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Daptomycin Susceptibility of Unusual Gram-Positive Bacteria: Comparison of Results Obtained by the Etest and the Broth Microdilution Method▽

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MICs of daptomycin, linezolid, and vancomycin against 212 isolates, including *Listeria monocytogenes* and *Pediococcus*, *Leuconostoc*, *Rhodococcus*, and *Nocardia* spp., were determined by the broth microdilution method; daptomycin MICs were also determined by the Etest. Except with those for *Leuconostoc* spp., daptomycin Etest MICs showed >90% agreement with MICs obtained by the broth microdilution method.

Daptomycin is a cyclic lipopeptide antibiotic, produced by *Streptomyces roseosporus*, with rapid bactericidal activity against a wide spectrum of gram-positive organisms, including multidrug-resistant strains such as vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, and penicillin-resistant streptococci (2, 8). Daptomycin also has in vitro activity against several anaerobic gram-positive pathogens, including *Clostridium perfringens*, *C. difficile*, *Finegoldia magna*, *Propionibacterium acnes*, *Peptoniphilus asaccharolyticus*, and *Anaerococcus prevotii* (12, 19). However, data about its in vitro activity against unusual yet clinically relevant gram-positive microbes with reduced susceptibility to vancomycin, including *Listeria monocytogenes* and *Pediococcus*, *Leuconostoc*, *Rhodococcus*, and *Nocardia* spp., remain scarce (3, 11, 13, 14, 18). All of these pathogens can cause invasive diseases, including bacteremia, pulmonary infections, and soft tissue infections, which usually occur in immunocompromised but occasionally in immunocompetent hosts (1, 3, 9, 13, 17).

In this study, we investigated the in vitro activities of daptomycin, vancomycin, and linezolid against five unusual gram-positive pathogens by determining the MICs by the broth microdilution method and comparing the daptomycin MIC results with those obtained by a daptomycin Etest (AB Biodisk, Solna, Sweden).

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A total of 212 nonduplicate unusual gram-positive bacterial isolates were tested. These bacteria included *Listeria monocytogenes* ($n = 31$), *Pediococcus* spp. ($n = 13$), *Leuconostoc* spp. ($n = 68$), *Rhodococcus* spp. ($n = 21$), *Nocardia asteroides* ($n = 19$), *Nocardia brasiliensis* ($n = 34$), and other *Nocardia* species

TABLE 1. Antimicrobial susceptibilities of 212 unusual gram-positive bacteria to daptomycin, linezolid, and vancomycin determined by the broth microdilution method and the Etest[a]

Organism (no. of isolates) and antimicrobial agent	MIC (μg/ml)		
	Range	50%	90%
Listeria monocytogenes (31)			
Vancomycin	0.5–1	1	1
Linezolid	1–2	2	2
Daptomycin	0.5–8	4	4
Daptomycin (Etest)	0.75–4	3	4
Pediococcus spp. (13)			
Vancomycin	>64	>64	>64
Linezolid	1–4	2	4
Daptomycin	0.06–0.5	0.25	0.5
Daptomycin (Etest)	0.032–0.5	0.25	0.5
Leuconostoc spp. (68)			
Vancomycin	0.5–>64	>64	>64
Linezolid	0.5–8	4	4
Daptomycin	0.06–2	0.12	0.25
Daptomycin (Etest)	0.032–2	0.25	0.5
Rhodococcus spp. (21)			
Vancomycin	0.25–>64	1	>64
Linezolid	0.5–16	1	2
Daptomycin	2–>64	>64	>64
Daptomycin (Etest)	3–>256	96	>256
Nocardia brasiliensis (34)			
Vancomycin	0.25–>64	>64	>64
Linezolid	0.5–16	8	8
Daptomycin	0.5–>64	>64	>64
Daptomycin (Etest)	0.19–>256	>256	>256
Nocardia asteroides (19)			
Vancomycin	32–>64	>64	>64
Linezolid	0.5–4	4	4
Daptomycin	64–>64	>64	>64
Daptomycin (Etest)	24–>256	>256	>256
Other *Nocardia* species (26)			
Vancomycin	0.25–>64	>64	>64
Linezolid	2–8	4	4
Daptomycin	1–>64	>64	>64
Daptomycin (Etest)	0.75–>256	>256	>256

[a] Susceptibilities were tested by the broth microdilution method unless stated otherwise.

