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Baseline Study To Determine In Vitro Activities of Daptomycin against Gram-Positive Pathogens Isolated in the United States in 2000-2001

Ian A. Critchley,[1] Renée S. Blosser-Middleton,[1] Mark E. Jones,[2] Clyde Thornsberry,[3] Daniel F. Sahm,[1] and James A. Karlowsky[1]*

Focus Technologies, Inc., Herndon, Virginia,[1] Focus Technologies, Hilversum, The Netherlands,[2] and Focus Technologies, Franklin, Tennessee[3]

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The activity of daptomycin was assessed by using 6,973 gram-positive bacteria isolated at 50 United States hospitals in 2000 and 2001. Among the isolates of *Streptococcus pneumoniae* ($n = 1,163$) collected, the rate of penicillin resistance was 16.1%; rates of oxacillin resistance among *Staphylococcus aureus* isolates ($n = 1,018$) and vancomycin resistance among *Enterococcus faecium* isolates ($n = 368$) were 30.0 and 59.5%, respectively. Multidrug-resistant (MDR) phenotypes (isolates resistant to three or more different chemical classes of antimicrobial agents) accounted for 14.2% of *S. pneumoniae* isolates, 27.1% of *S. aureus* isolates, and 58.4% of *E. faecium* isolates. For all gram-positive species tested, MICs at which 90% of the isolates tested were inhibited (MIC_{90}s) and MIC ranges for directed-spectrum agents (daptomycin, quinupristin-dalfopristin, and linezolid) were identical or highly similar for isolates susceptible or resistant to other agents or MDR. Daptomycin had a MIC_{90} of 0.12 μg/ml for both penicillin-susceptible and -resistant isolates of *S. pneumoniae*. Against oxacillin-resistant *S. aureus* daptomycin had a MIC_{90} of 0.5 μg/ml, and it had a MIC_{90} of 4 μg/ml against both vancomycin-susceptible and -resistant *E. faecium*. The MIC_{90}s for daptomycin and other directed-spectrum agents were unaffected by the regional or anatomical origin of isolates or patient demographic parameters (patient age, gender, and inpatient or outpatient care). Our results confirm the gram-positive spectrum of activity of daptomycin and that its activity is independent of susceptibility or resistance to commonly prescribed and tested antimicrobial agents. This study may serve as a baseline to monitor future changes in the susceptibility of gram-positive species to daptomycin following its introduction into clinical use.

Increasing resistance among gram-positive pathogens is limiting the use of presently available antimicrobial agents and is driving the search for new agents with novel mechanisms of action that are capable of circumventing present mechanisms of resistance (4, 5, 10, 16, 18, 20, 27). Resistance is often not restricted to a single agent or antimicrobial class but involves multiple agents belonging to several different antimicrobial classes (multidrug resistance). A new agent being introduced to the market for the therapy of gram-positive infections not only must cover the spectrum of clinically relevant pathogens (streptococci, staphylococci, and enterococci) but also must maintain its potency against multidrug-resistant (MDR) isolates. Quinupristin-dalfopristin and linezolid are two directed-spectrum agents recently marketed in the United States and elsewhere for the treatment of gram-positive infections (23). Another directed-spectrum agent, daptomycin, is presently in phase III clinical trials for the treatment of gram-positive infections in hospitalized patients (27). Phase II clinical trials with daptomycin demonstrated that it was efficacious in the treatment of skin and soft tissue infections and bacteremia (26). Several in vitro studies have shown that daptomycin, a cyclic lipopeptide antimicrobial, is active against gram-positive bacteria, including isolates that are resistant to presently available agents (1, 3, 17, 21, 25, 26). During 2000 and 2001, we collected 6,973 gram-positive pathogens from a geographically distributed network of 50 hospitals in the United States to assess susceptibilities to presently marketed and tested antimicrobial agents and newer directed-spectrum agents, including daptomycin, linezolid, and quinupristin-dalfopristin. This study was designed to benchmark the activity of daptomycin against a contemporary, representative collection of clinical isolates and to provide a baseline for monitoring any future changes in the susceptibility of gram-positive isolates to daptomycin following its launch into clinical use.

