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Vancomycin Creep and Daptomycin Minimum Inhibitory Concentration in Methicillin-Resistant Staphylococcus aureus

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Abstract

Objective: To determine the in vitro activity of daptomycin and vancomycin against 50 strains of methicillin-resistant Staphylococcus aureus (MRSA) isolated from blood and pus specimens. Material and methods: Fifty consecutive MRSA were isolated from pus (n=25) and blood (n=25) were included in the study. Oxacillin susceptibility was determined by cefoxitin disc diffusion, green colored colonies on chromogenic media. Susceptibility testing for 18 antimicrobial agents was determined by a disc diffusion method. The minimum inhibitory concentration (MIC) of daptomycin and vancomycin was determined by the Etest as recommended by the Clinical and Laboratory Standards Institute (CLSI). Results: Antibiotic susceptibility pattern of the MRSA isolates showed that 38% were multi-drug resistant overall and 52% in blood and 24% in pus isolates when expressed separately. The MIC50 and MIC90 of daptomycin were 0.08 and 0.09 mg/L and of vancomycin were 1.2 mg/L and 1.3 mg/L, respectively. Ten percent of the isolates had vancomycin MIC of 2 mg/L which is the upper limit of CLSI breakpoint for sensitive isolates. None of the isolates showed intermediate susceptibility or resistance to vancomycin or daptomycin. Conclusion: Creeping MIC of vancomycin is a matter of concern and MIC of 1.5–2 mg/L of vancomycin in MRSA increases the risk of development of complicated bacteraemia. MIC’s of vancomycin should be reported for all S. aureus isolates and should be used to guide treatment. Otherwise, daptomycin can be considered as an alternative antibiotic for therapy of MRSA infections in India.

Key words: Vancomycin, Daptomycin, Methicillin-resistant Staphylococcus aureus (MRSA), Minimum Inhibitory Concentration (MIC),

Introduction

Among Gram positive organisms, methicillin-resistant Staphylococcus aureus (MRSA) is one of the most common bacteria causing infections in hospitalized
patients. Empirical treatment of hospitalized patients suspected of having *S. aureus* infections should include an anti-MRSA antibiotic if the prevalence of MRSA is greater than 10% (1). The conventional agent for therapy of MRSA infections is vancomycin. Creeping minimum inhibitory concentration (MIC) of vancomycin in MRSA isolates have raised doubts on the rationale of using it for treatment. Treatment failure is associated with an increase in the MIC as well as a decrease in the rate of bacterial killing. The Clinical and Laboratory Standards Institute (CLSI) has done away with disc diffusion testing and recommends only MIC testing for vancomycin (2). *S. aureus* with reduced susceptibility to vancomycin was first reported in 1997 from Japan (3). Since then vancomycin-resistant *S. aureus* (VRSA) have been reported from various parts of the world including India (4). Daptomycin is a novel cyclic lipopeptide which has demonstrated a concentration-dependent bactericidal activity against most Gram-positive bacteria (5,6). Daptomycin’s mode of action is primarily to abolish ion gradients across the cytoplasmic membrane, thus compromising energy metabolism and inhibiting synthesis of macromolecules (6). The spectrum of antibacterial activity of daptomycin includes MRSA, vancomycin-resistant enterococci, and penicillin-resistant streptococci. The objective of the study was to determine the in vitro activity of vancomycin and daptomycin against MRSA isolated from hospitalized patients.

**Materials and Methods**

**Bacterial isolates**

Between August and December 2011, 50 consecutive *S. aureus* isolates, sporadically recovered from blood and pus from hospitalized patients in a university hospital in South India were included. Bacteria were identified as *S. aureus* by Gram stain and standard biochemical testing. Oxacillin susceptibility was determined by cefoxitin disc diffusion (2) and green colored colonies on chromogenic media, MRSA ID agar (bioMerieux, Marcy l’Etoile, France). *S. aureus* strain ATCC 29213 and ATCC 43300 were used as negative positive controls respectively.

**Susceptibility testing**

Susceptibility testing for 15 antimicrobial agents was determined by disc diffusion method according to CLSI guidelines of 2012 (2). The MIC of daptomycin (0.016 to 256 mg/L) and vancomycin (0.016 to 256 mg/L) was determined by the Etest (BioMerieux, Marcy l’Etoile, France) as recommended by the CLSI (2). 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 and vancomycin was defined as an MIC of ≤ ug1 mg/L and ≤ 2 mg/L respectively (2).

