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Comparative bactericidal activities of daptomycin, glycopeptides, linezolid and tigecycline against blood isolates of Gram-positive bacteria in Taiwan

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ABSTRACT

In-vitro MICs and minimum bactericidal concentrations (MBCs) of daptomycin, linezolid, tigecycline, vancomycin and teicoplanin against Gram-positive bacteria were determined using the broth microdilution method for ten blood isolates each of methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), including two vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*. One strain of VISA was tested in a time-kill synergism assay of daptomycin combined with oxacillin, imipenem, rifampicin and isepamicin. Daptomycin showed excellent in-vitro bactericidal activity against all the isolates tested, with no tolerance or synergism effects when combined with other agents, except with rifampicin against VISA. Vancomycin had better bactericidal activity against MRSA and MSSA than did teicoplanin. Linezolid had the poorest bactericidal activity against the isolates tested, with 100% tolerance by the MSSA and VRE isolates, and 80% tolerance by the MRSA isolates. Tolerance towards tigecycline was exhibited by 40% of the MRSA isolates, 100% of the MSSA and vancomycin-resistant *E. faecalis* isolates, and 90% of the vancomycin-resistant *E. faecium* isolates.

Keywords Bactericidal activity, daptomycin, enterococci, MRSA, *Staphylococcus aureus*, time-kill experiments

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INTRODUCTION

The unsatisfactory response to vancomycin treatment associated with vancomycin-tolerant or vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *S. aureus* in cases of bacteraemia and endocarditis caused by methicillin-resistant *S. aureus* (MRSA) necessitates the development of new treatment options [1–3]. Rapidly increasing rates of MRSA in nosocomial infections have been reported in Taiwan (from 26.3% in 1986 to 77% in 2001), with the emergence of vancomycin-heteroresistant *S. aureus* since 2000 [2,4,5]. A high rate of non-susceptibility to quinupristin–dalfopristin has been revealed among MRSA (31%) and vancomycin-resistant *Enterococcus faecium* (66%) isolates, despite the unavailability of this agent in Taiwan [6], and a high frequency of linezolid-associated thrombocytopenia and anaemia has been reported among patients with renal insufficiency and end-stage renal disease, associated with a high incidence of MRSA bacteraemia [7,8]. These findings illustrate the urgent need in Taiwan for alternative treatments with activity against these pathogens.

Daptomycin is a cyclic lipopeptide antibiotic with rapid bactericidal activity against vancomycin-resistant *E. faecium* and *Enterococcus faecalis* (VRE), MRSA, vancomycin-heteroresistant *S. aureus* and VISA because of its calcium-dependent alteration of the bacterial membrane potential [9]. Reduced susceptibility to daptomycin in *S. aureus* has been reported in association with an increased thickness of the cell wall [10]. Synergism between daptomycin and other antibiotics, including β-lactams and aminoglycosides, has been

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reported, but data concerning the efficacy of combinations with other antibiotics remain scarce [11,12].

Accordingly, the aim of the present study was to determine the in-vitro bactericidal activity of daptomycin, linezolid, tigecycline, vancomycin and teicoplanin against blood isolates of methicillin-susceptible *S. aureus* (MSSA), MRSA (including VISA) and VRE. Time-kill experiments involving combinations of daptomycin with oxacillin, imipenem, rifampicin and isepamicin were performed with one VISA strain.

MATERIALS AND METHODS

Bacterial isolates

Ten isolates each of non-duplicate blood isolates of MSSA, MRSA (including two VISA isolates [5]), vancomycin-resistant *E. faecium* and vancomycin-resistant *E. faecalis* were selected from among isolates collected during 2000–2005. The vancomycin MICs for the two VISA isolates were 6 mg/L according to Etests (AB Biodisk, Solna, Sweden) performed using the high inoculum (2 × McFarland standard) method [5]. Eight of ten MRSA isolates had oxacillin MICs >28 mg/L (range 32 - >128 mg/L). All except one of the 20 VRE isolates had vancomycin MICs >128 mg/L (range 64 - >128 mg/L), and all VRE isolates had teicoplanin MICs >64 mg/L (all expressed the VanA phenotype). The imipenem, rifampicin and isepamicin MICs for the VISA isolate used in the time-kill experiment were 16 mg/L, >128 mg/L and 32 mg/L, respectively. The isolates were stored at −70°C in trypticase soy broth (BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with glycerol 15% v/v. *S. aureus* ATCC 29213, *E. faecium* ATCC 19434 and *E. faecalis* ATCC 29212 were included as control strains.

