In vitro and in vivo activities of linezolid alone and combined with vancomycin and imipenem against *Staphylococcus aureus* with reduced susceptibility to glycopeptides

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Abstract The objective of this study was to evaluate the in vitro and in vivo efficacies of linezolid (35 mg/kg/5 h), vancomycin (60 mg/kg/5 h), imipenem (30 mg/kg/5 h), linezolid+imipenem, linezolid+vancomycin and vancomycin+imipenem against two clinical *Staphylococcus aureus* isolates with reduced susceptibility to glycopeptides using time–kill curves and the murine peritonitis model. Time–kill curves were performed over 24 h. For the murine peritonitis model, peritonitis was induced by the intraperitoneal inoculation of 10^8 CFU/ml of each bacterial strain. Four hours later (0 h), the mice were randomly assigned to a control group or to therapeutic groups receiving subcutaneous treatment for 25 h. Bacterial counts in peritoneal fluid, bacteraemia and mortality rates were determined. The time–kill curves showed that the addition of linezolid to imipenem yielded synergistic results after 24 h. The addition of linezolid decreased vancomycin activity. In the animal model, vancomycin and linezolid monotherapies produced comparable bacterial decreases in mice infected with each strain but linezolid achieved higher rates of blood sterilisation. Linezolid tested either in monotherapy or in combination showed similar efficacy against both strains in terms of bacterial killing, number of negative blood cultures and survival. Linezolid and vancomycin were moderately bactericidal and similar in efficacy against glycopeptide-intermediate or -resistant *S. aureus*. Linezolid combinations, as effective as linezolid tested alone, could be considered as alternative options for the treatment of glycopeptide-intermediate *S. aureus* (GISA) infections.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most important cause of antibiotic-resistant healthcare-associated infections [1]. In addition, clinical isolates of *S. aureus* with heterogeneous resistance to vancomycin (hGISA) and, more rarely, glycopeptide-intermediate-resistant strains (GISA) have emerged worldwide over the past several years [2–4]. Reduced vancomycin and teicoplanin activities against hGISA and GISA isolates have been reported in experimental studies [5–7], while in the clinical setting, vancomycin has appeared to be sub-optimal in deep-seated and difficult-to-treat infections caused by these strains [7, 8]. Furthermore, the antagonistic effect or false synergy showed by in vitro studies with the combination of glycopeptides and β-lactams refuses its use as a potentially promising alternative to glycopeptide monotherapy [9, 10]. The oxazolidinone linezolid, one of the new treatment options for multidrug-resistant Gram-positive bacteria [11–13], shows high in vitro activity against resistant staphylococcal strains [13, 14].
patients with MRSA infections, linezolid has shown comparable efficacy to vancomycin [15, 16]. Moreover, it shows excellent oral bioavailability and does not require dose adjustment for renal insufficiency [14]. Its unique mechanism of action by inhibiting ribosomal protein synthesis at an early stage of bacterial replication leads to the absence of cross-resistance with other antimicrobials [15]. Although linezolid-nonsusceptible strains are unusual [17], long courses of oxazolidinone therapy could select resistant mutants [18, 32]; hence, the use of a combined strategy might be considered in clinical practice. To date, the efficacy of linezolid as part of a combination has been studied against MRSA strains, but very few data has been reported against hGISA or GISA strains [19, 20]. Linezolid plus β-lactams exhibited bactericidal and synergistic activity against MRSA and GISA strains in experimental models of endocarditis and meningitis [19]. Linezolid plus rifampicin was an effective prophylactic regimen for preventing staphylococcal prosthetic vascular graft infection, although the combination did not show higher efficacy compared to linezolid monotherapy [20].

We aimed to evaluate and compare the efficacies of linezolid alone and in combination with either vancomycin or imipenem against two S. aureus strains with reduced susceptibility to glycopeptides.

