

# In Vitro Activity of Daptomycin against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Hetero-Resistant MRSA (hVRSA) Isolated from Patients at Siriraj Hospital

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## ABSTRACT

*In vitro* activity of daptomycin against 101 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and 9 strains of vancomycin-hetero-resistant MRSA (hVRSA) isolated from different patients hospitalized at Siriraj Hospital, Thailand, was determined by the Etest method. The minimum inhibitory concentration (MIC)<sub>50</sub> and MIC<sub>90</sub> of daptomycin against MRSA were 0.5 and 0.75 mg/L, respectively. The range of MIC of daptomycin against hVRSA was 0.5 to 1.5 mg/L. Daptomycin may be an alternative antibiotic for therapy of MRSA infections in Thailand. (*J Infect Dis Antimicrob Agents* 2008;25:57-61.)

## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common bacteria causing infections in hospitalized patients at Siriraj Hospital, Thailand.<sup>1,2</sup> The prevalence of MRSA was found to be 41.5 percent of all strains of *S. aureus* isolated from hospitalized patients at Siriraj Hospital from January to May 2005. Therefore, empirical treatment of hospitalized patients suspected of having *S. aureus* infections at Siriraj Hospital should now include an anti-

MRSA antibiotic since the prevalence of MRSA is greater than 10 percent.<sup>3</sup>

The conventional agent for therapy of MRSA infections is vancomycin. However, there are several limitations of treatment of MRSA infections with vancomycin, and its role in the management of serious infections is being reconsidered.<sup>4</sup> Vancomycin treatment failure is associated with an increase in the minimum inhibitory concentration (MIC) as well as a decrease in the rate of bacterial killing. Limitations of

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vancomycin include relatively poor tissue penetration (particularly in the lung), relatively slow bacterial killing, and the potential for its toxicity. Therefore, a search for new agents effective against MRSA is needed.

Daptomycin is a novel cyclic lipopeptide which has demonstrated a concentration-dependent bactericidal activity against most Gram-positive bacteria.<sup>5,6</sup> Daptomycin's mode of action is primarily to abolish ion gradients across the cytoplasmic membrane, thus compromising energy metabolism and inhibiting synthesis of macromolecules.<sup>6-8</sup> An *in vitro* activity of daptomycin is dependent upon physiological levels of free calcium ions.<sup>9</sup> It has been recently recognized that the disk-diffusion is not an adequate method for susceptibility testing. The daptomycin disks have been withdrawn, and the Clinical Laboratory Standard Institute (CLSI) does not recommend the disk-diffusion method.<sup>10</sup> The spectrum of antibacterial activity of daptomycin includes MRSA, vancomycin-resistant enterococci, and penicillin-resistant streptococci.<sup>5,6,11</sup>

The objective of the study was to determine the *in vitro* activity of daptomycin against MRSA and vancomycin-hetero-resistant MRSA (hVRSA) isolated from patients hospitalized at Siriraj Hospital.

## MATERIALS AND METHODS

### Bacterial isolates

One hundred and ten strains of MRSA were isolated from different infected patients hospitalized at Siriraj Hospital, Bangkok, Thailand from 2006 to 2008. These were isolated from lower respiratory tract (N=40), pus (N=36), blood (N=23), and urine (N=1). All isolates had oxacillin MIC of > 4 mg/L and vancomycin MIC of < 4 mg/L. One hundred and one isolates were vancomycin-susceptible MRSA, and 9 isolates were hVRSA. Vancomycin-susceptible MRSA and hVRSA were determined by a one-point population

analysis and confirmed by a population analysis.<sup>12</sup>

### Susceptibility testing

The MIC of daptomycin against the studied organisms was determined by the Etest. The daptomycin Etest strips (AB BIODISK, Sweden) with daptomycin concentration of 0.016 to 256 mg/L were used. A quality control was performed by testing the susceptibility of *S. aureus* ATCC 29213. The methodology for susceptibility testing was by direct colony suspension as recommended by the CLSI.<sup>13</sup> The test isolate was grown overnight on blood agar at 35°C, and then colonies were picked up and suspended in sterile normal saline equivalent to a 0.5 McFarland standard. The suspension was used to inoculate on Mueller-Hinton agar, and the Etest strip was placed according to the manufacturer's recommendations. The agar plates were incubated at 35°C for 16-18 hours before the MIC results were read. Susceptibility of *S. aureus* to daptomycin was defined as an MIC of ≤ 1 mg/L.<sup>13</sup>