Antimicrobial agents

The following antimicrobial agents were provided by their manufacturers for use in this study: vancomycin and rifampicin (Sigma, St Louis, MO, USA); teicoplanin, (Aventis Pharma, Romainville, France); linezolid (Pharmacia, Kalamazoo, MI, USA); tigecycline (Wyeth-Ayerst, Pearl River, NY, USA); and daptomycin (Cubist Pharmaceuticals, Lexington, MA, USA). The antimicrobial agents used in combination with daptomycin for synergism evaluation against the VISA isolate were rifampicin, isepamicin (Schering-Plough, Osaka, Japan), imipenem–cilastatin (Merck Sharp & Dohme, West Point, PA, USA) and oxacillin (Bristol-Myers Squibb, Princeton, NJ, USA).

Susceptibility testing

MICs and minimum bactericidal concentrations (MBCs) were determined according to CLSI guidelines using the broth microdilution method with an initial inoculum of 5×10^5 CFU/mL [13]. When testing daptomycin MICs and MBCs, the medium used was Mueller–Hinton broth containing physiological levels of calcium (50 mg/L) [14]. Susceptibility to glycopeptides, linezolid and daptomycin was determined according to CLSI guidelines [13]. Rates of susceptibility to tigecycline were determined on the basis of the recommended breakpoints for staphylococci (≤0.5 mg/L) and enterococci (≤0.25 mg/L) [15]. Tolerance was defined as an MBC/MIC ratio of ≥32 [16].

Time-kill studies

Time-kill bactericidal activity was determined using a starting inoculum of 5×10^6 CFU/mL as described previously [17]. Daptomycin concentrations of 4 × MIC, 2 × MIC, 1 × MIC, 0.5 × MIC and 0.25 × MIC for each isolate were analysed. The MICs of oxacillin and rifampicin for the two VISA isolates were both >128 mg/L, so an initial drug concentration of 128 mg/L was chosen when testing these two agents in combination with daptomycin. A physiological level of calcium (50 mg/L) was added to Mueller–Hinton broth when testing daptomycin alone and in synergy time-kill assays. Viable counts of dilutions of cultures containing antibiotics were performed at 0, 1, 2, 4, 8 and 24 h. Plates yielding 30–300 bacterial colonies were counted. Each study was performed in duplicate with appropriate growth controls, with the results presented as an average value. Bactericidal activity was defined as a ≥3 $\log_{10}$ decrease in the bacterial count at 8 and 24 h as compared with the count at 0 h. Synergy was defined as a ≥2 $\log_{10}$ reduction in CFU with the combination as compared with the single more active agent at 8 and 24 h. Additivity was defined as a CFU decrease of 1–2 $\log_{10}$, and indifference as an increase or decrease within 1 $\log_{10}$ of growth at 8 and 24 h. Antagonism was defined as an increase in CFU of ≥2 $\log_{10}$ after incubation with the combination, as compared with the single more active agent at 24 h.

RESULTS

Antimicrobial susceptibility

The in-vitro activity of vancomycin, teicoplanin, linezolid, tigecycline and daptomycin against MSSA, MRSA (including VISA) and VRE is summarised in Table 1. The MICs and MBCs of control strains were within the expected ranges [13]. Tigecycline had the lowest MICs among the antibiotics tested against MSSA, MRSA and VRE isolates, followed by daptomycin (Table 1). All of the isolates were susceptible to daptomycin, linezolid and tigecycline.

