In Vitro Activities of Linezolid, Vancomycin, Fosfomycin and Fusidic Acid Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

The *in vitro* activities of linezolid and other antigram-positive agents which have been used in Songklanagarind Hospital were evaluated against methicillin-resistant *Staphylococcus aureus* (MRSA). One hundred clinical isolates of MRSA were collected during the period of October 2000 and December 2001. The minimum inhibitory concentrations (MICs) of linezolid, vancomycin, fosfomycin and fusidic acid was determined by the Epsilon-test method. The results showed 100 percent susceptibility of linezolid, vancomycin and fusidic acid, 70 percent susceptibility of fosfomycin against MRSA isolates. According to the MIC₉₀ values, linezolid was the most active agent. The heterogeneity of methicillin resistance determined by the dropped plate count revealed homogeneous resistance to methicillin of all isolates. (*J Infect Dis Antimicrob Agents* 2004;21:7-10.)

INTRODUCTION

Nosocomial infections due to methicillin-resistant strains of *Staphylococcus aureus* (MRSA) have been reported worldwide.¹,² Among antigram-positive agents that are available in Thailand, vancomycin is generally recommended as the drug of choice in treating serious MRSA infections. However, since reporting of vancomycin-intermediate *Staphylococcus aureus* (VISA) from Japan³, vancomycin-resistant *Staphylococcus aureus* (VRSA) from the USA², potential adverse effects and relatively high cost of vancomycin, other alternative antibiotics are being investigated.⁴

Linezolid, the first available oxazolidinone antibiotic, has a broad spectrum of activity against gram-positive bacteria including drug-resistant strains. Linezolid inhibits bacterial protein synthesis by binding to the 50 S ribosomal subunit near to the interface with the 30 S subunit, causing inhibition of 70 S initiation complex formation. It is active against both methicillin-sensitive *S. aureus* (MSSA) and MRSA, and inhibits virtually all strains at a concentration of 4 mg/L or less.⁵,⁶ This study purposed to evaluate the *in vitro* activity of linezolid compared to other anti-MRSA drugs which have been used in Songklanagarind Hospital.

MATERIALS AND METHODS

Bacterial isolates

A total of 100 clinical isolates obtained from

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patients hospitalized in Songklanagarind Hospital were studied. Each isolate represented a single isolation from each patient. The identification of isolates was confirmed by colonial morphology, coagulase test and oxacillin disc diffusion test, as described by the National Committee for Clinical Laboratory Standards (NCCLS).

Susceptibility testing

Four antimicrobial agents, linezolid, vancomycin, fosfomycin and fusidic acid, were tested. The MIC of each isolate was determined by using the Epsilon-test (E-test) method (AB Biodisk, Sweden). S. aureus ATCC 29213 was used as a quality control.

The MICs of each drug were reported as an MIC range, MIC<sub>50</sub>, and MIC<sub>90</sub>. The MIC<sub>50</sub> and MIC<sub>90</sub> were expressed as the nearest log 2 concentration of antibiotic that inhibits 50 percent and 90 percent of the strains. The percentage of susceptibility to MRSA was obtained by using the following breakpoint concentrations: linezolid ≤ 4 mg/L, vancomycin ≤ 4 mg/L, fosfomycin ≤ 64 mg/L <sup>9</sup> and fusidic acid ≤ 0.5 mg/L. <sup>11</sup>

Detection of heterogeneity and homogeneity of MRSA

The heterogeneity of methicillin resistance was determined by the dropped plate count as described by Hartman and Tomasz. <sup>12</sup> The number of colonies of MRSA on 50 mg/L-methicillin-containing and methicillin-free plates incubated at 37°C were counted, and interpreted as the efficacy of plating (EOP). EOP was defined as the ratio of the colony forming unit (CFU) on methicillin-containing plates and the CFU on methicillin-free plate. A strain was considered heterogeneous of methicillin-resistance if the EOP was less than 0.1, and homogeneity of methicillin resistance when the EOP was between 0.1 and 1.