## RESULTS

The MIC of daptomycin against the quality control organism, *S. aureus* ATCC 29213 was 0.38 mg/L. The distribution of the MICs of daptomycin against the MRSA strains is shown in Table 1. The MICs at which 50 percent (MIC<sub>50</sub>) and 90 percent (MIC<sub>90</sub>) of MRSA strains were inhibited were 0.5 and 0.75 mg/L, respectively. The distribution of MICs of daptomycin against the hVRSA is shown in Table 2. The range of MIC of daptomycin for hVRSA was 0.5 to 1.5 mg/L. The modes of MIC of daptomycin for MRSA and hVRSA were 0.5 and 1 mg/L, respectively.

## DISCUSSION

The mechanism of action of daptomycin is calcium-dependent depolarization of the cell membrane,

**Table 1. The distribution of minimum inhibitory concentration (MIC) of daptomycin against 101 strains of methicillin-resistant *Staphylococcus aureus* (MRSA).**

Number of isolates with MIC (mg/L)							
0.125	0.19	0.25	0.38	0.5	0.75	1	1.5
0	2	13	17	38	25	6	0

**Table 2. The distribution of minimum inhibitory concentration (MIC) of daptomycin against 9 strains of vancomycin-hetero-resistant methicillin-resistant *Staphylococcus aureus* (hVISA).**

Number of isolates with MIC (mg/L)							
0.125	0.19	0.25	0.38	0.5	0.75	1	1.5
0	0	0	0	2	2	4	1

therefore its susceptibility test requires medium supplemented with a physiological level of calcium<sup>9</sup> or calcium-supplemented daptomycin Etest strips.<sup>14</sup> In our study, the MIC of daptomycin against the quality control organism (*S. aureus* ATCC 29213) was 0.38 mg/L, which was within the reference limits. Therefore, the MIC results from our study should be valid. We found that all isolates of MRSA were susceptible to daptomycin (MIC of  $\leq 1$  mg/L), but that one strain of hVISA had a MIC of  $>1$  mg/L. The MIC mode of hVISA was also higher than that of MRSA. However, the number of hVISA strains examined was small, so it will be preferable to confirm these data with larger collections of isolates. The results of our study are generally comparable with other data on European and North American resistant *S. aureus* clinical isolates.<sup>15-19</sup> It appears that *S. aureus* with reduced susceptibility to vancomycin also exhibits reduced susceptibility to daptomycin, and a positive correlation between reduced daptomycin susceptibility and reduced susceptibility to glycopeptides was observed.<sup>18,19</sup> In the study by Cui

and colleagues, the level of daptomycin susceptibility correlated strongly and positively with that of vancomycin susceptibility and with the cell wall thickness.<sup>20</sup> It is conceivable that the changes mediating reduced susceptibility to vancomycin in *S. aureus* also interfere with the antimicrobial action of daptomycin, and our observations are consistent with this postulation.

The efficacy of daptomycin has been found to be comparable to anti-staphylococcal penicillins or vancomycin for therapy of complicated skin and skin-structure infections including infected diabetic foot ulcers as well as *S. aureus* bacteraemia and right-sided endocarditis.<sup>21-23</sup>

However, daptomycin is not indicated for pneumonia because of its high degree of protein binding and it is inactivated by the pulmonary surfactant. The dosage of daptomycin is 4-6 mg/kg, given as intravenous administration once daily. The important adverse effects of daptomycin include creatine kinase elevation and myopathy.<sup>24</sup> Daptomycin is being registered with Thai Food and Drug Administration, and it should be

available for use in Thailand shortly. Based on the results of our in vitro activity study of daptomycin against resistant *S. aureus*, daptomycin may be an alternative to anti-MRSA antibiotics but the MICs should be confirmed before clinical use for treatment of infections caused by hVISA.

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