ABSTRACT

Objective: The present cross-sectional study was conducted to determine minimum inhibitory concentration (MIC) of daptomycin and vancomycin to clinical isolates of healthcare-associated-methicillin-resistant Staphylococcus aureus (HA-MRSA).

Methods: Centers for Disease Control and Prevention Criteria were used to define MR infections due to MRSA. Antibiotic susceptibility testing was done by Kirby–Bauer disk diffusion method. MIC of vancomycin and daptomycin was determined by Agar dilution method and E-test, respectively. Results of antibiotic susceptibility testing and MIC were interpreted as per Clinical Laboratory Standard Institute guidelines.

Results: A total of 110 strains of MRSA were isolated from healthcare-associated infections. All were susceptible to daptomycin, linezolid, and teicoplanin. A total of 106 isolates were vancomycin susceptible and four were vancomycin-intermediate S. aureus (VISA). MIC<sub>90</sub> and MIC<sub>50</sub> of vancomycin were 2 µg/ml. All MRSA isolates were susceptible to daptomycin. Four VISA strains had daptomycin MIC 1 µg/ml.

Conclusion: The present study showed the emergence of VISA among HA-MRSA isolates with high MIC<sub>90</sub> for vancomycin. Although all HA-MRSA isolates were susceptible to daptomycin, VISA isolates had high daptomycin MIC. This indicates that daptomycin may not be used as an alternative choice for VISA infections.

Keywords: Healthcare-associated methicillin-resistant Staphylococcus aureus, Vancomycin, Daptomycin.

IN VITRO ACTIVITY OF VANCOMYCIN AND DAPTOMYCIN AGAINST HEALTHCARE-ASSOCIATED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM CLINICAL SPECIMENS

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Methicillin-resistant Staphylococcus aureus (MRSA) can cause infection of varying severity in hospitalized patients. The prevalence of such healthcare-associated-MRSA (HA-MRSA) varies in different geographical areas [1]. A multicenter study from India shows that the prevalence of HA-MRSA varies from 19% to 64% [2]. Most HA-MRSA strains exhibit multidrug resistance causing problems in selection of the antibiotics for treatment. This is due to staphylococcus cassette chromosome mec I-III which are large mobile genetic elements encoding resistance to multiple non-β lactam antibiotics in addition to methicillin resistance. Severe infections caused by HA-MRSA require treatment with vancomycin. There is concern over the effectiveness of vancomycin because of minimum inhibitory concentration (MIC) creep, increasing resistance and problems in achieving pharmacokinetic/pharmacodynamic (PK/PD) profile [3]. Therefore, there is a need for alternative antibiotics in such cases.

Daptomycin, teicoplanin, dalbavancin, linezolid, cefepime, quinupristin/dalfopristin, and tigecycline are found to be effective against MRSA [4]. The use of these newer antibiotics will depend on many factors such as MIC, PK/PD profile, safety, availability, and cost. A literature search revealed that there are not many studies from the geographical area of present investigation with regards to determination of MIC of daptomycin and vancomycin to HA-MRSA. This information is critical for understanding the susceptibility pattern of HA-MRSA and selection of these antibiotics for treatment. In the present study, we determined the MIC of daptomycin and vancomycin to clinical isolates of HA-MRSA.

The present cross-sectional study was carried out using MRSA isolates from healthcare-associated infections in four tertiary care hospitals of Coastal Karnataka, South India. Healthcare-associated infections were defined as per Centers for Disease Control and Prevention Criteria (CDC), Atlanta [5]. The present study had approval of the Institutional Ethics Committee. These hospitals included two government hospitals of bed strength 600 and 250; two private tertiary care hospitals of bed strength 510 and 251. A total of 110 non-repetitive clinical isolate of HA-MRSA including 75 (pus), 15 (blood), 8 (intravascular catheter tip), 5 (endotracheal aspirate), 4 (sputum), 1 (dialysis central line tip), 1 (bronchoalveolar lavage), and 1 (pleural fluid) were used. The identification of S. aureus was done using standard bacteriological methods [6]. MRSA was detected using the Cefoxitin (30 µg) disk diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines [7].

Kirby–Bauer disk diffusion method was used for antibiotics susceptibility testing of MRSA isolates and results were interpreted based on CLSI guidelines [7]. The antibiotics used were ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (30 µg), linezolid (30 µg), rifampicin (5 µg), teicoplanin (30 µg), and trimethoprim/sulfamethoxazole (1.25 µg/23.75 µg). Antibiotics were purchased from Hi media Laboratories, Mumbai, Maharashtra, India. S. aureus ATCC 25923 was used as the quality control.