Tolerance

Tolerance to teicoplanin among MSSA and MRSA isolates was more common than tolerance to vancomycin (MSSA, 90% and 20%, and MRSA, 90% and 10%, respectively). Tolerance to tigecycline was noted for 40% of MRSA, 100% of MSSA and vancomycin-resistant *E. faecalis*, and 90% of vancomycin-resistant *E. faecium* isolates. For

Table 1. In-vitro activity of daptomycin, vancomycin, teicoplanin, linezolid and tigecycline against 40 isolates of Gram-positive bacteria

Bacteria	No. of isolates	Antimicrobial agent	MIC (mg/L)			MBC (mg/L)			No. (%) of isolates with tolerance[a]
			Range	50%	90%	Range	50%	90%	
MSSA	10	Daptomycin	0.25–1	0.25	0.5	0.25–4	0.5	2	0
		Linezolid	1	1	1	≥32	≥32	≥32	10 (100)
		Tigecycline	0.12–0.25	0.12	0.12	≥4–≥8	4	≥8	10 (100)
		Vancomycin	0.5–2	1	1	16–≥64	4	≥64	2 (20)
		Teicoplanin	0.5–4	0.5	1	8–≥128	≥16	≥32	9 (90)
MRSA[b]	10	Daptomycin	0.25–1	0.25	1	0.25–2	0.25	2	0
		Linezolid	1–2	1	2	8–≥64	64	≥64	8 (80)
		Tigecycline	0.12–1	0.12	1	1–≥8	2	≥4	4 (40)
		Vancomycin	0.5–4	1	4	4–≥128	8	≥128	1 (10)
		Teicoplanin	0.5–8	1	4	8–≥256	≥32	≥128	9 (90)
VRE (*Enterococcus faecalis*)	10	Daptomycin	1–2	1	2	4–8	8	8	0
		Linezolid	1–2	2	2	≥32–≥64	≥64	≥64	10 (100)
		Tigecycline	0.12–0.25	0.12	0.25	≥4–≥8	≥8	≥8	10 (100)
VRE (*Enterococcus faecium*)	10	Daptomycin	1–4	2	4	1–8	4	8	0
		Linezolid	1–2	2	2	≥32–≥64	≥64	≥64	10 (100)
		Tigecycline	0.06–0.12	0.06	0.06	0.5–≥4	≥2	≥2	9 (90)

[a]Tolerance was defined as MBC/MIC ≥32.
[b]Two isolates of vancomycin-intermediate *Staphylococcus aureus* were included.
MBC, minimum bactericidal concentration; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *S. aureus*; VRE, vancomycin-resistant enterococci.

linezolid, tolerance was exhibited by 100% of MSSA and VRE isolates, and 80% of MRSA isolates. No daptomycin tolerance was observed for any of the isolates studied.

Time-kill studies

Daptomycin showed dose-dependent bactericidal activity against MSSA, VISA and VRE in time-kill experiments (Fig. 1). Bactericidal activity was observed after 8 h for MSSA at ≥1 × MIC, for VISA at ≥2 × MIC, for *E. faecalis* at ≥1 × MIC, and for *E. faecium* at ≥4 × MIC. At 24 h, regrowth was observed for *E. faecalis* at 1 × MIC, and for VISA at 1 × MIC and 2 × MIC. For all isolates, there was little difference at all time points between the growth of the isolate at 0.25 × MIC and the control. Bactericidal synergism was observed at 0.5 × MIC (5.42 $\log_{10}$ reduction at both 8 and 24 h) and 1 × MIC (5.26 $\log_{10}$ reduction at both 8 and 24 h) for daptomycin combined with oxacillin (Fig. 2a). Synergism was also observed at 0.25 × MIC (3.05 $\log_{10}$ and 5.41 $\log_{10}$ reduction at 8 and 24 h, respectively), at 0.5 × MIC (3.64 $\log_{10}$ and 5.34 $\log_{10}$ reduction at 8 and 24 h, respectively) and at 1 × MIC (5.45 $\log_{10}$ reduction at both 8 and 24 h) for daptomycin combined with imipenem (Fig. 2b). The combination of daptomycin and isepamicin showed synergism at 1 × MIC (5.26 $\log_{10}$ reduction at both 8 and 24 h) and at 0.5 × MIC (2.14 $\log_{10}$ reduction at 8 h), but indifference at 0.25 × MIC. Regrowth was observed after 24 h at 0.5 × MIC, but with retained synergism (Fig. 2d). Daptomycin combined with rifampicin demonstrated indifference at 0.25 × MIC and early additivity at 0.5 × MIC (8 h); however, antagonism was noted for the combination of daptomycin plus rifampicin at 24 h (Fig. 2c).

