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In Vitro Activity of Daptomycin against Gram-Positive European Clinical Isolates with Defined Resistance Determinants

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The in vitro activity of daptomycin against 337 gram-positive European clinical isolates with known resistance genes was determined. The MIC ranges for *Staphylococcus aureus*, enterococci, pneunococci, and streptococci were 0.03 to 1, 0.25 to 8, 0.12 to 1, and 0.06 to 8 μg/ml, respectively. For only one streptococcus isolate and seven enterococcus isolates was the MIC 8 μg/ml.

The emergence of multiple-antibiotic-resistant gram-positive isolates continues to provide challenges. Daptomycin is a novel lipopeptide that exhibits rapid in vitro bactericidal activity against gram-positive pathogens (3, 8, 12, 13, 16, 19, 22), probably by disrupting bacterial membrane potential (1, 2, 4). The objective of this study was to determine the in vitro activities of daptomycin and other antibiotics against 337 antibiotic-resistant gram-positive clinical isolates with genetically characterized resistance determinants.

The isolates were collected between 1997 and 2000 from 23 European centers as described previously (6). No center or country provided a disproportionate number of isolates.

PCR analysis and, if necessary, DNA sequencing were used to identify the resistance determinants. In staphylococci, the *mecA* gene and mutations associated with quinolone resistance were determined as described previously (7, 18). The tetracycline resistance genes in *Staphylococcus aureus* and *Streptococcus pneumoniae* were detected as described by Warsa et al. (21). Fluoroquinolone resistance genes and macrolide, lincosamide, and streptogramin resistance genes in *S. pneumoniae* and other streptococci were detected as described earlier (9–11, 15, 18, 20). In enterococci, primers used to identify the *ant(4′)-Ia* gene have been described previously (17). Primers used to identify *aac(6′)-Ie+aph(2″)* were 5′-GAACATGAAT TACACGAGGG and 5′-CCATTTTCGATAAATTCCTG; the primers used for the detection of the *aph(3′)-IIIa* gene were 5′-AAATGACGGACAGCCGGTAT and 5′-CGATGG AGTGAAAGAGCCTG. Vancomycin resistance encoded by *vanA* in enterococci was determined as described previously (5).

MICs were determined by National Committee for Clinical Laboratory Standards methodology (14). Antimicrobial agents and frozen microtiter plates containing antibiotic solutions and physiologic concentrations of Ca^{2+} (50 μg/ml) were supplied by TREK Diagnostic Systems, Inc. (Westlake, Ohio).

For 38 *mecA*-positive, methicillin-resistant *S. aureus* isolates, the MIC range for daptomycin was 0.03 to 0.5 μg/ml (Table 1), with MICs at which 50 and 90% of the isolates tested are inhibited (MIC_{50} and MIC_{90}, respectively) of 0.25 and 0.5 μg/ml, respectively. Four types of amino acid changes were detected in the quinolone resistance-determining region of *grlA/gyrA* of 49 *S. aureus* isolates. The MIC range for daptomycin against these resistant isolates was 0.06 to 0.5 μg/ml (Table 1).

A tetracycline-resistant phenotype in *S. aureus* was caused by the presence of either *tetK* ($n = 18$), *tetM* ($n = 18$), or both genes ($n = 7$). The MIC ranges of daptomycin for these isolates were 0.06 to 1, 0.06 to 0.25, and 0.12 to 0.5 μg/ml, respectively (Table 1).

Seven *Enterococcus faecalis* and 27 *Enterococcus faecium* *vanA*-positive isolates were tested for sensitivity to a number of antibiotics (Table 1). The MICs for daptomycin ranged from 0.5 to 4 μg/ml for *E. faecalis* and from 0.25 to 8 μg/ml for *E. faecium*; the MIC_{50}s for these species were 0.5 and 4 μg/ml, respectively; and the MIC_{90} was 4 μg/ml for both species.

A total of 32 *E. faecalis* and 16 *E. faecium* isolates exhibited high-level gentamicin resistance. The daptomycin MICs ranged from 0.5 to 8 μg/ml for both species (Table 1); the MIC_{50}s for *E. faecalis* and *E. faecium* were 2 and 4 μg/ml, respectively; and the MIC_{90}s were 2 and 8 μg/ml, respectively.

In this study, daptomycin MICs for *E. faecium* appeared slightly higher than those in other published data. A recent study reported MIC_{50}s and MIC_{90}s of 2 μg/ml for 25 *E. faecium* isolates (both vancomycin resistant and susceptible) at physiologic calcium concentrations (50 μg/ml) (22). One explanation for these results may be the limited number of isolates tested in our study.

The MICs of daptomycin and other antibiotics were determined for pneumococci with known resistance mechanisms for fluoroquinolones, tetracycline, clindamycin, and erythromycin. Two fluoroquinolone-susceptible isolates lacking amino acid changes in GyrA, ParC, GyrB, or ParE were tested, and for both, the daptomycin MIC was 0.12 μg/ml (Table 1).