Materials and methods

Bacterial strains

Two clinical isolates of S. aureus with different degrees of resistance to glycopeptides were included: an hGISA strain isolated in our hospital and belonging to the Iberian clone, growing on 4 mg/l vancomycin Mueller–Hinton plates with a sub-population frequency of $3.6 \times 10^{-6}$ CFU/ml (this strain was equivalent to the Mu3 heteroresistant strain) [8], and a GISA strain (Mu50, ATCC 700699) reported as the first GISA strain [3]. Minimum inhibitory concentrations (MICs, mg/l), determined using the Etest and the macrodilution method [21], were as follows for the GISA strain: cloxacillin, 1,024; cefotaxime, 1,024; teicoplanin 8; vancomycin (VAN), 2; linezolid (LZD), 1; and imipenem (IMP), 32. MICs for the GISA strain were: cloxacillin, 1,024; cefotaxime, 1,024; teicoplanin, 8; VAN, 2; LZD, 2; and IMP, 64.

In vitro time–kill studies

The bactericidal activities of the drugs were tested in glass tubes containing Mueller–Hinton broth and a final inoculum of $1 \times 10^5$ to $1 \times 10^6$ CFU/ml [21]. Linezolid (Pfizer, Madrid, Spain), imipenem–cilastatin (Merck, Sharp & Dohme, Madrid, Spain) and vancomycin (Normon, Madrid, Spain) were provided by the manufacturers. Antibiotics were tested for a range of concentrations according to their MICs and their achievable levels in human serum: linezolid concentrations ranging from $1/4 \times$ to $8 \times$ MIC, vancomycin concentrations ranging from $1/4 \times$ to $1 \times$ MIC, imipenem levels from $1/8 \times$ to $1 \times$ MIC, as well as concentrations of $1/4 \times$, $1/2 \times$ and $1 \times$ MIC of each drug in combination. In all experiments, growth control was assessed using an extra tube without antibiotic. At 0, 6 and 24 h of incubation, aliquots of 100 μl were taken from each tube to perform direct and 10-fold dilutions, and were cultured onto 5% sheep blood agar plates (SBA) at 37°C for 24 h. Experiments were performed in duplicate. The following effects were studied in combinations after 24 h of incubation: a bactericidal effect was defined as a decrease in the initial inoculum of $\geq 3 \log_{10}$CFU/ml; the synergy of a combination was defined as a $> 2 \log_{10}$CFU/ml reduction over the most active agent alone, with one of the drugs at subinhibitory concentration; an indifferent effect was defined as $< 1 \log$ (increase or decrease) in killing.

Pharmacokinetics

Pharmacokinetic studies were performed to select dose regimens that result in serum concentrations similar to those found in humans [6, 22]. Groups of 21 healthy mice were used for each pharmacokinetic study. A single weight-adjusted antibiotic dose was administered subcutaneously (sc) to each animal. At different time points, sets of three animals were anaesthetised intraperitoneally (ip), and blood samples (0.5 ml) were obtained by an intracardiac puncture. Blood was centrifuged and serum stored at $-80^\circ$C. Pharmacokinetic and pharmacodynamic parameters were obtained by a computer-assisted method (PK Functions for Microsoft Excel; Usansky, Desai and Tang-Liu, Pharmacokinetics and Drug Metabolism Dept., Allergan, Irvine, CA 92606, USA) after the determination of antibiotic concentrations over time. Based on the obtained parameters, the final selected doses were: vancomycin 60 mg/kg every 5 h (300 mg/kg/day), linezolid 35 mg/kg every 5 h (175 mg/kg/day) and imipenem 30 mg/kg every 5 h (150 mg/kg/day).