* Corresponding author. Mailing address: Focus Technologies, Inc., 13665 Dulles Technology Dr., Suite 200, Herndon, VA 20171. Phone: (703) 480-2500. Fax: (703) 480-2670. E-mail: jkarlowsky@focusanswers.com.

MATERIALS AND METHODS

Organism collection and identification. During 2000 and 2001, 6,973 gram-positive isolates were collected from patient specimens at 50 hospitals distributed throughout the nine U.S. Bureau of the Census regions. Five to six hospitals were enrolled in each region and included children's, community, teaching, university, and Veterans Administration hospitals. Each hospital laboratory was requested to submit 25 *Streptococcus pneumoniae*, 10 viridans streptococcus, 10 *Streptococcus pyogenes*, 5 *Streptococcus agalactiae*, 20 *Staphylococcus aureus*, 25 coagulase-negative staphylococci, and 55 *Enterococcus* species isolates. No more than 15 of the *Enterococcus* species isolates submitted by each site could be collected from urine specimens. Isolates were limited to one per patient and were collected sequentially by each laboratory. Isolates were deemed to be clinically significant according to algorithms present in participating laboratories. Isolates were collected from both inpatients ($n = 1,766$) and outpatients ($n = 5,031$), and 176 isolates were collected from patients with an unknown patient location. Isolates were collected from blood ($n = 1,840$), wound ($n = 1,666$), respiratory ($n = 1,572$), urine ($n = 1,073$), cerebrospinal fluid ($n = 56$), and other or unknown ($n = 766$) specimen sources. In total, 1,163 *S. pneumoniae*, 273 *S. agalactiae*, 484 *S. pyogenes*, 369 viridans streptococci, 1,018 *S. aureus*, 1,126 coagulase-negative

staphylococci, 2,092 *Enterococcus faecalis*, 368 *Enterococcus faecium*, and 80 *Enterococcus* (defined as non-*E. faecalis* and non-*E. faecium*) species or strains were collected. Isolates were transported by using Amies swabs (Technical Consultants Ltd., Lancashire, United Kingdom) and were submitted to the central laboratory (Focus Technologies, Inc., Herndon, Va.) for in vitro antimicrobial susceptibility testing. Upon receipt, isolates were subcultured onto 5% sheep blood agar and their identities were confirmed by using standard methods. *S. pneumoniae* isolates were confirmed by using optochin disk testing. *S. agalactiae* isolates were confirmed by the observation of β-hemolysis and by use of the PathoDx Strep Grouping agglutination test (Remel, Lenexa, Kans.). Confirmation of *S. pyogenes* was through observation of colony morphology and β-hemolysis on blood agar and L-pyrrolidonyl-β-naphthylamide (PYR) hydrolysis. Viridans streptococci were prescreened by using optochin disk testing and were speciated by using Vitek (bioMerieux, Hazelwood, Mo.). *S. aureus* isolates were confirmed by using the coagulase test. The coagulase test and Vitek confirmed coagulase-negative staphylococci. All enterococci were confirmed by using the PYR test and were speciated by using Vitek. Enterococci with unique phenotypes (e.g., quinupristin-dalfopristin-resistant *E. faecium*) were verified through PCR by using D-Ala-D-Ala ligase gene (*ddl*) primers specific for *E. faecalis* and *E. faecium* (6).

Antimicrobial testing. All isolates were tested by broth microdilution according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (19). Isolates were subcultured onto 5% sheep blood agar and were grown overnight (20 to 24 h) prior to testing. Sensititre microdilution panels (TREK Diagnostics, Westlake, Ohio) containing frozen antimicrobials over an appropriate range of concentrations were used. The agents tested against streptococci, staphylococci, and enterococci varied slightly for each species and are listed in Table 1. MICs were interpreted as susceptible, intermediate, or resistant according to NCCLS M100-S12 guidelines where available (19). NCCLS breakpoints were not available to interpret daptomycin MICs (19). Wells containing daptomycin were supplemented with 50 μg of Ca^{2+}/ml. The results of antimicrobial susceptibility testing for daptomycin, linezolid, and quinupristin-dalfopristin were compared against data for isolates susceptible and resistant to presently marketed antimicrobial agents.