DISCUSSION

Bactericidal activity appears to be necessary for clinical efficacy in certain circumstances, e.g., endocarditis, meningitis, osteomyelitis and severe infections involving neutropenic patients [18]. Vancomycin remains the treatment of choice for severe MRSA infection, but its efficacy is inferior to that of anti-staphylococcal penicillins against MSSA [19]. In the present study, daptomycin had the most potent in-vitro bactericidal activity among the agents studied, with rapid bactericidal activity against MSSA, VISA and VRE. A recent study revealed that daptomycin showed non-inferiority, as compared with the standard regimens, for treating both MSSA and MRSA bacteraemia and right-sided endocarditis [20]. Further studies are required to evaluate its clinical efficacy against VISA.

An increase in vancomycin MIC, even within the susceptible range, has raised the risk of treatment failure in cases of MRSA bacteraemia [21]. Tolerance to antibiotics could increase bacterial survival and regrowth after antibiotic removal without altering the MIC. This phenomenon may provide a degree of antibiotic resistance

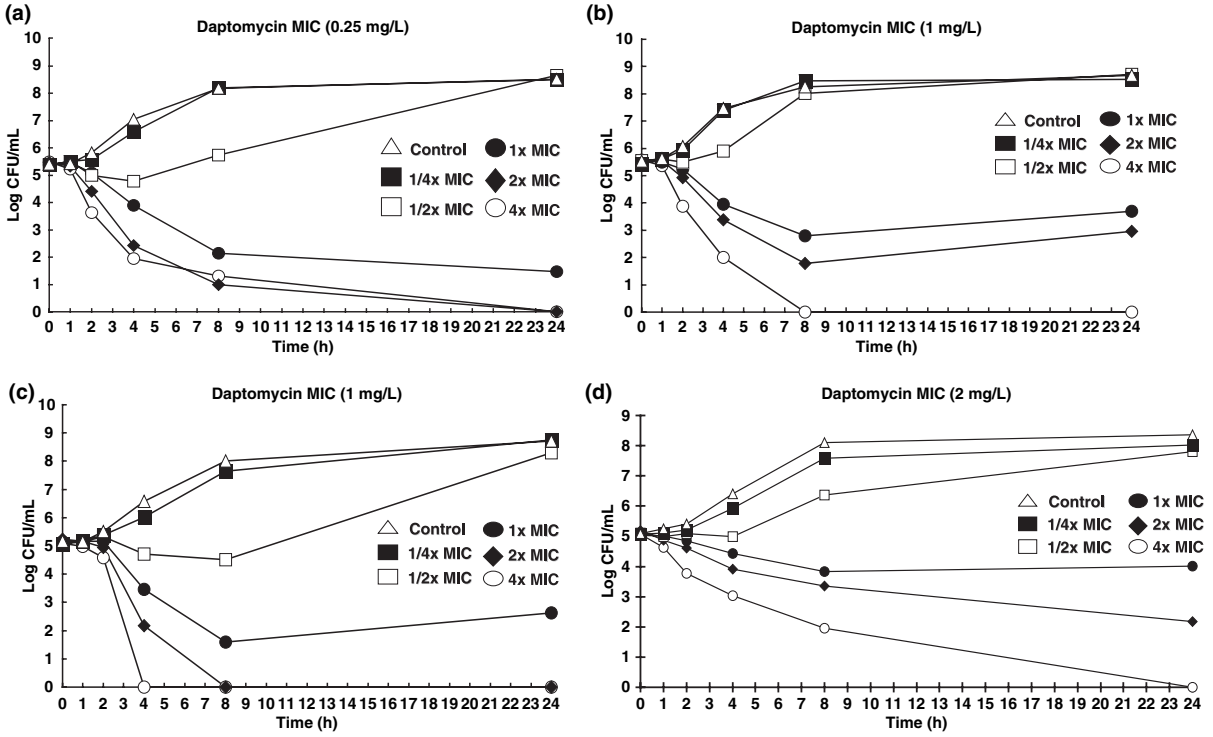

Fig. 1. Time-kill curves for (a) methicillin-susceptible *Staphylococcus aureus*, (b) methicillin-resistant vancomycin-intermediate *S. aureus* (vancomycin MIC 6 mg/L), (c) vancomycin-resistant *Enterococcus faecalis*, and (d) vancomycin-resistant *Enterococcus faecium*.