Mouse peritonitis model

This mouse peritonitis model has been previously characterised in our laboratory [6, 23]. Inbred, female C57BL/6 mice (6 weeks; 14–16 g) were used (Harlan Int. Ibérica, S.A., Barcelona, Spain). Inoculation was performed via a 26-gauge syringe by ip injection of 0.5 ml of the inoculum consisting of a $5 \times 10^8$ CFU/ml staphylococcal suspension with 5% (w/v) mucin in sterile saline. A group of control mice (n≥18) were killed 4 h after inoculation (hour 0) and antibiotic sc therapy was initiated. The rest of the mice were randomised.
to the control group receiving saline \((n\geq25)\) or to one of the following therapeutic schedules \((n\geq10\) per therapy\): linezolid, vancomycin, imipenem, linezolid+vancomycin, linezolid+imipenem and vancomycin+imipenem, receiving sc treatment over 25 h. At 25 h of therapy \((5\) h after the last antibiotic dose\), mice were anaesthetised ip with ketamine/xylazine and peritoneal washes were performed by injecting 2 ml of sterile saline ip followed by a massage of the abdomen. Immediately, 0.1 ml of blood was withdrawn by cardiac puncture and the animals were then sacrificed by cervical dislocation. Next, the abdomen was opened and 0.2 ml of peritoneal fluid (PF) was recovered from the peritoneum. Undiluted and 10-fold-diluted PF samples \((0.1\) ml\) were plated on SBA plates to perform bacterial determinations. Mortality was recorded after 25 h of therapy. Blood samples were grown in tryptic soy broth \((TSB)\) at 37°C for 24 h and then 0.1 ml of broth was cultured on SBA plates to assess \(S.\) aureus bacteraemia.

Antibiotic assays

Vancomycin serum concentrations were determined by fluorescent polarisation immunoassay using a TDx analyser \((Abbott\ Científica, S.A., Diagnostics Division, Costa Brava 13, 28034 Madrid, Spain)\) with a detection limit of 2.0 μg/ml. Serum concentrations of linezolid and imipenem were measured using the agar disc diffusion method and Bacillus subtilis ATCC 12432 and Escherichia coli ATCC 25922, respectively, as assay organisms. Standard curves were constructed using mouse plasma. Assay validation indicated linearity \((r^2\) value\) of 0.9709 for imipenem and 0.9887 for linezolid. The detection limit was 0.5 μg/ml and 2 μg/ml for imipenem and linezolid, respectively.

Statistics

Statistical analysis was performed with SPSS 12.0. Analysis of variance \((ANOVA)\) with Dunnett’s post-hoc tests was used to analyse multiple bacterial count comparisons between therapeutic and control groups. Two-tailed Fisher’s exact test was used for analysing the survival and bacteraemia data. A \(p\)-value of <0.05 was considered to be statistically significant.

Results

In vitro time–kill studies

Linezolid achieved a bacterial decrease of up to \(2\log_{10}\) CFU/ml when tested at 4–16 mg/l against both strains. Vancomycin achieved a bacterial decrease of \(2\log_{10}\) CFU/ml when studied at 2 and 8 mg/l against the hGISA and the GISA strain, respectively. Imipenem failed to inhibit bacterial growth at any tested concentration \((8–64\) mg/l\).

In killing curves with the hGISA strain, linezolid combined with vancomycin showed lower activity than vancomycin alone. Vancomycin activity was decreased between \(1–1.5\log_{10}\) CFU/ml at 24 h. The same combination improved the activities of antibiotics tested alone against the GISA strain \((Fig. 1)\).

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The combination of linezolid at concentrations above the MIC and imipenem did not improve upon the activity of linezolid tested alone against either strain. The addition of sub-MIC concentrations of linezolid to imipenem produced a synergistic effect against both strains (Fig. 2).

The combination of vancomycin with imipenem was bactericidal and synergistic against the hGISA strain. Vancomycin tested at 2 mg/l in combination with imipenem (8–64 mg/l) was also bactericidal and improved upon the activity of vancomycin alone against the GISA strain.

Pharmacokinetics

The linezolid- and vancomycin-free maximum concentrations in serum were 18.16 and 37.73 mg/l, respectively (with a protein binding of 26% for linezolid and 25% for vancomycin [24, 25]). The imipenem-free maximum concentration found in serum was 38.26 mg/l. Drug serum concentrations in humans are 12–15 mg/l for linezolid (dose 600 mg/12 h), 30–40 mg/l for vancomycin (dose 1 g/12 h) [26] and 32.1 mg/L (dose 500 mg/6 h) for imipenem [27].