RESULTS

Activity against streptococci. Of the 1,163 isolates of *S. pneumoniae* collected and tested, 728 (62.6%) were penicillin susceptible, 248 (21.3%) were penicillin intermediate, and 187 (16.1%) were penicillin resistant (Table 1). Decreased susceptibility to penicillin was associated with decreased susceptibility to other β-lactams, erythromycin, clindamycin, and trimethoprim-sulfamethoxazole (SXT), as indicated by higher MICs at which 90% of the isolates tested were inhibited (MIC_{90}s) and lower percent susceptibilities for the penicillin-resistant isolates compared to those for penicillin-susceptible isolates. The MIC_{90}s and percent susceptibilities of levofloxacin, vancomycin, quinupristin-dalfopristin, and linezolid were unaffected by penicillin resistance. Daptomycin showed consistent activity against penicillin-susceptible (MIC_{90}, 0.12 μg/ml), -intermediate (MIC_{90}, 0.25 μg/ml), and -resistant (MIC_{90}, 0.12 μg/ml) isolates of pneumococci.

Of the 484 isolates of *S. pyogenes* collected and tested, 91.1% were susceptible to erythromycin and 98.6% were susceptible to clindamycin. Isolates resistant to β-lactams were not observed. Daptomycin and quinupristin-dalfopristin displayed MIC_{90}s of 0.06 and ≤0.12 μg/ml, respectively, compared with a MIC_{90} of 1 μg/ml for linezolid. All isolates of *S. agalactiae* (n = 273) were β-lactam susceptible; susceptibility was lowest for erythromycin (72.2%) and clindamycin (87.5%). Against *S. agalactiae*, daptomycin and quinupristin-dalfopristin had MIC_{90}s of 0.25 μg/ml, while that for linezolid was 1 μg/ml. Of the 369 isolates of viridans streptococci tested, susceptibility ranged from 51.8% for erythromycin to 100% for linezolid. Daptomycin, quinupristin-dalfopristin, and linezolid had MIC_{90}s of 1 μg/ml.

Activity against staphylococci. Of the 1,018 isolates of *S. aureus* collected and tested, 305 (30.0%) were resistant to oxacillin. Cross-resistance to oxacillin compromised the activities of erythromycin, clindamycin, gentamicin, ciprofloxacin, and SXT. Daptomycin and quinupristin-dalfopristin MIC_{90}s were one doubling dilution higher for oxacillin-resistant isolates (0.5 μg/ml) than for oxacillin-susceptible isolates (0.25 μg/ml). The MIC_{90} was 4 μg/ml for linezolid tested against both oxacillin-susceptible and -resistant isolates. Susceptibility rates for coagulase-negative staphylococci (n = 1,126) were lowest for erythromycin (28.2%), oxacillin (31.6%), and ciprofloxacin (53.1%). Daptomycin and quinupristin-dalfopristin had MIC_{90}s of 0.5 μg/ml against coagulase-negative staphylococci versus a MIC_{90} of 2 μg/ml for linezolid.

Activity against enterococci. Among the 368 isolates of *E. faecium* tested, 219 (59.5%) were resistant to vancomycin. Susceptibilities to ampicillin, ciprofloxacin, teicoplanin, and linezolid were lower for vancomycin-resistant *E. faecium* than for vancomycin-susceptible isolates. MIC_{90}s for daptomycin, quinupristin-dalfopristin, and linezolid were identical for both vancomycin-susceptible and -resistant isolates. The majority of *E. faecalis* isolates (97.9%) were susceptible to vancomycin; percent susceptibilities to ciprofloxacin and teicoplanin were lower for vancomycin-resistant isolates compared to those for vancomycin-susceptible isolates. Daptomycin and linezolid MIC_{90}s were 2 μg/ml against both vancomycin-susceptible and vancomycin-resistant isolates. Against other *Enterococcus* species, rates of susceptibility were lowest for quinupristin-dalfopristin (47.5%) and ciprofloxacin (75.0%). The linezolid MIC_{90} was 2 μg/ml against other *Enterococcus* species, while that for daptomycin was 4 μg/ml.