RESULTS

The specimens of the 100 MRSA isolates were blood (25), pus (33), sputum (36) and body fluid (6). The activities of linezolid, vancomycin, fosfomycin and fusidic acid against all isolates of MRSA are shown in Table 1. All isolates were susceptible to linezolid, vancomycin and fusidic acid while fosfomycin inhibited only 70 percent of the tested isolates. All isolates had vancomycin MIC ≤ 3 mg/L, and 57 percent were inhibited by vancomycin at concentration of 2-3 mg/L (Table 2). According to the MIC<sub>90</sub> values, linezolid was the most active agent. Phenotypic expression of all isolates showed homogeneous resistance to methicillin with the efficacy of plating of 0.12-1.

DISCUSSION

This study demonstrates that linezolid has an excellent in vitro activity against MRSA. In term of MIC<sub>90</sub> values, linezolid is more potent than vancomycin. This high activity of linezolid against MRSA is consistent with reports from Rybak et al<sup>7</sup> and Fines et al.<sup>4</sup> All isolates of MRSA in this study were susceptible to ≤ 1 mg/L of linezolid. The MIC of linezolid for MRSA is

<table>
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<tr>
<th>Antimicrobial agents</th>
<th>MIC Range</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>S *(%)</th>
<th>Susceptibility</th>
<th>Breakpoint</th>
</tr>
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<tbody>
<tr>
<td>Linezolid</td>
<td>0.023-0.75</td>
<td>0.25</td>
<td>0.5</td>
<td>100</td>
<td>≤ 4</td>
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<tr>
<td>Vancomycin</td>
<td>0.5-3</td>
<td>1.5</td>
<td>2</td>
<td>100</td>
<td>≤ 4</td>
<td></td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>0.38-&gt;1,024</td>
<td>1.5</td>
<td>&gt; 1,024</td>
<td>70</td>
<td>≤ 64</td>
<td></td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>0.016-0.094</td>
<td>0.016</td>
<td>0.064</td>
<td>100</td>
<td>≤ 0.5</td>
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</tr>
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</table>

Note: S* = susceptibility
0.023-0.75 mg/L whereas the MIC of vancomycin is 0.5-3 mg/L.

Linezolid has a unique mechanism of inhibitory action on the bacterial protein synthesis. It displays in vitro activity against MRSA, vancomycin-resistant enterococci (VRE) and penicillin-resistant S. pneumoniae (PRSP).4,5,13 Clinical results showed that linezolid and vancomycin have similar clinical efficacy. Linezolid therapy was shown to be successful for MRSA infection in patient with a severe allergic reaction to vancomycin.12 Plasma concentrations of intravenous and oral linezolid are equivalent, with average concentration exceeding the MIC for susceptible pathogens throughout the 12 h dosing interval.5

In Thailand, MRSA strains with reduced susceptibility to vancomycin have been first reported from Siriraj Hospital, Bangkok.14 They found three strains which contained subpopulation of cells that could grow in 4 mg/L of vancomycin. In our study, the MIC range of vancomycin was 0.5-3 mg/L, with a significant proportion of MIC range of 2-3 mg/L. There is no significant difference from the previous MRSA isolates during 1997-1998.15 And all isolates showed homogeneous resistance to methicillin.

Our study showed the decreased susceptibility of fosfomycin which has been used for ten years in Songklanagarind Hospital. Routine disc susceptibility test of MRSA isolates in 2001 also showed only 71 percent fosfomycin sensitivity. MRSA isolates remained 100 percent susceptible to fusidic acid.

In conclusion, MRSA isolates in Songklanagarind Hospital were still susceptible to vancomycin and fusidic acid, but there was an increased resistance to fosfomycin. Linezolid was found to be very active against the MRSA strains and appears to be a potentially useful drug for MRSA infections.

ACKNOWLEDGEMENT
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References
5. Diekema DJ, Jones RN. Oxazolidinone antibiotics.


