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Original Research Article

Assessment of minimum inhibitory concentration to vancomycin, tigecycline, linezolid, daptomycin, ceftaroline and mupirocin against methicillin resistant *Staphylococcus aureus* clinical isolates by antibiotic gradient strips

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ABSTRACT

Background: *Staphylococcus aureus* infections are one of the most common and serious hospital-acquired infections seen in developing countries. Methicillin resistant *Staphylococcus aureus* (MRSA) is an important human pathogen and normally colonized in body parts including skin, nose, perineum and throat. MRSA is resistant not only to all β-lactam groups but also other antibiotics including aminoglycosides, tetracycline and macrolides. In the present study the efficacy of agents used in the management of MRSA infections was determined by antibiotic gradient testing.
Methods: A total of 60 clinical isolates of MRSA strains were collected from various diagnostic labs in central Kerala. Clinical isolates were reconfirmed as MRSA by gram staining, yellow-coloured colonies on mannitol salt Agar (MSA). Antibiotic susceptibility testing was done by disc diffusion method as recommended by CLSI guidelines. *S. aureus* isolates resistant to cefoxitin (30 µg) was identified as MRSA. Antibiotic gradient testing was performed to determine the MIC of vancomycin, tigecycline, linezolid, daptomycin, ceftaroline and mupirocin against MRSA isolates.
Results: All the 60 MRSA isolates tested were sensitive to vancomycin, tigecycline, linezolid, daptomycin, ceftaroline and mupirocin (100%) and none of the MRSA isolates show resistance.
Conclusions: Results of present study indicates that these agents may be used alongside vancomycin in management of infection caused by MRSA.

Keywords: MRSA, Minimum inhibitory concentration, Multidrug resistance, Anti-MRSA drugs, Decolonization

INTRODUCTION

Staphylococcus aureus is recognized as one of the most common organisms causing nosocomial and community-acquired infections in every region of the world.[1] *S. aureus* is a ubiquitous microorganism that is able to colonize the anterior nares and other skin districts of healthy individuals.[2] It contributes significantly to the spread of infections, from minor illnesses to those that pose a threat to one's life, in both hospitals and the general population.[3] Today, a wide variety of medications are available to treat *S. aureus* infections due to its remarkable capacity to develop resistance to antimicrobial agents. Due to their great efficacy and safety, β-lactam antibiotics continue to be one of the main options for treating *S. aureus* infections. However, widespread β-lactam resistance among clinical strains of *S. aureus* limits their use.[4]

MRSA is a significant public health issue that affects people all over the world, leading to significant morbidity and mortality as well as increased medical expenses.[5] Methicillin was introduced in 1959 to treat infections

caused by penicillin-resistant *S. aureus*. MRSA, was 1st reported from the U. K. in 1961.[6] This time resistance was not due to hydrolysing enzyme, but to a more sophisticated mechanism. Methicillin, like all penicillin, exerts its action by blocking proteins called penicillin binding protein, which are responsible for construction and maintenance of bacterial cell wall. *S. aureus* resistant strains acquired a new protein, called PBP2a, which was not blocked by methicillin and could replace other PBP7, thus allowing survival of *S. aureus* in presence of methicillin. PBP2a is encoded by the gene *mecA*, which is hallmark of MRSA.[7]

Methicillin resistance first appeared together with the development of resistance to majority of non-beta-lactam antibiotics, which reduced number of alternatives for treating MRSA infections. Except for vancomycin, some MRSA strains were resistant to all available antibiotic.[8,9] The situation was made worse by the appearance of strains with decreased sensitivity to vancomycin followed by vancomycin-resistant *S. aureus* strains.[10] This stimulated the search for new antibiotics that were effective against MRSA strains. Since then, a number of antibiotics have been created and given clinical use approval, including daptomycin, linezolid, tedizolid, telavancin, oritavancin, tigecycline, dalbavancin, and ceftaroline. Mupirocin is a topical antibiotic used for treatment of skin and soft tissue infections caused by MRSA and decolonization of MRSA in carriers.[11,12] It inhibits protein synthesis by binding specifically to iso-leucyl-tRNA synthetase enzyme.[13]

Study aimed to determine min inhibitory concentration of currently used therapeutic agents in management of MRSA infections namely vancomycin, tigecycline, linezolid, daptomycin, ceftaroline and mupirocin.