Activities of daptomycin and comparative agents against MDR isolates. MDR was defined as resistance to three or more different chemical classes of antimicrobial agents. MDR phenotypes accounted for 14.2% of *S. pneumoniae* isolates, 27.1% of *S. aureus* isolates, and 58.4% of *E. faecium* isolates. Among *S. pneumoniae* isolates, the most common MDR phenotype included resistance to penicillin, erythromycin, and SXT, accounting for 86.0% of the MDR isolates; resistance to ceftriaxone, penicillin, erythromycin, and SXT was the next most common phenotype (6.1% of the MDR isolates). Among *S. aureus* strains, 69.6% of MDR isolates were resistant to oxacillin, erythromycin, and ciprofloxacin. Among *E. faecium* strains, 99.5% of the MDR isolates were resistant to ampicillin, vancomycin, and ciprofloxacin. No changes in the MIC_{90}s and MIC distributions for daptomycin, linezolid, or quinupristin-dalfoprisitin were observed for MDR and non-MDR isolates of *S. pneumoniae*, *S. aureus*, and *E. faecium* (data not shown). The MIC distribution for daptomycin against 11 levofloxacin nonsusceptible isolates of *S. pneumoniae* ranged from 0.06 to 0.25 μg/ml, highly similar to that for levofloxacin-susceptible isolates (≤0.015 to 0.5 μg/ml) (data not shown). Against high-level gentamicin-resistant isolates of *E. faecalis* and *E. faecium*, the MIC_{90}s for daptomycin were 2 and 4 μg/ml, respectively (data not shown). For daptomycin, the MIC_{90}s against all gram-positive pathogens remained consistent regardless of regional or anatomical origins of isolates or patient demographic