The MIC of vancomycin (Sigma-Aldrich Corporation, St. Louis, US) was done using Agar dilution method using vancomycin concentration ranging from 128 to 0.03125 µg/ml [8]. Vancomycin MIC ≤2 µg/ml was considered susceptible, 4-8 µg/ml intermediate, and ≥16 µg/ml resistant [7]. S. aureus ATCC 29213 and Enterococcus faecalis ATCC 29212 were used as negative control. E. faecalis ATCC 51299 was used as positive control. Daptomycin MIC was determined using E-test (BioMerieux, France) as per manufacturer’s instructions. HA-MRSA with an MIC of ≤1 µg/ml was considered susceptible. S. aureus ATCC 29213 was used as a control.

We studied a total of 110 HA-MRSA isolates, of which 75 (68.2%) were from male and remaining 35 (31.8%) were from female patients. Maximum strains were isolated from pus 75 (68.2%) followed by blood 15 (13.6%), intravascular catheter tip 8 (7.3%), endotracheal...
isolates with vancomycin MIC of more than 2 µg/ml an
of 9.5% when the MIC was 1-2 µg/ml. This difference was statistically
significant (p=0.03) [11]. Vancomycin MIC more than 1 µg/ml maybe
associated with treatment failure with MRSA infection. These results
clearly indicate usage of vancomycin for treatment of MRSA infections
should be based on MIC values, test for hVISA and PK/PD profile.

In the present study, we observed that all HA-MRSA isolate were
susceptible to daptomycin. However, high MIC (1 µg/ml) was observed
in 4 (3.6%) isolates. Previous studies from North India have also
reported 100% susceptibility to daptomycin [9,12,13]. Daptomycin was
approved by Food and Drug Administration in 2003 for the treatment of
bacteria and skin and soft tissue infections caused by S. aureus. S.
aureus isolates with vancomycin MIC ≥2 µg/ml may have higher
daptomycin MIC in the non-susceptible category (more than 1 µg/ml)
causing treatment failure [14]. In the present study, all the four VISA
isolates had daptomycin MIC 1 µg/ml. This indicates that daptomycin
may not be an alternative choice for treatment of VISA/VRSA infections.

In conclusion, the present study showed the emergence of VISA among
HA-MRSA clinical isolates and high MIC of vancomycin. All strains
were susceptible to daptomycin. However, VISA isolates had high
daptomycin MIC, indicating daptomycin may not be an alternative
choice for VISA infection.

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REFERENCES

1. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H,
et al. Metilicillin-resistant Staphylococcus aureus (MRSA): Global
epidemiology and harmonisation of typing methods. Int J Antimicrob

2. Indian Network for Surveillance of Antimicrobial Resistance (INSAR)
group. Methicillin resistant Staphylococcus aureus (MRSA) in India:

3. Hawser SP, Bouchillon SK, Hoban DJ, Dowzicky M, Babincak T. Rising
incidence of Staphylococcus aureus with reduced susceptibility
to vancomycin and susceptibility to antibiotics: A global analysis 2004-

4. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ,
et al. Clinical practice guidelines by the Infectious Diseases Society
of America for the treatment of methicillin-resistant Staphylococcus

5. Centers for Disease Control and Prevention. CDC/NHSN Surveillance
Definition for Specific Types of Infection. Available from: http://
www.cdc.gov/nhsn/pdfs/pscmanual/17pscnosinfdef-current.pdf. [Last
accessed 2015 Nov 13].

6. Bannerman TL, Staphylococci and other catalase positive cocci that
grow aerobically. In: Murray PR, Baron EJ, Jorgenson JH, editors.

7. Clinical and Laboratory Standards Institute. Performance Standards
For Antimicrobial Susceptibility Testing; Twenty Fifth Informational

For Antimicrobial Susceptibility Testing; Twenty –Second Informational

for methicillin-resistant Staphylococcus aureus from north India. Indian

10. Thati V, Shivamavar CT, Gaddad SM. Vancomycin resistance among
methicillin resistant Staphylococcus aureus isolates from intensive
care units of tertiary care hospitals in Hyderabad. Indian J Med Res

Eliopoulos GM. Relationship of MIC and bacterial activity to efficacy
of vancomycin for treatment of methicillin-resistant Staphylococcus


Kumari et al.