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TABLE 2. Comparison of daptomycin MIC results obtained by the Etest and the broth microdilution method against 212 unusual gram-positive bacterial isolates

Organism (no. of isolates)	No. (%) of isolates for which the Etest MIC differed from the broth microdilution MIC by the following number of $\log_2$ dilutions:							Mean difference	95% CI[a]	Agreement (%)[b]
	≤−3	−2	−1	0	1	2	≥3			
L. monocytogenes (31)	0 (0)	0 (0)	2 (6.5)	18 (58.1)	10 (32.3)	1 (3.2)	0 (0)	0.32	0.083–0.56	96.7
Pediococcus spp. (13)	0 (0)	0 (0)	5 (38.5)	7 (53.8)	0 (0)	1 (7.7)	0 (0)	−0.23	−0.73–0.27	92.3
Leuconostoc spp. (68)	3 (4.4)	8 (11.8)	26 (38.2)	25 (36.8)	5 (7.4)	1 (1.5)	0 (0)	−0.64	−0.88–−0.40	82.3
Rhodococcus spp. (21)	0 (0)	0 (0)	3 (14.3)	17 (81.0)	1 (4.8)	0 (0)	0 (0)	−0.10	−0.29–0.10	100
Nocardia spp. (79)	0 (0)	2 (2.5)	2 (2.5)	74 (93.7)	0 (0)	1 (1.3)	0 (0)	−0.05	−0.14–0.04	96.2

[a] CI, confidence interval.
[b] Percentage of isolates with Etest MICs within 1 $\log_2$ dilution of broth dilution MICs.

($n = 26$). These isolates were collected from various clinical specimens (blood, sterile tissues, airway secretions, nonsterile tissues, and wound cultures) of patients who were treated at the National Taiwan University Hospital, a 2,000-bed hospital located in northern Taiwan, from January 1995 to June 2006. The in vitro activities of drugs were determined using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute (5). The test medium for all drugs was Mueller-Hinton broth; for testing daptomycin, the broth contained physiological levels of calcium (50 μg/ml) as recommended previously (7). Standard powders of the three antimicrobial agents, daptomycin, vancomycin, and linezolid, were obtained from various manufacturers for broth microdilution testing. The Etest containing a gradient of daptomycin plus calcium was used according to the manufacturer's instructions, and the results were compared with MICs obtained by the broth microdilution method. *S. aureus* ATCC 29213 and *Enterococcus faecalis* 29212 were used as control strains.

Acceptable Etest accuracy for an antimicrobial agent was defined as >90% agreement (within 1 twofold dilution) with MICs determined by the broth microdilution method (6). The mean difference in $\log_2$ units between MICs obtained by the two methods was calculated using a one-sample *t* test.

The broth dilution MICs of the three drugs for the different bacteria are shown in Table 1. Elevated linezolid MICs were found for isolates of *Leuconostoc* spp. and some *Nocardia* spp. MICs were higher for *N. brasiliensis* isolates than for *N. asteroides* and other *Nocardia* species. The results of the daptomycin Etest correlated well with those obtained by the broth microdilution method, except for *Leuconostoc* spp. (agreement, 82.3%) (Table 2). For four isolates, MICs were 2 dilutions higher by the Etest than by the broth microdilution method; these included one *L. monocytogenes*, one *Leuconostoc* sp., one *Pediococcus* sp., and one *Nocardia* sp. isolate. The daptomycin MICs for *Leuconostoc* spp. by the Etest tended to be lower than those by the broth microdilution method and ≥2-fold lower for 16.2% of the isolates. Among all isolates tested, MICs determined by the Etest were more than 2 dilutions lower than MICs determined by the broth microdilution method for 12 isolates (17.6%) and more than 3 dilutions lower for 4 isolates (5.8%).

Our data further support the potential clinical application of daptomycin against infections caused by *Leuconostoc* and *Pediococcus* spp., though only limited clinical data have been reported (11). Infections caused by *L. monocytogenes* are rare in Taiwan and usually present as bloodstream infections in patients with underlying malignancies and as meningitis in healthy persons (16). The MICs of daptomycin against *L. monocytogenes* were higher in this study than in recent reports (19). *Rhodococcus* spp. typically cause bacteremia and pulmonary infection in immunocompromised hosts; occasionally, they may cause pulmonary infection or localized infection in immunocompetent patients (17). Effective treatment often requires a combination of several agents and prolonged usage. In Taiwan, multidrug-resistant strains causing invasive diseases have been reported, limiting treatment options (15). The in vitro activity of daptomycin against *Rhodococcus* spp. was poor, a finding that has not been reported previously. The MIC data for vancomycin against *Rhodococcus* spp. differed in this study, indicating that any treatment with this agent should be based on individualized MIC data.

Sulfonamides combined with a carbapenem or an expanded-spectrum cephalosporin are regarded as the drugs of choice for severely ill patients (3). All of the 79 *Nocardia* isolates tested in this study were also inhibited by linezolid at a concentration of ≤8 μg/ml, but the MICs at which 50 and 90% of isolates were inhibited (MIC_{50} and MIC_{90}, respectively) were higher than those previously reported (4, 10, 20). Linezolid MICs were higher for *N. brasiliensis* isolates than for *N. asteroides* and other *Nocardia* species in this study. The mechanisms responsible for high linezolid MICs against *Nocardia* species in Taiwan require further clarification.

Daptomycin Etest MIC results correlated well with results obtained by the broth microdilution method for *L. monocytogenes* and for *Pediococcus*, *Rhodococcus*, and *Nocardia* spp. in this study, suggesting the clinical usefulness of the Etest method. For *L. monocytogenes*, there is a trend toward overestimation of MICs determined by the Etest (Table 2). For *Leuconostoc*, a very major error occurred between these two methods, indicating that the Etest should not be used clinically to detect the daptomycin MICs for this organism.

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