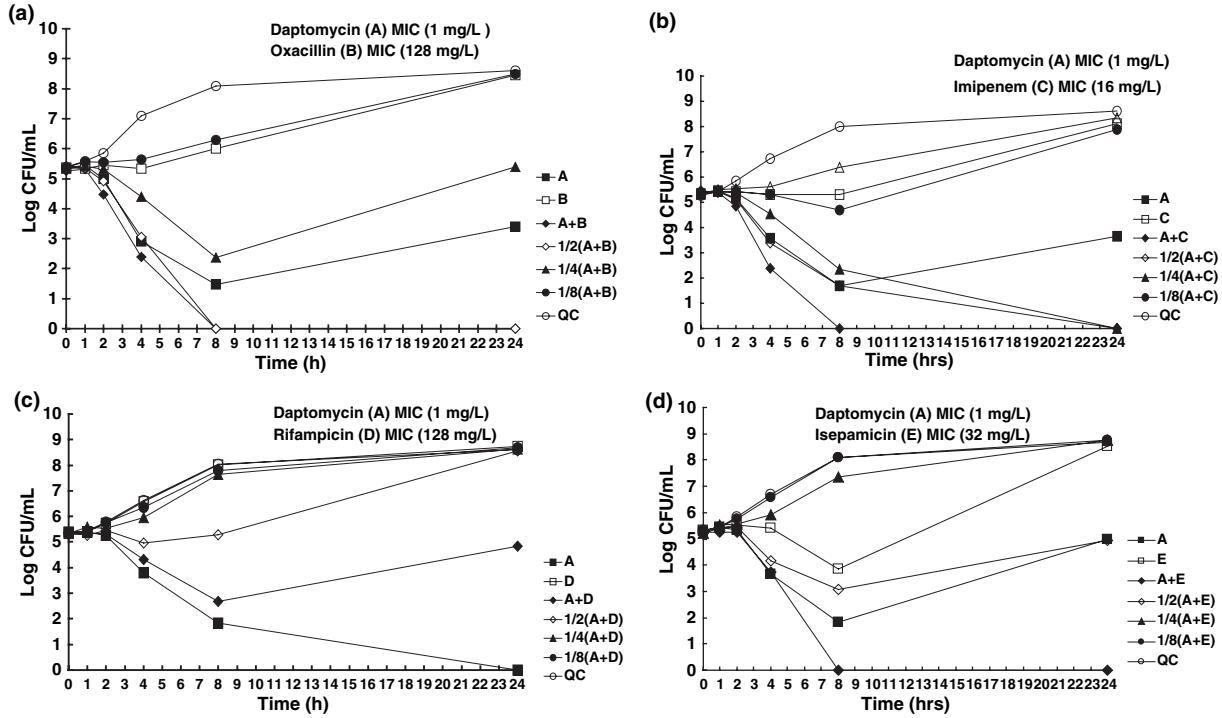

Fig. 2. Time-kill curves of daptomycin in combination with (a) oxacillin, (b) imipenem, (c) rifampicin, and (d) isepamicin against methicillin-resistant vancomycin-intermediate *Staphylococcus aureus* (vancomycin MIC 6 mg/L).

and increase the difficulty of treating infections, e.g., endocarditis, that require effective bactericidal action. Indeed, it has been suggested that tolerance of MRSA to vancomycin should be evaluated to assess the risk of clinical failure during treatment [2]. Vancomycin tolerance has been observed previously in ≤15.2% of wild-type MRSA and 73.95% of vancomycin-heteroresistant *S. aureus* isolates [2]. In the present study, 90% of both MSSA and MRSA isolates were more susceptible to teicoplanin than to vancomycin (20% and 10%, respectively), which is a finding similar to that reported previously [22]. It is not surprising that a high proportion of MSSA, MRSA and VRE isolates had an MBC/MIC ratio of ≥32 for linezolid and tigecycline, because of the bacteriostatic characteristics of these agents.