Twenty isolates, categorized into four different groups according to amino acid changes associated with quinolone resistance, were tested for activity against a number of antibiotics (Table 1). The daptomycin MICs for the 20 isolates ranged from 0.12 to 1 μg/ml, and the MIC_{50}s and MIC_{90}s were 0.12 and 0.25 μg/ml, respectively.

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TABLE 1. MIC range and defined resistance determinants of various gram-positive pathogens

Species	Resistance determinant(s)	*n*	Antibiotic	MIC range (μg/ml)
Staphylococcus aureus	*mecA*	38	Daptomycin Oxacillin	0.03–0.5 >8–>8
	Ser80Phe/Glu88Lys[a]	14	Daptomycin Ciprofloxacin	0.12–0.5 8–>8
	Ser80Phe/Ser84Leu	13	Daptomycin Ciprofloxacin	0.12–0.5 4–>8
	Ser80Tyr/Glu88Lys	9	Daptomycin Ciprofloxacin	0.12–0.5 8–>8
	Ser80Tyr/Ser84Leu	13	Daptomycin Ciprofloxacin	0.06–0.5 8–>8
	tetK	18	Daptomycin	0.06–1
	tetM	18	Daptomycin	0.06–0.25
	tetK+tetM	7	Daptomycin	0.12–0.5
Enterococcus faecalis	*vanA*	7	Daptomycin Teicoplanin Vancomycin	0.5–4 32–>64 256–>256
	aph(3′)IIIa	4	Daptomycin Gentamicin	0.5–1 >500–>500
	aac(6′)-aph2″	11	Daptomycin Gentamicin	0.5–2 >500–>500
	aac(6′)-aph2″ + aph(3′)IIIa	17	Daptomycin Gentamicin	0.5–8 >500–>500
Enterococcus faecium	*vanA*	27	Daptomycin Teicoplanin Vancomycin	0.25–8 8–>64 64–>256
	aph(3′)IIIa	2	Daptomycin Gentamicin	4–4 >500–>500
	aac(6′)-aph2″	5	Daptomycin Gentamicin	4–8 >500–>500
	aac(6′)-aph2″ + aph(3′)IIIa	9	Daptomycin Gentamicin	0.5–8 >500–>500
Streptococcus pneumoniae	nc/nc/nc/nc[b]	2	Daptomycin Levofloxacin	0.12–0.12 1–1
	nc/nc/nc/Ile460Val	5	Daptomycin Levofloxacin	0.12–1 1
	Ser81Phe/Ser79Phe/nc/nc	12	Daptomycin Levofloxacin	0.12–0.25 4–>8
	Ser81Tyr/Ser79Phe/nc/Ile460Val	1	Daptomycin Levofloxacin	0.12 4
	Ser81Phe/Asp83Asn/nc/nc	1	Daptomycin Levofloxacin	0.25 8
	Ser81Phe/Asp83Asn/nc/Ile460Val	1	Daptomycin Levofloxacin	0.06 8
	tetM	12	Daptomycin	0.12–1
	ermB	9	Daptomycin Clindamycin Erythromycin	0.12–0.12 >1–>1 >2–>2

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TABLE 1—*Continued*

Species	Resistance determinant(s)	*n*	Antibiotic	MIC range (μg/ml)
	mefE	7	Daptomycin	0.12–0.25
			Erythromycin	>2–>2
	tetM+*ermB*	7	Daptomycin	0.12–0.25
			Erythromycin	>2–>2
	tetM+*mefE*	6	Daptomycin	0.06–0.25
			Erythromycin	>2–>2
Streptococcus mitis	nc/nc/nc/nc[c]	3	Daptomycin	0.5–1
			Levofloxacin	1–1
			Erythromycin	0.06–>2
	nc/nc/Ser494Thr/nc	5	Daptomycin	1–8
			Levofloxacin	1–1
			Erythromycin	0.06–>2
	Ser81Phe/Ser79Ile/Ser494Thr/nc	1	Daptomycin	0.5
			Levofloxacin	4
			Erythromycin	>2
	Ser81Phe/Ser79Phe/nc/nc	1	Daptomycin	2
			Levofloxacin	0.5
			Erythromycin	>2
	Ser81Tyr/Ser79Ile/nc/nc	1	Daptomycin	0.25
			Levofloxacin	8
			Erythromycin	2
	Ser81Tyr/Ser79Ile/Ser494Thr/nc	1	Daptomycin	1
			Levofloxacin	2
			Erythromycin	2
Streptococcus sanguis	nc/nc/nc/nc	1	Daptomycin	2
			Levofloxacin	1
			Erythromycin	0.03
	nc/nc/Ser494/Thrnc	2	Daptomycin	1–1
			Levofloxacin	1–1
			Erythromycin	0.03–>2
	Ser81Phe/Ser79Ile/nc/nc	1	Daptomycin	0.5
			Levofloxacin	1
			Erythromycin	0.03
Streptococcus agalactiae	*ermA*	1	Daptomycin	0.25
			Clindamycin	≤0.25
			Erythromycin	>2
	ermA+*ermTR*	2	Daptomycin	0.25–0.5
			Clindamycin	≤0.25–>1
			Erythromycin	2–>2
	ermA+*ermC*+*ermTR*	1	Daptomycin	0.25
			Clindamycin	>1
			Erythromycin	>2
	ermB	3	Daptomycin	0.06–0.25
			Clindamycin	≤0.25–>1
			Erythromycin	1–>2
Streptococcus bovis	*ermB*	11	Daptomycin	0.03–0.12
			Clindamycin	1–>1
			Erythromycin	>2–>2
	ermB+*ermC*	1	Daptomycin	0.06
			Clindamycin	>1
			Erythromycin	>2