TABLE 1. In vitro activities of daptomycin and comparative agents

Species, phenotype (no. of isolates tested), and antimicrobial agent	MIC range (μg/ml)	MIC_{90} (μg/ml)	% Susceptible (NCCLS susceptible breakpoint)
S. pneumoniae			
Penicillin susceptible (728)			
Penicillin	≤0.03–0.06	≤0.03	100 (≤0.06)
Amoxicillin-clavulanate	≤0.015–0.25	≤0.015	100 (≤2)
Cefuroxime	≤0.015–0.25	0.03	100 (≤1)
Ceftriaxone	≤0.12–0.5	≤0.12	100 (≤1)
Vancomycin	≤0.06–0.5	0.25	100 (≤1)
Erythromycin	≤0.015–>2	0.06	93.1 (≤0.25)
Clindamycin	≤0.25–>1	≤0.25	98.1 (≤0.25)
Levofloxacin	≤0.25–>8	1	99.3 (≤2)
SXT	≤0.06–>4	1	89.3 (≤0.5)
Quinupristin-dalfopristin	≤0.12–2	0.5	99.0 (≤1)
Linezolid	≤0.25–2	1	100 (≤2)
Daptomycin	≤0.015–0.25	0.12	(NA)[a]
Penicillin intermediate (248)			
Penicillin	0.12–1	1	0
Amoxicillin-clavulanate	≤0.015–4	1	99.6
Cefuroxime	≤0.015–2	0.5	98.8
Ceftriaxone	≤0.12–>4	4	31.0
Vancomycin	≤0.06–0.25	0.25	100
Erythromycin	0.03–>2	>2	35.5
Clindamycin	≤0.25–>1	>1	79.8
Levofloxacin	≤0.25–>8	1	98.8
SXT	0.12–>4	>4	33.9
Quinupristin-dalfopristin	≤0.12–1	0.5	100
Linezolid	≤0.25–1	1	100
Daptomycin	≤0.015–0.5	0.25	
Penicillin resistant (187)			
Penicillin	2–>8	4	0
Amoxicillin-clavulanate	0.25–8	8	46.0
Ceftriaxone	0.25–>4	4	81.8
Cefuroxime	2–>4	>4	0
Vancomycin	≤0.06–0.25	0.25	100
Erythromycin	0.03–>2	>2	13.9
Clindamycin	≤0.25–>1	>1	74.3
Levofloxacin	≤0.25–8	1	98.4
SXT	0.25–>4	>4	2.1
Quinupristin-dalfopristin	≤0.12–1	0.5	100
Linezolid	0.5–1	1	100
Daptomycin	≤0.015–0.25	0.12	
S. pyogenes (484)			
Penicillin	≤0.03–0.12	≤0.03	100 (≤0.12)
Amoxicillin-clavulanate	≤0.015–0.25	≤0.015	(NA)
Ceftriaxone	≤0.015–0.25	≤0.015	100 (≤0.5)
Cefuroxime	≤0.12–0.5	≤0.12	(NA)
Vancomycin	≤0.06–0.5	0.25	100 (≤1)
Erythromycin	≤0.015–<2	0.12	91.1 (≤0.25)
Clindamycin	≤0.25–>1	≤0.25	98.6 (≤0.25)
Levofloxacin	≤0.25–8	0.5	99.8 (≤2)
SXT	≤0.06–>4	0.25	(NA)
Quinupristin-dalfopristin	≤0.12–1	≤0.12	100 (≤1)
Linezolid	≤0.25–2	1	100 (≤2)
Daptomycin	≤0.015–0.5	0.06	(NA)
S. agalactiae (273)			
Penicillin	≤0.03–0.12	0.06	100 (≤0.12)
Amoxicillin-clavulanate	≤0.015–0.12	0.06	(NA)
Ceftriaxone	≤0.015–0.12	0.06	100 (≤0.5)
Cefuroxime	≤0.12–0.5	≤0.12	(NA)
Vancomycin	0.12–0.5	0.25	100 (≤1)
Erythromycin	0.03–>2	>2	72.2 (≤0.25)
Clindamycin	≤0.25–>1	>1	87.5 (≤0.25)
Levofloxacin	≤0.25–>8	1	98.9 (≤2)
SXT	≤0.06–4	0.12	(NA)
Quinupristin-dalfopristin	≤0.12–1	0.25	100 (≤1)
Linezolid	0.5–1	1	100 (≤2)
Daptomycin	0.03–0.5	0.25	(NA)
Viridans streptococci (369)			
Penicillin	≤0.03–8	2	71.3 (≤0.12)
Amoxicillin-clauvulanate	≤0.015–16	2	(NA)
Ceftriaxone	≤0.015–>4	1	87.5 (≤1)
Cefuroxime	≤0.12–>4	4	(NA)
Vancomycin	≤0.06–2	0.5	99.7 (≤1)
Erythromycin	≤0.015–>2	>2	51.8 (≤0.25)
Clindamycin	≤0.25–>1	≤0.25	93.8 (≤0.25)
Levofloxacin	≤0.25–>8	1	97.6 (≤2)
SXT	≤0.06–>4	2	(NA)
Quinupristin-dalfopristin	≤0.12–2	1	99.5 (≤1)
Linezolid	≤0.25–2	1	100 (≤2)
Daptomycin	≤0.015–2	1	(NA)
S. aureus			
Oxacillin susceptible (713)			
Oxacillin	≤0.06–2	0.5	100 (≤2)
Vancomycin	≤0.25–1	1	100 (≤4)
Erythromycin	≤0.12–16	>16	34.6 (≤0.5)
Clindamycin	0.06–>4	0.5	92.7 (≤0.5)
Gentamicin	≤0.06–>16	1	97.2 (≤4)
Teicoplanin	≤0.12–16	0.5	99.9 (≤8)
Ciprofloxacin	≤0.03–>8	2	89.3 (≤1)
SXT	≤0.25–>4	≤0.25	96.9 (≤2)
Quinupristin-dalfopristin	≤0.06–0.5	0.25	100 (≤1)
Linezolid	0.5–4	4	100 (≤4)
Daptomycin	0.03–0.5	0.25	(NA)
Oxacillin resistant (305)			
Oxacillin	4–>8	>8	0
Vancomycin	≤0.25–2	1	100
Erythromycin	0.5–>16	>16	2.6
Clindamycin	0.06–>4	>4	36.4
Gentamicin	≤0.06–>16	>16	79.3
Teicoplanin	≤0.12–4	1	100
Ciprofloxacin	0.06–>8	>8	12.5
SXT	≤0.25–>4	>4	87.9
Quinupristin-dalfopristin	≤0.06–1	0.5	100
Linezolid	≤0.25–4	4	100
Daptomycin	0.12–0.5	0.5	
Coagulase-negative staphylococci (1,126)			
Oxacillin	≤0.06–>8	>8	31.6 (≤0.25)
Vancomycin	≤0.25–4	2	100 (≤4)
Erythromycin	≤0.12–>16	>16	28.2 (≤0.5)
Clindamycin	≤0.03–>4	>4	67.5 (≤0.5)
Gentamicin	≤0.06–>16	>16	71.4 (≤4)
Teicoplanin	≤0.12–>32	8	96.0 (≤8)
Ciprofloxacin	≤0.03–>8	>8	53.1 (≤1)
SXT	≤0.25–>4	>4	61.5 (≤2)
Quinupristin-dalfopristin	≤0.06–4	0.5	99.5 (≤1)
Linezolid	≤0.25–4	2	100 (≤4)
Daptomycin	≤0.015–2	0.5	(NA)
E. faecium			
Vancomycin susceptible (147)			
Vancomycin	≤0.5–4	1	100 (≤4)

