

In Vitro Activity of Daptomycin against Methicillin-Resistant *Staphylococcus aureus*, Including Heterogeneously Glycopeptide-Resistant Strains

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The purpose of the present study was to assess the in vitro activity of daptomycin against a well-defined collection of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates ($n = 98$), including heterogeneously glycopeptide-resistant MRSA (hGISA) strains. Susceptibility testing was performed by using the Etest system. Daptomycin was potent against both glycopeptide-susceptible and hGISA strains.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important nosocomial pathogen (1). According to a report from the National Nosocomial Infection Surveillance System, ca. 60% of all *S. aureus* isolated from patients in intensive care units in U.S. hospitals were methicillin resistant in 2003 (12). Infections caused by MRSA strains are associated with longer hospital stay, more days of antibiotic administration, and higher costs than infections caused by methicillin-susceptible *Staphylococcus aureus* (MSSA) strains (1, 5). Reports of vancomycin intermediately susceptible *S. aureus* (VISA), first isolated in Japan in 1997 (9), and vancomycin-resistant *S. aureus* in the United States (4) caused widespread alarm among physicians since it was feared that we are entering an era of untreatable *S. aureus* infections. Vancomycin remains the standard for treating most MRSA infections; however, concerns over increases in the rates of heteroresistance and tolerance to this agent, combined with its pharmacodynamic and clinical shortcomings and the increasingly important role of gram-positive bacterial infections in clinical medicine, have motivated the development of newer agents. Daptomycin is a cyclic lipopeptide antibiotic that is rapidly bactericidal in vitro against a broad spectrum of gram-positive bacteria. Its unique mechanism of action involves calcium-dependent binding to the bacterial plasma membrane and disruption of membrane function (3).

The purpose of the present study was to assess the in vitro activity of daptomycin against MRSA isolates collected in The Netherlands using a well-defined collection of strains that included heterogeneously glycopeptide-resistant MRSA strains (hGISA).

The MRSA isolates were collected in The Netherlands between 1989 and 1998 and are part of the MRSA strain collection of the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. Identification as *S. aureus* and methicillin resistance were determined by duplex PCR for the *mecA* gene and coagulase gene as de-

scribed previously (10, 19). In the present study a total of 98 strains were tested, including 19 hGISA strains, which were determined by using modified population analysis profiles (20, 21). The MIC of daptomycin was determined by using the Etest system (AB Biodisk, Solna, Sweden) with a concentration range of 0.016 to 256 $\mu\text{g/ml}$. The daptomycin Etest contained a concentration gradient of daptomycin with a standard amount of calcium throughout the strip. E-test strips were applied to the surface of 150-mm Mueller-Hinton agar plates. Plates were incubated at 35°C in ambient air for 18 to 24 h prior to reading the MIC results. The MICs of the following antimicrobial agents were determined simultaneously: vancomycin, teicoplanin, gentamicin, cotrimoxazole, ciprofloxacin, erythromycin, clindamycin, and rifampin. All MICs were determined with the Etest system. For vancomycin and teicoplanin, the Etest strips were placed on brain heart infusion agar, and a large inoculum (no. 2 McFarland standard) and an extended incubation time (48 h) were used to be able to detect hGISA isolates. Isolates were categorized as susceptible or resistant to an antimicrobial agent according to the breakpoints published by the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical

TABLE 1. In vitro activities of daptomycin and other antimicrobial agents against 98 MRSA strains isolated in The Netherlands

Antibiotic	MIC (mg/liter)			% Susceptibility ^a	
	Range	MIC ₅₀	MIC ₉₀	S	R
Daptomycin	0.125–1	0.38	0.75	100	0
Oxacillin	0.19– \geq 256	256	256	0	100 ^b
Vancomycin ^c	2–6	3.0	4.0		
Teicoplanin ^c	1–16	3.0	12.0		
Gentamicin	0.38– \geq 256	1.0	256	54.1	43.9
Cotrimoxazole	0.032– \geq 32	0.094	32.0	87.8	11.2
Ciprofloxacin	0.25– \geq 32	32.0	32.0	24.5	72.4
Erythromycin	0.19– \geq 256	256	256	26.5	72.4
Clindamycin	0.047– \geq 256	0.19	256	58.2	41.8
Rifampin	0.004– \geq 32	0.012	32.0	69.4	21.3

^a S, sensitive; R, resistant.

^b Identification as *S. aureus* and methicillin resistance were determined by duplex PCR for the *mecA* gene and coagulase gene (19).

^c The Etest system with a large inoculum and 48 h of incubation was used.

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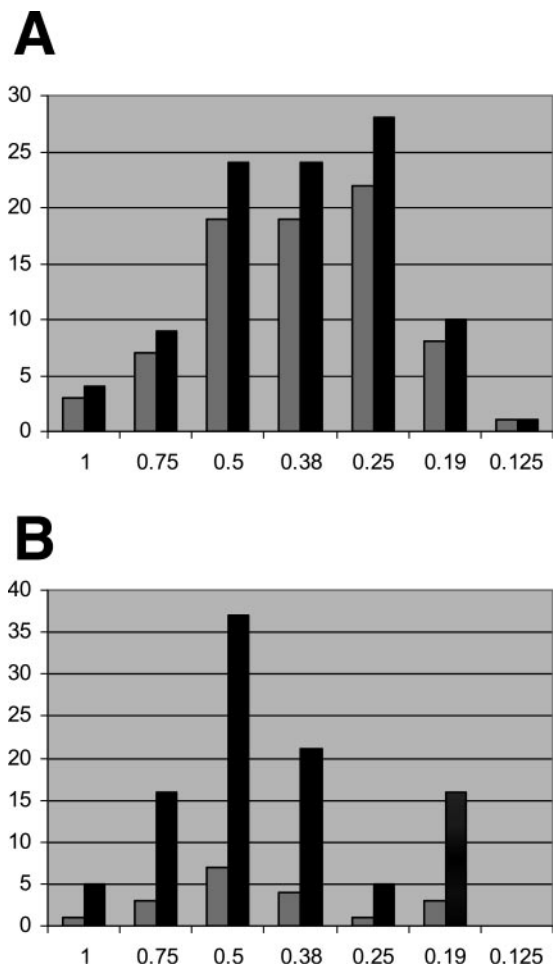