Table 1: Antibiotic susceptibility pattern of HA-MRSA (n=110)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>HA-MRSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>22 (20.0)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>55 (50.0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>34 (30.9)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>60 (54.5)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>50 (100.0)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>108 (98.1)</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>110 (100.0)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>75 (68.1)</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>51 (46.4)</td>
</tr>
</tbody>
</table>

HA-MRSA: Healthcare-associated-methicillin-resistant Staphylococcus aureus

Table 2: MIC of vancomycin and daptomycin to HA-MRSA (n=110)

<table>
<thead>
<tr>
<th>MIC of vancomycin (µg/ml)</th>
<th>Number of HA-MRSA (%)</th>
<th>MIC of daptomycin (µg/ml)</th>
<th>Number of HA-MRSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>0</td>
<td>1</td>
<td>4 (3.6)</td>
</tr>
<tr>
<td>64</td>
<td>0</td>
<td>0.75</td>
<td>3 (2.7)</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>0.5</td>
<td>13 (11.8)</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>0.47</td>
<td>20 (18.2)</td>
</tr>
<tr>
<td>08</td>
<td>0</td>
<td>0.39</td>
<td>10 (9.1)</td>
</tr>
<tr>
<td>04</td>
<td>4 (3.6)</td>
<td>0.25</td>
<td>5 (4.5)</td>
</tr>
<tr>
<td>02</td>
<td>61 (55.5)</td>
<td>0.23</td>
<td>8 (7.2)</td>
</tr>
<tr>
<td>01</td>
<td>37 (33.6)</td>
<td>0.19</td>
<td>6 (5.5)</td>
</tr>
<tr>
<td>0.5</td>
<td>8 (7.3)</td>
<td>0.125</td>
<td>12 (10.9)</td>
</tr>
<tr>
<td>0.25</td>
<td>0</td>
<td>0.094</td>
<td>9 (8.2)</td>
</tr>
<tr>
<td>0.125</td>
<td>0</td>
<td>0.064</td>
<td>10 (9.1)</td>
</tr>
<tr>
<td>0.0625</td>
<td>0</td>
<td>0.047</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>0.03125</td>
<td>0</td>
<td>0.032</td>
<td>1 (0.9)</td>
</tr>
</tbody>
</table>

Vancomycin: MIC<sub>min</sub> and MIC<sub>C</sub> (2 µg/ml), Daptomycin: MIC<sub>C</sub> (0.5 µg/ml),
MIC<sub>min</sub> (0.38 µg/ml), MIC: Minimum inhibitory concentration,
HA-MRSA: Healthcare-associated-methicillin-resistant Staphylococcus aureus

aspire 5 (4.5%), tissue 4 (3.6%), dialysis central line tip 1 (0.9%),
bronchioalveolar lavage 1 (0.9%), and pleural fluid 1 (0.9%).

All the isolates were susceptible to daptomycin, linezolid, and
teicoplanin. Antibiotic susceptibility testing revealed that 96.1% were
susceptible to rifampicin, 68.1% to tetracycline, 54.5% to gentamicin,
50% to clindamycin, 46.4% to trimethoprim/sulfamethoxazole, and
20% to ciprofloxacin (Table 1).

With regards to vancomycin, 106/110 (96.4%) were susceptible
(MIC≤2 µg/ml) and 04/110 (3.6%) were intermediate
(MIC=4 µg/ml); vancomycin-intermediate S. aureus (VISA). MIC<sub>C</sub> and
MIC<sub>min</sub> of vancomycin were 2 µg/ml. All the isolates were susceptible
to daptomycin (MIC<sub>C</sub><1 µg/ml). MIC<sub>C</sub> and MIC<sub>min</sub> of daptomycin were
0.5 µg/ml and 0.38 µg/ml, respectively (Table 2).

In the present study, on 110 HA-MRSA isolates, we observed high MIC<sub>C</sub>
and MIC<sub>min</sub> of vancomycin (2 µg/ml) and four VISA. A previous study
from North India showed one VISA isolate and high MIC<sub>C</sub> and MIC<sub>min</sub>
of vancomycin. Vancomycin-resistant S. aureus (VRSA) was not reported
in this study [9]. However, another study from South India reported
seven VRSA isolates with vancomycin MIC range 16-64 µg/ml [10].
Another problem with the usage of vancomycin is the heteroresistant
VISA (hVISA). These are the strains with MIC value 0.5-2 µg/ml in
patients where the therapy with the standard usage of vancomycin may
fail. The standard antibiotic susceptibility testing methods fail to detect
hVISA strains.

The Infectious Diseases Society of America guidelines states that
for S. aureus isolates with vancomycin MIC of more than 2 µg/ml an
alternative to vancomycin should be considered [4]. A previous study
showed that treatment of MRSA bacteremia with vancomycin MIC
≤0.5 µg/ml had a success rate of 55.6% as opposed to a success rate
susceptibility of methicillin resistant *Staphylococcus aureus* (MRSA).