**Results**

The distributions of the MICs of daptomycin and vancomycin against the MRSA strains are shown in figure 1. The minimum inhibitory concentration MIC50

<table>
<thead>
<tr>
<th>Isolates</th>
<th>MIC50 (µg/ml)</th>
<th>MIC90 (µg/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Vancomycin</td>
<td>Daptomycin</td>
</tr>
<tr>
<td>Blood</td>
<td>1.11</td>
<td>0.08</td>
</tr>
<tr>
<td>Pus</td>
<td>1.57</td>
<td>0.08</td>
</tr>
<tr>
<td>All</td>
<td>1.19</td>
<td>0.08</td>
</tr>
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MRSA isolated from hospitalized patients.

**Discussion**

Determination of vancomycin resistance in *S. aureus*
Figure 1. The Distribution of minimum inhibitory concentration (MIC) of Vancomycin (A) and Daptomycin (B) against MRSA isolates (n=50).

Figure 2. Antibiotic Susceptibility of MRSA isolates (n=50). *GM, gentamicin; CIP, ciprofloxacin; COT, cotrimoxazole; TE, tetracycline; LEVO, levofloxacin; DOXY, doxycycline; E, erythromycin; VA, vancomycin; C, chloramphenicol; CD, clindamycin; TGC, tigecycline; RIF, rifampicin; LZ, linezolid; TEC, teicoplanin.
is difficult due to methodological difficulties (7). Staphylococci with a vancomycin MIC ≤ 2 mg/L is considered susceptible while those with MICs ≥ 4 mg/L are resistant which is based on the fact that the serum trough concentration of 5 to 10 mg/L can be achieved using the normal dosage. Infections due to S. aureus can involve different sites and concentrations of vancomycin at these sites are lower than serum concentration (8,9). The problem is further compounded by the fact that vancomycin intermediate isolates (hVISA) are not reliably distinguished from susceptible isolates by rapid automated methods like Microscan and Vitek (10). Due to inaccuracies of disc diffusion methods CLSI has withdrawn disc diffusion diameters and recommends only MIC determination for vancomycin (2,11).

VRSA is being increasingly reported from India (9). Previous studies have documented that vancomycin MIC90 S. aureus in India is 1 mg/L (9,10,). Although among S. aureus clinical isolates VRSA and VISA strains are rare, the bigger concern is about the emergence of S. aureus with reduced susceptibility to vancomycin. In 2004, Moise-Broder and co-workers reported a direct relationship between and clinical failure rates and vancomycin MIC as 47.6% at 0.5 mg/L, 70% at 1 mg/L and 90% at 2 mg/L (12). In January 2006, CLSI revised the vancomycin MIC interpretive criteria for S. aureus. Consequently, isolates with vancomycin MICs of ≤2 mg/L and ≥16 mg/L are currently defined as vancomycin susceptible and vancomycin resistant, respectively, and isolates for which vancomycin MICs are 4 to 8 mg/L being classified as vancomycin intermediate (2). Since vancomycin brings about concentration-dependent bacterial killing, it is necessary to achieve high trough concentrations to bring effective eradication of the pathogen. Using Montecarlo simulation, it was found that the probability of achieving an AUC/MIC ratio of >400 for organism with MIC of 2 mg/L and 0.5 mg/L were 0 and 100% respectively (13). A novel cyclic lipopeptide daptomycin has recently been introduced as an anti-MRSA agent. The mechanism of action of daptomycin, is calcium-dependent depolarization of the cell membrane, therefore its susceptibility test requires medium supplemented with a physiological level of calcium or calcium-supplemented daptomycin Etest strips (14). We found that for all isolates of MRSA were susceptible to daptomycin with MIC defined as ≤ 1 mg/L (2).

In conclusion, creeping MIC of vancomycin is a matter of concern and studies have documented in vitro and in vivo disconnect between isolates classified as vancomycin susceptible by disc diffusion. An MIC of 1.5–2 mg/L of vancomycin in increases the risk of development of complicated bacteraemia. MIC’s of vancomycin should be reported for all S. aureus isolates and should be used to guide treatment or else daptomycin can be considered as an alternative antibiotic for therapy of MRSA infections in India.

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Disclosure
None of the authors disclosed any conflicts of interest.

References


