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In Vitro Activities of Daptomycin against 2,789 Clinical Isolates from 11 North American Medical Centers

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The in vitro activity of daptomycin is affected by the concentration of calcium cations in the test medium. Mueller-Hinton broth is currently adjusted to contain 10 to 12.5 mg of magnesium per liter and 20 to 25 mg of calcium per liter, but for testing of daptomycin, greater concentrations of calcium (50 mg/liter) are recommended to better resemble the normal concentration of ionized calcium in human serum. Two levels of calcium were used for broth microdilution tests of 2,789 recent clinical isolates of gram-positive bacterial pathogens. MICs of daptomycin were two- to fourfold lower when the broth contained additional calcium. For most species, however, the percentages of strains that were inhibited by 2.0 μg of daptomycin per ml were essentially identical with the two broth media. Enterococci were the important exception; i.e., 92% were inhibited when tested in calcium-supplemented broth but only 35% were inhibited by 2.0 μg/ml without the additional calcium. This type of information should be considered when selecting criteria for defining in vitro susceptibility to daptomycin.

Daptomycin is a lipopeptide which is bactericidal against a wide range of gram-positive bacterial pathogens, including the antibiotic-resistant pneumococci, enterococci, and staphylococci that are currently presenting a challenge for the design of empirical chemotherapy. Daptomycin is now being evaluated for possible use in situations in which there may be a high prevalence of antibiotic-resistant gram-positive bacteria (10).

The antibacterial activity of daptomycin requires the presence of calcium cations. Previous studies (1–3, 5, 6) have led to the recommendation that when testing daptomycin, the broth medium should contain additional calcium approaching the concentration of ionized calcium that is normally found in human serum (ca. 50 mg/liter). For broth microdilution susceptibility tests of other agents, the National Committee for Clinical Laboratory Standards (NCCLS) recommends cation-adjusted Mueller-Hinton broth (CAMHB) that is adjusted to contain 20 to 25 mg of Ca^{2+} per liter and 10 to 12.5 mg of Mg^{2+} per liter (7). Those concentrations were selected in order to standardize microdilution tests of the aminoglycosides and tetracyclines, but when testing daptomycin, calcium supplemented CAMHB is recommended (1–3, 6). In the present study, we further assessed the impact of that recommendation by testing more than 2,700 consecutive clinical isolates in both types of broth medium in order to determine which species are likely to be most profoundly affected by the additional calcium. Others have proposed provisional MIC breakpoints of ≤2 μg/ml for susceptible, 4 μg/ml for intermediate, and ≥8 μg/ml for resistant (2, 3, 6, 10). Interpretive criteria have not yet been officially established, but we have focused our attention on MICs ranging from 2.0 to 4.0 μg/ml when assessing the effect of adding additional calcium to the broth medium.

Consecutive clinical isolates of gram-positive pathogens were collected from 11 medical centers throughout North America. The 2,789 isolates were gathered in the late winter and early spring of 1999, each isolate was from a different patient, and each was judged to be clinically significant at the time it was first recovered. The medical centers contributing to this collection are identified in the acknowledgments at the end of the text, and the species that were represented are listed in Table 1.

Broth microdilution tests were performed as outlined by the NCCLS (7). Daptomycin was serially diluted in CAMHB and in calcium supplemented CAMHB. The latter contained 50 mg of Ca^{2+} per liter, and the former contained 25 mg/liter. Vancomycin was the control agent serially diluted in CAMHB only. Lysed horse blood (ca. 3%) was added when testing streptococci. The daptomycin concentrations ranged from 0.008 to 16 μg/ml, and vancomycin concentrations ranged from 1.0 to 16 μg/ml.

The results of microdilution tests in two broth media are presented in Table 1. As reported elsewhere (1–3, 5, 6), the in vitro activity of daptomycin was enhanced when the concentration of calcium was increased. In calcium-supplemented CAMHB, daptomycin was more potent than vancomycin. For most species, the added calcium increased the activity of daptomycin two- to fourfold. The proportion of strains that were inhibited by ≤2 μg/ml or by ≤4 μg/ml in the two broth media varied considerably with different species.

Table 2 summarizes the results of such tests with three genera. Among the 550 enterococci, 35% of the strains tested were inhibited by 2.0 μg/ml in CAMHB but when additional calcium was added to the broth, 92% were susceptible to that concentration. Among 38 viridans group streptococci, only 24 (63%) were inhibited by 2 μg of daptomycin per ml when diluted in CAMHB, but 34 (89%) were inhibited in calcium-supplemented CAMHB. For staphylococci and streptococci other than those of the viridans group, >99% of the strains were inhibited by daptomycin at 2 μg/ml in CAMHB and all but one were inhibited by that concentration when tested in calcium-supplemented CAMHB.