Mortality and bacteraemia rates

In control mice, the mortality rates were 90% and 69% after 25 h of infection with the hGISA and GISA strains, respectively. At the same time point, the mortality in mice infected with the hGISA strain was 0% in all therapeutic groups, except for the imipenem (20%, 2/10) and the vancomycin plus imipenem (7%, 1/14) groups. In mice infected with the GISA strain, the mortality was 0% in all treated animals except for those receiving imipenem monotherapy (45.5%, 5/11). Data from GISA-infected animals treated with imipenem monotherapy were not considered for any statistical analysis because of the low number of animals that survived after 25 h of therapy (n=6).

Bacteraemia in control animals at 0 h, expressed as a percentage of positive blood cultures, was 100% for each strain. Bacteraemia rates in control and therapeutic groups after 25 h of therapy are shown in Table 1. Imipenem alone and in combination with vancomycin failed in blood bacterial clearance. Linezolid alone and its combinations

**Table 1** Bacteraemia rates of control and therapeutic groups of infected mice after 25 h of subcutaneous therapy. Data are expressed as percentages of positive blood cultures (n≥10 mice/group except for imipenem against the GISA strain, where n=6). LZD, linezolid; VAN, vancomycin; IMP, imipenem

| Therapy (25h) | % positive blood cultures |
|--------------|--------------------------|
| hGISA strain | GISA strain              |
| Control      | 100                      | 100                      |
| LZD 35 mg/kg/5 h | 73<sup>a</sup> | 67<sup>a, c</sup>       |
| VAN 60 mg/kg/5 h | 93            | 81                      |
| IMP 30 mg/kg/5 h | 100         | 81<sup>*</sup>          |
| LZD + VAN    | 64<sup>a, b</sup>     | 79                      |
| LZD + IMP    | 64<sup>a, b</sup>     | 67<sup>a, c</sup>       |
| VAN + IMP    | 93            | 100                     |

<sup>a</sup>Small n; this group was excluded from the statistical studies

<sup>p<0.04 vs. control group</sup>

<sup>b</sup>p<0.04 vs. IMP group

<sup>c</sup>p<0.05 vs. VAN + IMP group
significantly reduced the bacteraemia rates achieved by the control group in hGISA-infected mice \( (p<0.04) \). Mice treated with linezolid combinations also showed a lower number of positive blood cultures than the imipenem-treated group \( (p<0.04) \). In GISA-infected mice, linezolid alone and in combination with imipenem significantly reduced the bacteraemia rates reached by the control \( (p \leq 0.02) \) and the vancomycin plus imipenem \( (p<0.05) \) groups.

**Murine peritonitis model: therapeutic efficacy**

Bacterial counts in PF (mean log\(_{10}\) CFU/ml ± SD) of control animals at hour 0 were 8.17±0.81 for the hGISA strain \( (n=25) \) and 7.82±0.57 for the GISA strain \( (n=18) \). Bacterial counts in PF of control and treated mice after 25 h are shown in Table 2. The efficacy of an antibiotic therapy was defined as the decrease in the number of CFU \( (\Delta \log \text{CFU/ml}) \) in PF between 0 and 25 h. All regimens were statistically more effective than the control group for both strains \( (p<0.001) \). Linezolid monotherapy produced similar bacterial decreases against both isolates. Linezolid was as effective as vancomycin against the hGISA strain but slightly improved upon the vancomycin activity against the GISA strain.

Linezolid combinations showed comparable efficacies to linezolid monotherapy against both strains. The association of linezolid with vancomycin showed more active in reducing bacterial counts than vancomycin alone in mice infected with the GISA strain. The addition of linezolid to imipenem showed enhanced activity against vancomycin alone against both strains. The association of linezolid with either vancomycin or imipenem showed higher activity than vancomycin plus imipenem against both strains \( (p=0.048, \text{linezolid plus vancomycin vs vancomycin plus imipenem against the GISA strain}) \).

**Discussion**

The increasing incidence of nosocomial infections due to *S. aureus* antibiotic-resistant strains and the report of therapeutic failures associated with standard glycopeptide therapy highlight the importance of identifying new synergistic drug combinations [1, 7, 8]. Linezolid has demonstrated good activity against most staphylococci, including methicillin-resistant strains [12, 13].