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TABLE 1—*Continued*

Species	Resistance determinant(s)	*n*	Antibiotic	MIC range (μg/ml)
	ermB+*ermTR*	1	Daptomycin	0.03
			Clindamycin	>1
			Erythromycin	>2
Streptococcus equisimilis	*ermB*+*ermTR*	1	Daptomycin	0.06
			Clindamycin	>1
			Erythromycin	>2
	ermC+*ermTR*	1	Daptomycin	0.03
			Clindamycin	≤0.25
			Erythromycin	>2
Streptococcus intermedius	*ermB*+*ermTR*	1	Daptomycin	1
			Clindamycin	>1
			Erythromycin	>2
Streptococcus mitis	*ermA*+*mefA*	1	Daptomycin	0.5
			Clindamycin	≤0.25
			Erythromycin	2
	ermB	5	Daptomycin	0.5–2
			Clindamycin	≤0.25–>1
			Erythromycin	>2–>2
	mefA	5	Daptomycin	0.25–1
			Clindamycin	≤0.25–≤0.25
			Erythromycin	>2–>2
Streptococcus oralis	*mefA*	4	Daptomycin	0.5–2
			Clindamycin	≤0.25–≤0.25
			Erythromycin	2–>2
Streptococcus pyogenes	*ermB*	2	Daptomycin	0.03–0.06
			Clindamycin	≤0.25–>1
			Erythromycin	2–>2
	ermTR	2	Daptomycin	0.06–0.06
			Clindamycin	≤0.25–≤0.25
			Erythromycin	>2–>2
	mefA	1	Daptomycin	0.03
			Clindamycin	≤0.25
			Erythromycin	>2
Streptococcus salivarius	*ermB*	2	Daptomycin	0.12–0.12
			Clindamycin	1–>1
			Erythromycin	2–>2
	mefA	1	Daptomycin	0.12
			Clindamycin	≤0.25
			Erythromycin	>2

[a] Mutations in *grlA*/*gyrA* of *S. aureus*, respectively.
[b] Mutations in *gyrA*/*gyrB*/*parC*/*parE* of *S. pneumoniae*, respectively. nc, no change in amino acids
[c] Mutations in *gyrA*/*gyrB*/*parC*/*parE* of other streptococci, respectively.

The *S. pneumoniae* isolates were divided into five groups based on the presence of tetracycline and erythromycin/clindamycin resistance determinants (Table 1). The lowest daptomycin MIC obtained in four of the five groups of isolates was 0.12 μg/ml; the *tetM mefE* group had the lowest MIC, 0.06 μg/ml. The highest daptomycin MIC obtained in the five groups was 1.0 μg/ml for isolates with *tetM*, compared with 0.12 μg/ml for *ermB*-containing isolates or 0.25 μg/ml for isolates in the remaining three groups.

Daptomycin and a number of comparator agents were tested for activity in 12 *Streptococcus mitis* and four *Streptococcus sanguis* isolates containing an assortment of different amino acid changes associated with quinolone resistance (Table 1). The daptomycin MICs ranged from 0.25 to 8 μg/ml. The daptomycin MIC of 8 μg/ml occurred with a single *S. mitis* isolate and was somewhat higher than what was observed in other streptococcal species.

The activities of daptomycin and three additional antibiotics

were tested in eight streptococcal species ($n = 46$) with different clindamycin and/or erythromycin resistance-encoding genes (Table 1). The daptomycin MICs ranged from 0.03 to 2 μg/ml. No major differences were observed between streptococcal isolates with characterized resistance determinants, with the exception of a single fluoroquinolone-resistant *S. mitis* isolate.

The results were generally comparable with other data on European and North American antibiotic-resistant clinical isolates that were phenotypically but not genetically characterized (3, 8, 12, 16, 21).

The Food and Drug Administration has defined susceptibility interpretive criteria for the approved indications. *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae* subsp. *equisimilis* isolates as well as all *S. aureus* isolates are susceptible to a MIC of ≤1 μg/ml, whereas vancomycin-susceptible *E. faecalis* are susceptible to MICs of ≤4 μg/ml. Based on these breakpoints, all isolates belonging to these species tested were susceptible to daptomycin. Furthermore, daptomycin also shows excellent activity against the other resistant gram-positive isolates.

In conclusion, daptomycin exhibits broad in vitro activity against a wide range of antibiotic-resistant, gram-positive pathogens containing different resistance determinants.

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