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TABLE 1. In vitro activities of daptomycin and vancomycin in CAMHB and of daptomycin in calcium-supplemented CAMHB

Microorganism[a] (no. of isolates tested) and antimicrobial agent	Broth medium[b]	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus				
Oxacillin-S (375)				
Daptomycin	Ca^{2+} suppl.	0.03–1.0	0.25	0.5
Daptomycin	CAMHB	0.12–4.0	1.0	1.0
Vancomycin	CAMHB	≤1.0–2.0	≤1.0	≤1.0
Oxacillin-R (172)				
Daptomycin	Ca^{2+} suppl.	0.12–2.0	0.25	0.5
Daptomycin	CAMHB	0.5–8.0	1.0	1.0
Vancomycin	CAMHB	≤1.0–2.0	≤1.0	≤1.0
Coagulase-negative *Staphylococcus* sp.				
Oxacillin-S (204)				
Daptomycin	Ca^{2+} suppl.	0.016–2.0	0.25	0.5
Daptomycin	CAMHB	0.016–4.0	1.0	1.0
Vancomycin	CAMHB	≤1.0–4.0	≤1.0	2.0
Coagulase-negative				
Oxacillin-R (339)				
Daptomycin	Ca^{2+} suppl.	0.12–2.0	0.25	0.5
Daptomycin	CAMHB	0.25–8.0	1.0	1.0
Vancomycin	CAMHB	≤1.0–4.0	2.0	2.0
Micrococcus sp. (10)				
Daptomycin	Ca^{2+} suppl.	0.03–0.25	0.06	0.12
Daptomycin	CAMHB	0.06–0.5	0.12	0.25
Vancomycin	CAMHB	≤1.0–2.0	≤1.0	≤1.0
Enterococcus faecalis				
Vancomycin-S (377)				
Daptomycin	Ca^{2+} suppl.	0.06–4.0	1.0	2.0
Daptomycin	CAMHB	0.25–16	4.0	8.0
Vancomycin	CAMHB	≤1.0–4.0	≤1.0	2.0
Vancomycin-R (10)				
Daptomycin	Ca^{2+} Suppl.	0.12–4.0	2.0	4.0
Daptomycin	CAMHB	0.25–8.0	8.0	8.0
Vancomycin	CAMHB	8.0–>16	>16	>16
Enterococcus faecium				
Vancomycin-S (50)				
Daptomycin	Ca^{2+} suppl.	0.12–8.0	2.0	4.0
Daptomycin	CAMHB	0.12–>16	8.0	16
Vancomycin	CAMHB	≤1.0–2.0	≤1.0	2.0
Vancomycin-R (91)				
Daptomycin	Ca^{2+} suppl.	0.25–8.0	2.0	4.0
Daptomycin	CAMHB	0.5–16	4.0	8.0
Vancomycin	CAMHB	8.0–>16	>16	>16
Other *Enterococcus* spp. (22)[c]				
Daptomycin	Ca^{2+} suppl.	0.25–8.0	1.0	4.0
Daptomycin	CAMHB	1.0–16	4.0	16
Vancomycin	CAMHB	≤1.0–>16	2.0	>16
Streptococcus agalactiae (208)				
Daptomycin	Ca^{2+} suppl.	0.03–0.5	0.12	0.25
Daptomycin	CAMHB	0.06–1.0	0.5	1.0
Vancomycin	CAMHB	≤1.0	≤1.0	≤1.0