It is interesting to consider the implications of regrowth in the presence of daptomycin at 1 × MIC for the VISA and VRE isolates in the present study, which has also been noted previously [23]. This phenomenon disappeared after increasing the daptomycin concentration to 4 × MIC for VISA and 2 × MIC for VRE, thereby further illustrating the concentration-dependent killing effecct of daptomycin [23,24]. The reasons why daptomycin showed lower efficacy against *E. faecium* when compared with the other isolates (at 4 × MIC) in the present study remain unclear. The inferior activity of daptomycin against *E. faecium*, as compared with *E. faecalis* and MRSA (including vancomycin-intermediate isolates), might partly explain this phenomenon [23,25]. The daptomycin MIC for the VISA isolate studied was 1 mg/L, which is at the upper limit of the CLSI breakpoint [13]. As the trough free drug concentration may fall below the MIC (*c.* 0.6 mg/L) [12], caution is required when treating pathogens with relatively high daptomycin MICs.

Daptomycin showed bactericidal synergy at 24 h when combined with oxacillin, imipenem and isepamicin. Previous studies have reported synergism between daptomycin and β-lactams, despite the high MICs of β-lactams for MRSA and VRE isolates [11,12,26]. In the present study, daptomycin combined with imipenem showed greater in-vitro synergy than when combined with oxacillin and isepamicin, and maintained a bactericidal effect against VISA, even at 0.25 × MIC. A serum concentration of 61.2 ± 9.8 mg/L was clinically achievable 30 min after an infusion of 1 g imipenem plus 1 g cilastatin [27]. More data are required concerning the efficacy of the combination of daptomycin and imipinem against *S. aureus* isolates with a higher daptomycin MIC.

The reasons for the difference in effects seen when combining rifampicin with daptomycin against the VISA isolate in the present study remain to be clarified. A previous study found that daptomycin combined with rifampicin had an indifferent effect against 82% of Gram-positive isolates studied [11], and no synergism was observed between daptomycin and rifampicin for rifampicin-susceptible or -resistant MRSA [12]. However, additivity was reported against two, and synergy against one, of the three VISA isolates for which no antagonism was detected [28]. The isolate that showed synergy was susceptible to rifampicin, whereas the VISA isolate in the present study was resistant. Furthermore, the rifampicin concentration used in the present study was higher than that used previously (128 mg/L and 0.004 mg/L, respectively). The extent to which the rifampicin concentration, or VISA susceptibility to rifampicin, affects the response to daptomycin plus rifampicin remains to be clarified.

In conclusion, this study showed that daptomycin had the most potent in-vitro bactericidal activity against the MSSA, MRSA (including VISA) and VRE isolates tested. Daptomycin plus imipinem–cilastatin had the most effective in-vitro synergy against a VISA isolate.

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REFERENCES

1. Chang FY, Peacock JE, Musher DM *et al. Staphylococcus aureus* bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study. *Medicine (Baltimore)* 2003; **82**: 333–339.
2. Jones RN. Microbiological features of vancomycin in the 21st century: minimum inhibitory concentration creep, bactericidal/static activity, and applied breakpoints to predict clinical outcomes or detect resistant strains. *Clin Infect Dis* 2006; **1** (suppl): S13–S24.
3. Srinivasan A, Dick JD, Perl TM. Vancomycin resistance in staphylococci. *Clin Microbiol Rev* 2002; **15**: 430–438.
4. Hsueh PR, Teng LJ, Chen WH *et al.* Increasing prevalence of methicillin-resistant *Staphylococcus aureus* causing nosocomial infections at a university hospital in Taiwan from 1986 to 2001. *Antimicrob Agents Chemother* 2004; **48**: 1361–1364.

