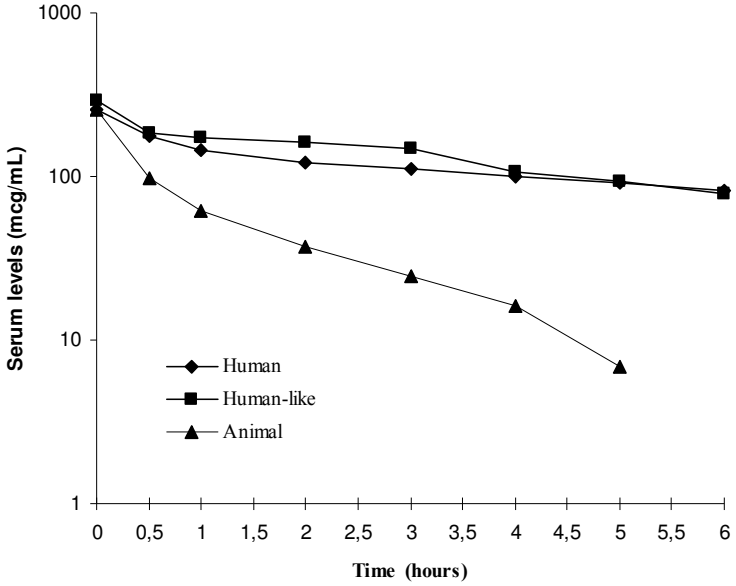
A) Fosfomycin human-like pharmacokinetics profile.



B) Imipenem human-like pharmacokinetics profile.



C) Ceftriaxone human-like pharmacokinetics profile.



1

2

3