Continued on following page

TABLE 1—*Continued*

Microorganism[a] (no. of isolates tested) and antimicrobial agent	Broth medium[b]	MIC (μg/ml)		
		Range	50%	90%
Streptococcus pyogenes (239)				
Daptomycin	Ca^{2+} suppl.	0.016–0.5	0.03	0.06
Daptomycin	CAMHB	0.06–2.0	0.12	0.25
Vancomycin	CAMHB	≤1.0–2.0	≤1.0	<1.0
Streptococcus pneumoniae				
Penicillin-S (373)[d]				
Daptomycin	Ca^{2+} suppl.	0.06–0.5	0.12	0.25
Daptomycin	CAMHB	0.06–1.0	0.5	0.5
Vancomycin	CAMHB	≤1.0	≤1.0	≤1.0
Penicillin-I (92)[d]				
Daptomycin	Ca^{2+} suppl.	0.06–0.5	0.12	0.25
Daptomycin	CAMHB	0.25–1.0	0.5	1.0
Vancomycin	CAMHB	≤1.0–2.0	≤1.0	≤1.0
Penicillin-R (110)[d]				
Daptomycin	Ca^{2+} suppl.	0.06–0.5	0.12	0.25
Daptomycin	CAMHB	0.25–2.0	0.5	1.0
Vancomycin	CAMHB	≤1.0	≤1.0	≤1.0
Beta-hemolytic *Streptococcus* sp. (32)[e]				
Daptomycin	Ca^{2+} suppl.	0.03–0.5	0.06	0.5
Daptomycin	CAMHB	0.12–1.0	0.12	1.0
Vancomycin	CAMHB	≤1.0	≤1.0	≤1.0
Streptococcus viridans group (38)[f]				
Daptomycin	Ca^{2+} suppl.	0.03–8.0	0.5	2.0
Daptomycin	CAMHB	0.06–8.0	1.0	2.0
Vancomycin	CAMHB	≤1.0	≤1.0	≤1.0
Lactobacillus sp. (10)				
Daptomycin	Ca^{2+} suppl.	≤0.008–8.0	0.5	4.0
Daptomycin	CAMHB	≤0.008–16	0.5	8.0
Vancomycin	CAMHB	≤1.0–>16	≤1.0	>16
Corynebacterium sp. (29)[g]				
Daptomycin	Ca^{2+} suppl.	0.016–0.5	0.25	0.5
Daptomycin	CAMHB	0.06–1.0	0.25	1.0
Vancomycin	CAMHB	≤1.0	≤1.0	≤1.0
Bacillus sp. (8)				
Daptomycin	Ca^{2+} suppl.	0.12–4.0	0.25	
Daptomycin	CAMHB	0.06–16	1.0	
Vancomycin	CAMHB	≤1.0–2.0	≤1.0	

[a] S, susceptible; R, resistant; I, intermediate.
[b] CAMHB contained Ca^{2+} at 25 mg/liter, and Ca^{2+} suppl. was the same medium adjusted to 50 mg of Ca^{2+} per liter.
[c] Includes nine isolates of *E. avium*, eight of *E. gallinarum*, three of *E. casseliflavus*, 1 of *E. durans*, and 1 of *E. raffinosus*.
[d] Penicillin S, MIC of ≤0.06 μg/ml; penicillin I, MIC of 0.12 to 1.0 μg/ml; penicillin R, MIC of ≥2.0 μg/ml.
[e] Includes 5 serogroup C, 21 serogroup G, and 6 serogroup F isolates.
[f] Includes 12 *S. milleri*, 3 *S. salivarius*, 2 *S. mitis*, 4 *S. sanguis*, 2 *S. mutans*, and 15 unidentified-species isolates.
[g] Includes 7 *C. jeikeium* isolates and 22 with no species identified.

Daptomycin is equally active against vancomycin-susceptible and vancomycin-resistant enterococci. In the recommended calcium-supplemented CAMHB, 92% of the strains tested were inhibited by ≤2.0 μg/ml whereas 40 (7%) required 4.0 μg/ml for inhibition and for only 4 (0.7%) strains was the MIC 8.0 μg/ml. MICs of >8 μg/ml were not recorded for the 550 enterococci. This distribution of MICs appears to represent a normally bell-shaped curve that can be bisected if arbitrary MIC breakpoints are assigned without considering the population statistics. The critical question is whether enterococcal infections will respond to appropriate dosages of daptomycin. That can only be answered by clinical data, and such information is currently being gathered. Animal studies (4, 8, 10) and early clinical experiences (9, 10) suggest that daptomycin might

TABLE 2. Distribution of daptomycin MICs for three different genera of bacteria tested in two different broth media

Daptomycin MIC (μg/ml)	No. of strains at each MIC and 2 levels of calcium[a]					
	1,094 *Staphylococcus* sp. strains		1,096 *Streptococcus* sp. strains		550 *Enterococcus* sp. strains	
	25 mg/liter	50 mg/liter	25 mg/liter	50 mg/liter	25 mg/liter	50 mg/liter
>16					1	
16					14	
8	2		1	1	109	4
4	2		3	1	232	40
2	39	3	12	2	130	172
1	741	16	155	6	51	201
0.5	251	339	597	38	5	106
0.25	53	656	82	351	6	18
0.12	5	73	216	430	1	6
0.06		5	28	114		2
0.03		1	1	140	1	1
0.016	1	1	1	13		
0.008						
Median MIC	1.0	0.25	0.5	0.12	4.0	1.0
Geometric mean MIC	0.81	0.30	0.38	0.13	3.34	1.12

[a] Cation-adjusted Mueller-Hinton broth with calcium adjusted to 25 or 50 mg/liter was used.

be useful for treating serious enterococcal infections. The next question is whether strains for which the MIC is 4.0 or 8.0 μg/ml respond any differently than those for which the MIC is ≤2.0 μg/ml. Pneumococci, hemolytic streptococci, and staphylococci are nearly all susceptible to ≤2.0 μg/ml in vitro, and there is no reason to believe that they will not respond to daptomycin chemotherapy, but that remains to be proven.

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