Continued on following page

TABLE 1—*Continued*

Species, phenotype (no. of isolates tested), and antimicrobial agent	MIC range (μg/ml)	MIC_{90} (μg/ml)	% Susceptible (NCCLS susceptible breakpoint)
Ampicillin	≤0.25–128	64	44.2 (≤8)
Teicoplanin	≤0.03–8	0.5	100 (≤8)
Ciprofloxacin	≤0.12–>64	>64	32.7 (≤1)
Quinupristin-dalfopristin	≤0.12–8	2	85.7 (≤1)
Linezolid	≤0.5–2	2	100 (≤2)
Daptomycin	0.06–8	4	(NA)
Vancomycin resistant (219)			
Vancomycin	32–>256	>256	0
Ampicillin	0.5–>128	128	0.9
Teicoplanin	≤0.03–>64	>64	14.2
Ciprofloxacin	0.25–>64	>64	1.4
Quinupristin-dalfopristin	0.25–2	1	97.3
Linezolid	1–4	2	99.5
Daptomycin	0.25–4	4	
E. faecalis			
Vancomycin susceptible (2,049)			
Vancomycin	≤0.5–4	2	100 (≤4)
Ampicillin	≤0.25–8	1	100 (≤8)
Teicoplanin	≤0.03–1	0.12	100 (≤8)
Ciprofloxacin	≤0.12–>64	64	59.3 (≤1)
Quinupristin-dalfopristin	≤0.12–32	8	3.9 (≤1)
Linezolid	≤0.5–2	2	100 (≤2)
Daptomycin	0.03–4	2	(NA)
Vancomycin resistant (40)			
Vancomycin	32–>256	>256	0
Ampicillin	≤0.25–2	2	100
Teicoplanin	≤0.03–>64	>64	67.5
Ciprofloxacin	0.5–>64	>64	7.5
Quinupristin-dalfopristin	≤0.12–32	16	2.5
Linezolid	1–2	2	100
Daptomycin	≤0.015–2	2	
Other *Enterococcus* spp. (80)			
Vancomycin	≤0.5–256	4	98.8 (≤4)
Ampicillin	≤0.25–128	4	93.8 (≤8)
Teicoplanin	≤0.03–16	0.25	98.8 (≤8)
Ciprofloxacin	≤0.12–>64	16	75.0 (≤1)
Quinupristin-dalfopristin	0.25–32	4	47.5 (≤1)
Linezolid	1–4	2	98.8 (≤2)
Daptomycin	0.03–8	4	(NA)

[a] NA, NCCLS published breakpoints unavailable for interpretation (19).

parameters (patient age, gender, and inpatient or outpatient care) (data not shown).

DISCUSSION

Daptomycin demonstrated potent antimicrobial activity against all of the clinically relevant gram-positive pathogens collected and tested in this study, including those resistant to presently marketed agents. Although daptomycin is presently in phase III clinical trials, several investigators first reported the in vitro activity of this agent against gram-positive organisms more than 15 years ago (7–9, 13, 15, 28). Comparing the results described in historical studies with those generated in the present study shows that there has been little or no change in the susceptibility of gram-positive organisms to daptomycin during the last 15 years. The early studies also documented that the activity of daptomycin was dependent on the concentration of calcium in the growth medium (8, 11, 12, 14). Many of these studies tested the activity of daptomycin in cation-supplemented Mueller-Hinton broth containing 50 μg of Ca^{2+}/ml. Daptomycin is two- to fourfold more active in cation-adjusted Mueller-Hinton broth containing 50 μg of Ca^{2+}/ml than in medium containing 20 to 25 μg of Ca^{2+}/ml, the concentration of Ca^{2+} presently recommended by the NCCLS (11). To generate optimal results on the activity of daptomycin, all isolates in this study were tested in cation-supplemented Mueller-Hinton broth containing 50 μg of Ca^{2+}/ml, and this likely explains the agreement of our results with those generated by studies conducted in the late 1980s that used similarly supplemented media. For example, the susceptibility of oxacillin-resistant *S. aureus* to daptomycin remained unchanged (MIC_{90} of 0.25 μg/ml) for isolates tested in 1986 and 1987 (8, 15) compared to those of isolates collected and tested in 2000 and 2001. More recently, Barry et al. reported the in vitro activity of daptomycin against 2,789 clinical isolates from 11 centers in North America during 1999 (3). The MIC_{90}s and MIC ranges reported for daptomycin for all key pathogens reported in the 1999 study were similar, if not identical, to the values reported in this study.