Linezolid was tested in vitro at achievable concentrations in human serum after oral administration of 500 and 600 mg regimens [22]. At 4–16 mg/L, linezolid was effective against both hGISA and GISA strains. Its association with different drugs exerted distinct effects. Linezolid combined with imipenem was synergistic against both strains. The synergistic interaction between low concentrations of linezolid and imipenem has been previously reported against MRSA strains [28]. An indifferent effect was the most common result achieved with the interaction between linezolid and vancomycin according to previous studies involving MRSA and hGISA strains [29, 30]. Of particular interest was our finding of a synergistic killing with sub-MIC concentrations of both antibiotics in combination against the GISA strain. This enhanced effect has been reported on another GISA strain in an in vitro pharmacodynamic model [31].

In the murine peritonitis model caused by the hGISA strain, no differences were found between linezolid and vancomycin monotherapies in terms of bacterial counts in peritoneal fluid and survival. In contrast, in GISA-infected mice, linezolid showed a slightly higher activity than vancomycin, although this did not reach statistical significance. Moreover, linezolid achieved higher percentages of blood culture sterilisation in comparison to vancomycin against both isolates. The use of combined regimens would be a good approach to improve the effectiveness of linezolid in the management of drug-resistant infections. In our experimental setting, the addition of linezolid to vancomycin showed similar efficacy but decreased the bacteraemia rates in comparison to vancomycin and linezolid monotherapies against the hGISA strain. The same combination enhanced vancomycin activity against the GISA strain but did not improve the rates of blood sterilisation achieved with monotherapy regimens.

Even though β-lactam antibiotics do not show any activity against MRSA and hGISA strains, their use in combination with linezolid has been shown to be highly effective against MRSA strains in vitro and in experimental endocarditis [28]. In our study, linezolid combined with imipenem was an effective therapy in mice infected with hGISA/GISA strains in terms of bacterial and bacteraemia reduction.

Our study found some discrepancies between in vitro and in vivo results. It should be emphasised that in vitro

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**Table 2** Bacterial counts in peritoneal fluid (PF) for therapeutic and control groups after 25 h of subcutaneous therapy

| Therapy (25h)   | PF bacterial counts ± SD (log CFU/ml) \( [n] \) | hGISA strain | GISA strain |
|-----------------|-----------------------------------------------|--------------|-------------|
| Control         | 8.19±0.57 \( [29] \)                          | 8.29±0.9     | [29]        |
| LZD 35 mg/kg/5 h| 5.88±0.61 \( [15]^{a} \), 6.20±0.61 \( [18]^{a,b} \) |              |             |
| VAN 60 mg/kg/5 h| 5.90±0.31 \( [14]^{a} \)                      | 6.02±0.56    | [16]^{a}    |
| IMP 30 mg/kg/5 h| 6.40±0.80 \( [10]^{a} \)                      | 7.38±1.38    | [6]^{a}     |
| LZD + VAN       | 5.94±0.30 \( [14]^{a} \)                      | 5.61±0.56    | [14]^{a,b}  |
| LZD + IMP       | 5.81±0.41 \( [14]^{a} \)                      | 5.74±0.52    | [15]^{a}    |
| VAN + IMP       | 6.15±0.68 \( [14]^{a} \)                      | 6.28±0.49    | [14]^{a}    |

*Small \( n \); this group was excluded from the statistical studies

\( ^{a}p<0.001 \) vs. control group

\( ^{b}p<0.05 \) vs. VAN + IMP group
interaction may not translate into clinical efficacy, mainly because of the diversity of mechanisms involved in in vivo antibiotic interactions which cannot be analysed by the use of in vitro techniques [33]. Indeed, experts recommend to use the in vivo efficacy more than the in vitro data when selecting an anti-staphylococcal drug as a therapeutic option [34].

To date, few studies have addressed the role of linezolid combinations against glycopeptide-resistant *S. aureus*. The present study confirms the anti-staphylococcal activity of linezolid in association with vancomycin or.imipenem, indicating that linezolid combinations preserve the activity of linezolid alone and might be considered as therapeutic options in the management of infections caused by *S. aureus* strains with reduced susceptibility to glycopeptides.