5. Wang JL, Tseng SP, Hsueh PR, Hiramastu K. Vancomycin heteroresistance in methicillin-resistant *Staphylococcus aureus*, Taiwan. *Emerg Infect Dis* 2004; **10**: 1702–1704.
6. Luh KT, Hsueh PR, Teng LJ *et al.* Quinupristin–dalfopristin resistance among gram-positive bacteria in Taiwan. *Antimicrob Agents Chemother* 2000; **44**: 3374–3380.
7. Wu VC, Wang YT, Wang CY *et al.* High frequency of linezolid-associated thrombocytopenia and anemia among patients with end-stage renal disease. *Clin Infect Dis* 2006; **42**: 66–72.
8. Lin YH, Wu VC, Tsai IJ *et al.* High frequency of linezolid-associated thrombocytopenia among patients with renal insufficiency. *Int J Antimicrob Agents* 2006; **28**: 345–351.
9. Hancock RE. Mechanisms of action of newer antibiotics for Gram-positive pathogens. *Lancet Infect Dis* 2005; **5**: 209–218.
10. Cui L, Tominaga E, Neoh HM, Hiramatsu K. Correlation between reduced daptomycin susceptibility and vancomycin resistance in vancomycin-intermediate *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2006; **50**: 1079–1082.
11. Debbia E, Pesce A, Schito GC. In vitro activity of LY146032 alone and in combination with other antibiotics against gram-positive bacteria. *Antimicrob Agents Chemother* 1988; **32**: 279–281.
12. Rand KH, Houck HJ. Synergy of daptomycin with oxacillin and other beta-lactams against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2004; **48**: 2871–2875.
13. Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, 17th edn, informational supplement. Wayne, PA: CLSI, 2007.
14. Fuchs PC, Barry AL, Brown SD. Daptomycin susceptibility tests: interpretive criteria, quality control, and effect of calcium on in vitro tests. *Diagn Microbiol Infect Dis* 2000; **38**: 51–58.
15. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) Steering Committee. EUCAST technical note on tigecycline. *Clin Microbiol Infect* 2006; **12**: 1147–1149.
16. Sherris JC. Problems in in vitro determination of antibiotic tolerance in clinical isolates. *Antimicrob Agents Chemother* 1986; **30**: 633–637.
17. Eliopoulos GM, Mollering RC. Antimicrobial combinations. In: Lorian V, ed., *Antibiotics in laboratory medicine*, 4th edn. Baltimore: Williams & Wilkins, 1996; 330–396.
18. Pankey GA, Sabath LD. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clin Infect Dis* 2004; **38**: 864–870.
19. Karchmer AW. *Staphylococcus aureus* and vancomycin: the sequel. *Ann Intern Med* 1991; **115**: 739–741.
20. Fowler VG, Boucher HW, Corey GR *et al.* Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. *N Engl J Med* 2006; **355**: 653–665.
21. Sakoulas G, Moise-Broder PA, Schentag J, Forrest A, Moellering RC, Eliopoulos GM. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *J Clin Microbiol* 2004; **42**: 2398–2402.
22. Perry JD, Jones AL, Gould FK. Glycopeptide tolerance in bacteria causing endocarditis. *J Antimicrob Chemother* 1999; **44**: 121–124.
23. Rybak MJ, Hershberger E, Moldovan T, Grucz RG. In vitro activities of daptomycin, vancomycin, linezolid, and quinupristin–dalfopristin against staphylococci and enterococci, including vancomycin-intermediate and -resistant strains. *Antimicrob Agents Chemother* 2000; **44**: 1062–1066.
24. Akins RL, Rybak MJ. Bactericidal activities of two daptomycin regimens against clinical strains of glycopeptide intermediate-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, and methicillin-resistant *Staphylococcus aureus* isolates in an in vitro pharmacodynamic model with simulated endocardial vegetations. *Antimicrob Agents Chemother* 2001; **45**: 454–459.
25. Biedenbach DJ, Bell JM, Sader HS, Fritsche TR, Jones RN, Turnidge JD. Antimicrobial susceptibility of Gram-positive bacterial isolates from the Asia-Pacific region and an in vitro evaluation of the bactericidal activity of daptomycin, vancomycin, and teicoplanin: a SENTRY Program Report (2003–2004). *Int J Antimicrob Agents* 2007; **30**: 143–149.
26. Rand KH, Houck H. Daptomycin synergy with rifampicin and ampicillin against vancomycin-resistant enterococci. *J Antimicrob Chemother* 2004; **53**: 530–532.
27. Dreetz M, Hamacher J, Eller J *et al.* Serum bactericidal activities and comparative pharmacokinetics of meropenem and imipenem–cilastatin. *Antimicrob Agents Chemother* 1996; **40**: 105–109.
28. Credito K, Lin G, Appelbaum PC. Activity of daptomycin alone and in combination with rifampin and gentamicin against *Staphylococcus aureus* by time-kill methodology. *Antimicrob Agents Chemother* 2007; **51**: 1504–1507.