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Short Communication

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

JYOTI KUMARI[1], SHALINI SHENOY M[1], CHAKRAPANI M[2], VIDYALAKSHMI K[1], GOPALKRISHNA BHAT K[1]*

[1]Department of Microbiology, Kasturba Medical College, Manipal University, Mangalore, Karnataka, India. [2]Department of Medicine, Kasturba Medical College, Manipal University, Mangalore, Karnataka, India. Email: gopalkrishna.bhat@manipal.edu

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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 HR 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_{90} and MIC_{50} 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_{90} 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.

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, ceftaroline, 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 Costal 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 (tissue), 1 (dialysis central line tip), 1 (bronchioalveolar 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 antibiotic 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

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

Antibiotic	HA-MRSA (%)
Ciprofloxacin	22 (20.0)
Clindamycin	55 (50.0)
Erythromycin	34 (30.9)
Gentamicin	60 (54.5)
Linezolid	110 (100.0)
Rifampicin	108 (98.1)
Teicoplanin	110 (100.0)
Tetracycline	75 (68.1)
Trimethoprim/sulfamethoxazole	51 (46.4)

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

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

MIC of vancomycin (µg/ml)	Number of HA-MRSA (%)	MIC of daptomycin (µg/ml)	Number of HA-MRSA (%)
128	0	1	4 (3.6)
64	0	0.75	3 (2.7)
32	0	0.5	13 (11.8)
16	0	0.47	20 (18.2)
08	0	0.38	18 (16.4)
04	4 (3.6)	0.25	5 (4.5)
02	61 (55.5)	0.23	8 (7.2)
01	37 (33.6)	0.19	6 (5.5)
0.5	8 (7.3)	0.125	12 (10.9)
0.25	0	0.094	9 (8.2)
0.125	0	0.064	10 (9.1)
0.0625	0	0.047	1 (0.9)
0.03125	0	0.032	1 (0.9)

Vancomycin: MIC_{90} and MIC_{50} (2 µg/ml), Daptomycin: MIC_{90} (0.5 µg/ml), MIC_{50} (0.38 µg/ml), MIC: Minimum inhibitory concentration, HA-MRSA: Healthcare-associated-methicillin-resistant *Staphylococcus aureus*

aspirate 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 98.1% 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_{90} and MIC_{50} of vancomycin were 2 µg/ml. All the isolates were susceptible to daptomycin (MIC≤1 µg/ml). MIC_{90} and MIC_{50} 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_{90} and MIC_{50} of vancomycin (2 µg/ml) and four VISA. A previous study from North India showed one VISA isolate and high MIC_{90} and MIC_{50} 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 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 bacteremia 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_{90} for 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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