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References

1. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J et al (2003) Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 290:2976–2984

2. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y (2003) Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. Clin Infect Dis 36:53–59

3. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC (1997) Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother* 40:135–136

4. Mao Y, Rahav G, Belausov N, Ben-David D, Smollan G, Keller N (2007) Prevalence and characteristics of heteroresistant vancomycin-intermediate *Staphylococcus aureus* bacteremia in a tertiary care center. *J Clin Microbiol* 45:1511–1514

5. Climo MW, Patron RL, Archer GL (1999) Combinations of vancomycin and beta-lactams are synergistic against staphylococci with reduced susceptibilities to vancomycin. *Antimicrob Agents Chemother* 43:1747–1753

6. Domenech A, Ribes S, Caballero C, Domínguez MA, Montero A, Liñares J et al (2004) A mouse peritonitis model for the study of glycopeptide efficacy in GISA infections. *Microb Drug Resist* 10:346–353

7. Moore MR, Perdrea-Remington F, Chambers HF (2003) Vancomycin treatment failure associated with heterogeneous vancomycin-intermediate *Staphylococcus aureus* in a patient with endocarditis and in the rabbit model of endocarditis. *Antimicrob Agents Chemother* 47:1262–1266

8. Ariza J, Pujol M, Cabo J, Peña C, Fernández N, Liñares J et al (1999) Vancomycin in surgical infections due to methicillin-resistant *Staphylococcus aureus* with heterogeneous resistance to vancomycin. *Lancet* 8:1587–1588

9. Aritaka N, Hanaki H, Cui L, Hiramatsu K (2001) Combination effect of vancomycin and beta-lactams against a *Staphylococcus aureus* strain, Mu3, with heterogeneous resistance to vancomycin. *Antimicrob Agents Chemother* 45:1292–1294

10. Goldstein FW, Atou R, Ben Ali A, Nguyen J, Ly A, Kitzis MD (2004) False synergy between vancomycin and beta-lactams against glycopeptide-intermediate *Staphylococcus aureus* (GISA) caused by inappropriate testing methods. *Clin Microbiol Infect* 10:342–345

11. Drew RH (2007) Emerging options for treatment of invasive, multidrug-resistant *Staphylococcus aureus* infections. *Pharmacotherapy* 27:227–249

12. Lentino JR, Narita M, Yu VL (2008) New antimicrobial agents as therapy for resistant gram-positive cocci. *Eur J Clin Microbiol Infect Dis* 27:3–15

13. Rybak MJ, Hershberger E, Moldovan T, Gruez RG (2000) In vitro activities of daptomycin, vancomycin, linezolid, and quinupristin–dalfopristin against *Staphylococci* and *Enterococci*, including vancomycin-intermediate and -resistant strains. *Antimicrob Agents Chemother* 44:1062–1066

14. Perry CM, Jarvis B (2001) Linezolid: a review of its use in the management of serious gram-positive infections. Drugs 61:525–551

15. Rubinstein E, Cammarata S, Oliphant T, Wunderink R (2001) Linezolid (PNU-100766) versus vancomycin in the treatment of hospitalized patients with nosocomial pneumonia: a randomized, double-blind, multicenter study. *Clin Infect Dis* 32:402–412

16. Stevens DL, Herr D, Lampiris H, Hunt JL, Batts DH, Hatkin B (2002) Linezolid versus vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* infections. *Clin Infect Dis* 34:1481–1490

17. Jones RN, Fritsche TR, Sader HS, Ross JE (2007) *Zyvox* Annual Appraisal of Potency and Spectrum Program Results for 2006: an activity and spectrum analysis of linezolid using clinical isolates from 16 countries. *Diagn Microbiol Infect Dis* 59:199–209

18. Wilson P, Andrews JA, Charlesworth R, Walesby R, Singer M, Farrell DJ et al (2003) Linezolid resistance in clinical isolates of *Staphylococcus aureus*. *J Antimicrob Chemother* 51:186–188