FIG. 1. MICs for daptomycin for 79 glycopeptide-susceptible MRSA strains (A) and 19 heterogeneously glycopeptide-resistant MRSA strains (hGISA) (B) collected in The Netherlands as determined by using the Etest system. Gray bars, total number of isolates tested; black bars, percentage of the total tested.

Laboratory Standards) (11). The breakpoint for daptomycin is 1 mg/liter for *S. aureus* (both methicillin-resistant and methicillin-susceptible strains).

For 98 MRSA isolates, the MIC range for daptomycin was 0.125 to 1.0 $\mu\text{g/ml}$ (Table 1 and Fig. 1), with MICs at which 50 and 90% of the isolates tested are inhibited (MIC_{50} and MIC_{90} , respectively) of 0.38 and 0.75 $\mu\text{g/ml}$, respectively. The MIC range for daptomycin against hGISA isolates was 0.19 to 1.0 $\mu\text{g/ml}$. The MICs of MRSA isolates for other antibiotics are outlined in Table 1. The MICs of daptomycin correlated weakly, but statistically significantly, with those of vancomycin ($r = 0.318$; $P = 0.001$) and teicoplanin ($r = 0.334$; $P = 0.001$) by using log-linear regression analysis.

The results are generally comparable with other data on European and North American antibiotic-resistant clinical isolates that were phenotypically characterized (2, 8, 14, 18). In a recent study, the in vitro activity of daptomycin against 337 gram-positive European clinical isolates with known resistance genes was determined (7). MICs were determined by CLSI methodology using microtiter plates containing antibiotic so-

lutions and physiologic concentrations of Ca^{2+} (50 $\mu\text{g/ml}$). For 38 MRSA isolates, the MIC range for daptomycin was 0.03 to 0.5 $\mu\text{g/ml}$, with MIC_{50} and MIC_{90} values for the isolates tested of 0.25 and 0.5 $\mu\text{g/ml}$, respectively. The daptomycin MICs determined in our study appear to be slightly higher. One explanation for these results may be the limited number of isolates tested in the study by Fluit et al. (7) and because we selected for hGISA in our study.

It appears that *S. aureus* with reduced susceptibility to vancomycin also exhibits reduced susceptibility to daptomycin (13, 16). In concordance with findings by Cui et al. (6), we found a positive correlation between reduced daptomycin susceptibility and reduced susceptibility to glycopeptides. Although the correlation coefficient is relatively weak, it is highly significant. In the study by Cui et al., the reduction of daptomycin susceptibility showed a strong correlation with the increment of cell wall thickness. The level of daptomycin susceptibility correlated strongly and positively with that of vancomycin susceptibility ($r = 0.814$; $P < 0.0001$) and with cell wall thickness ($r = 0.883$; $P < 0.0001$). It is conceivable that the changes mediating reduced susceptibility to vancomycin in *S. aureus*, including a thickened cell wall, changes in cellular metabolism, and enhanced cell wall turnover, interfere with the antimicrobial action of daptomycin. In a recent study, the bactericidal activity of vancomycin and daptomycin against 105 well-characterized *S. aureus* strains with decreased susceptibility against vancomycin (88 hGISA and 17 VISA), as well as 105 vancomycin-susceptible MRSA strains (VSSA) were tested by using a CLSI broth microdilution method (15). All VSSA and hGISA strains were inhibited by ≤ 1 mg of daptomycin/liter. However, a slight skewing toward higher daptomycin MIC results was noted when the hGISA ($\text{MIC}_{50} = 0.5$ mg/liter, and $\text{MIC}_{90} = 1$ mg/liter) and VISA ($\text{MIC}_{50} = 1$ mg/liter, and $\text{MIC}_{90} = 2$ mg/liter) were compared to VSSA strains (MIC_{50} and $\text{MIC}_{90} = 0.5$ mg/liter). Among the 17 VISA strains evaluated, 7 (41.2%) showed a daptomycin MIC of 2 mg/liter and 1 (5.9%) had a daptomycin MIC of 4 mg/liter. However, all daptomycin minimum bactericidal concentration (MBC) results were at the MIC or twofold higher than the MIC, and the MBC/MIC ratios were not significantly affected by the susceptibility to vancomycin. Conversely, 69.3% of hGISA strains and all VISA strains showed a vancomycin MBC/MIC ratio consistent with tolerance (tolerance was defined as an MBC/MIC ratio of ≥ 32 or ≥ 16 with an associated MBC at ≥ 32 mg/liter for vancomycin). The increased MIC of daptomycin associated with hGISA is therefore most likely not caused by an intrinsic resistance mechanism but by the thickening of the cell wall. Further studies are needed before final conclusions can be made.

In conclusion, daptomycin exhibits broad in vitro activity against a collection of MRSA strains collected in The Netherlands. The in vitro data presented in this and other studies, together with promising clinical results (17), show that daptomycin is a valuable new addition to the arsenal of antimicrobial agents for the treatment of infections caused by gram-positive microorganisms.

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