The results of our study demonstrated that significant resistance to selected agents exists among the most common, clinically relevant gram-positive pathogens. Unfortunately, isolates that are resistant to one agent are often resistant to other antimicrobial agents. To date, relatively few surveillance studies have either monitored or reported on the prevalence of MDR isolates. One recent report showed that the proportion of *S. pneumoniae* isolates in the United States that were resistant to three or more antimicrobial classes increased from 9 to 14% between 1995 and 1998 (29). Another report draws attention to the fact that penicillin-resistant isolates of *S. pneumoniae* also showed reduced susceptibility to other β-lactams, macrolides, and SXT (22), and this was also the case in this study. Furthermore, the study by Sahm et al. also showed that there was a notable increase in several MDR phenotypes of *S. pneumoniae* between surveillance studies conducted during the 1997-1998 and 1998-1999 respiratory seasons (22). A separate study conducted in 1999 showed that 13.3% of oxacillin-resistant isolates of *S. aureus* were also resistant to azithromycin, levofloxacin, and SXT (A. M. Staples, I. A. Critchley, C. Thornsberry, K. S. Murfitt, and D. F. Sahm, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 161, p. 70, 2000). Although resistance to many presently marketed agents is emerging, the results of this study suggest that there has been little or no selective pressure by these agents for the development of cross-resistance to daptomycin in clinical isolates. In

accordance with previous observations, we found that daptomycin retained equivalent activity against isolates that were susceptible and resistant to presently marketed agents. Despite regional and patient demographic parameter (e.g., age) variations in resistance to presently available antimicrobial agents (22), the activity of daptomycin was not affected by regional or anatomical origins of isolates or patient demographic parameters (patient age, gender, and inpatient or outpatient care).

Daptomycin retained equivalent activity against both MDR and non-MDR isolates. To date, relatively few studies have monitored the extent of MDR phenotypes in key gram-positive pathogens. The results of this study demonstrated that MDR phenotypes are prevalent among *S. pneumoniae* (14.2% of isolates), *S. aureus* (27.1%), and *E. faecium* (58.4%) isolates. Newer directed-spectrum agents, because of their novel modes of action, are likely to be clinically successful in treating patients infected with present MDR phenotypes. Daptomycin, for example, disrupts the function of bacterial plasma membranes (2, 24). In vitro studies to monitor the emergence of resistance to daptomycin in *S. aureus*, *S. epidermidis*, *E. faecalis*, and *E. faecium* by using three different methods were unsuccessful in generating spontaneously resistant mutants (24). Serial passage of *S. aureus* in the presence of daptomycin generated mutants with daptomycin MICs that were 8- to 32-fold higher than that of the parent strain. However, these mutants exhibited significant growth defects, had increased voltage difference across the plasma membrane, and had reduced virulence. Although the propensity for daptomycin to select for resistant mutants is low, any mutants arising during therapy would likely be unable to proliferate during infection because of the reduced virulence.

In conclusion, we found that antimicrobial resistance, including multidrug resistance, was prevalent in key gram-positive pathogens isolated in the United States during 2000 and 2001. Daptomycin is a novel agent with potent activity against gram-positive organisms responsible for serious hospital infections, including those that are resistant to presently available agents. This study represents one of the most extensive studies conducted to monitor the activity of daptomycin against clinical isolates and will serve as a baseline for future studies to monitor the activity of this agent following its launch and clinical use.

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