19. Jacqueline C, Caillon J, Le Mabecque V, Miègeville AF, Hamel A, Bagnon D et al (2007) In vivo efficacy of cefaroline (PPI-0903), a new broad-spectrum cephalosporin, compared with linezolid and vancomycin against meticillin-resistant and vancomycin-intermediate *Staphylococcus aureus* in a rabbit endocarditis model. *Antimicrob Agents Chemother* 51:3397–3400

20. Sacar M, Sacar S, Kaleli I, Onem G, Turgut H, Goksin I et al (2007) Linezolid alone and in combination with rifampicin prevents experimental vascular graft infection due to meticillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. *J Surg Res* 139:170–175

21. Clinical and Laboratory Standards Institute (CLSI) (2005) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6, 6th ed. CLSI, Wayne, PA

22. MacGowan AP (2003) Pharmacokinetic and pharmacodynamic profile of linezolid in healthy volunteers and patients with Gram-positive infections. *J Antimicrob Chemother* 51(Suppl 2a):i17–i25

23. Domenech A, Ribes S, Caballero C, Taberner F, Tubau F, Domínguez MA et al (2005) Experimental study on the efficacy of combinations of glycopeptides and beta-lactams against *Staphylococcus aureus* with reduced susceptibility to glycopeptides. *J Antimicrob Chemother* 56:709–716

24. Gentry-Nielsen MJ, Olsen KM, Preheim LC (2002) Pharmacodynamic activity and efficacy of linezolid in a rat model of pneumococcal pneumonia. *Antimicrob Agents Chemother* 46:1345–1351
25. Knudsen JD, Fuursted K, Esersen F, Frimodt-Møller N (1997) Activities of vancomycin and teicoplanin against penicillin-resistant pneumococci in vitro and in vivo and correlation to pharmacokinetic parameters in the mouse peritonitis model. Antimicrob Agents Chemother 41:1910–1915

26. Murillo O, Doménech A, Garcia A, Tubau F, Cabellos C, Gudiol F et al (2006) Efficacy of high doses of levofloxacin in experimental foreign-body infection by methicillin-susceptible Staphylococcus aureus. Antimicrob Agents and Chemother 50:4011–4017

27. Paradis D, Vallée F, Allard S, Bisson C, Daviau N, Drapeau Ch et al (1992) Comparative study of pharmacokinetics and serum bactericidal activities of cefpirome, ceftriaxime, ceftriaxone, imipenem, and ciprofloxacin. Antimicrob Agents Chemother 36:2085–2092

28. Jacqueline C, Navas D, Batard E, Miègeville AF, Le Mabecque V, Kergueris MF et al (2005) In vitro and in vivo synergistic activities of linezolid combined with subinhibitory concentrations of imipenem against methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 49:45–51

29. Jacqueline C, Caillon J, Le Mabecque V, Miègeville AF, Donnio PY, Bugnon D et al (2003) In vitro activity of linezolid alone and in combination with gentamicin, vancomycin or rifampicin against methicillin-resistant Staphylococcus aureus by time–kill curve methods. J Antimicrob Chemother 51:857–864

30. Sahuquillo Arce JM, Colombo Gainza E, Gil Brusola A, Ortiz Estévez R, Cantón E, Gobernado M (2006) In vitro activity of linezolid in combination with doxycycline, fosfomycin, levofloxacin, rifampicin and vancomycin against methicillin-susceptible Staphylococcus aureus. Rev Esp Quimioter 19:252–257

31. Allen GP, Cha R, Rybak MJ (2002) In vitro activities of quinupristin–dalfopristin and ceftazidime, alone and in combination with various antimicrobials, against multidrug-resistant staphylococci and enterococci in an in vitro pharmacodynamic model. Antimicrob Agents Chemother 46:2606–2612

32. Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L et al (2001) Linezolid resistance in a clinical isolate of Staphylococcus aureus. Lancet 358:207–208

33. Fantin B, Carbon C (1992) In vivo antibiotic synergism: contribution of animal models. Antimicrob Agents Chemother 36:907–912

34. Cunha BA (2005) Methicillin-resistant Staphylococcus aureus: clinical manifestations and antimicrobial therapy. Clin Microbiol Infect 11:33–42