METHODS

The present study was carried out at department of medical microbiology, school of medical education during the period of June 2022 to August 2022. 60 clinical isolates of MRSA strains were collected from various diagnostic labs in central Kerala. Clinical isolates were reconfirmed as MRSA by gram staining, yellow coloured colonies on MSA and based on susceptibility to cefoxitin (30 µg) as prescribed by CLSI (M100-S32).[14] Antibiotic susceptibility testing by modified Kirby Bauer's disc diffusion method was also done for penicillin (10 units), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), mupirocin (200 µg-for detection of high-level resistance), linezolid (30 µg), quinupristin/ dalfopristin (12 µg), linezolid (30 µg), tetracycline (10 µg), ciprofloxacin (5 µg), trimethoprim sulfamethoxazole (1.25/23.75 µg) as prescribed by CLSI standards (M02-A13).[15] Inducible resistance to clindamycin was tested by 'D test' as prescribed by CLSI (M100-S32).[14]

Tigecycline, linezolid, daptomycin, ceftaroline and mupirocin E-strips were purchased from bioMerieux India Pvt Ltd and vancomycin Ezy MIC™ Strip was purchased from Hi-media laboratories Pvt ltd.

Lawn culture was made on MHA medium. The MIC test strips were applied on to inoculated agar surface. After 18 hours incubation or longer, a symmetrical inhibition ellipse centered along the strip is formed. The MIC is read directly from the scale in terms of µg/mL, at the point where the edge of the inhibition ellipse intersects with the MIC test strips. MIC is directly interpreted by CLSI (M100-S32)[14] as shown in Table 1.

Table 1: Breakpoints of antibiotic tested based on CLSI (M100-S32).[14]

Antimicrobial agent	MIC Breakpoints µg/ml			
	Sensitive	Resistant	Intermediate	SDD*
Vancomycin	≤2	≥16	4-8	-
Tigecycline	≤0.5	>0.5	-	-
Linezolid	≤4	≥8	-	-
Daptomycin	≤1	-	-	-
Ceftaroline	≤1	≥8	-	2-4
Mupirocin	<256	-	-	-

*Susceptible-dose dependent

Determination of MIC by antibiotic gradient testing

The antibiotic susceptibility pattern of MRSA isolates obtained in the present study is given in Figure 1. Out of 60 clinical samples, 100% (n=60) were resistant to cefoxitin. 41.66% of isolates were sensitive to erythromycin while 8.33% isolates were of intermediate susceptibility. The remaining 50% were resistant. Clindamycin sensitive was observed in 60% of the isolates. 28.33% were resistant to clindamycin and the remaining 11.66% were of intermediate susceptibility. Ciprofloxacin sensitive was observed in 31.66% of isolates and 33.33% were classified as intermediate. 35% of the isolates were resistant to ciprofloxacin. 98.33% MRSA isolates were sensitive to linezolid while 1.66% exhibited resistance. 90% displayed sensitivity, 8.33% were resistant and 1.66% was classified as intermediate susceptibility to Trimethoprim-sulfamethoxazole. The 100% isolates were sensitive to mupirocin. Quinupristin/ dalfopristin resistance was observed in 73.33% of the isolates while 25% were sensitive and remaining 1.66% were of intermediate susceptibility, 68.33% of isolates were sensitive to tetracycline while 4% were resistant and the remaining 25% were of intermediate susceptibility. 38% displayed sensitivity 17% were resistant and 5% was classified as of intermediate susceptibility to gentamicin.

Out of 60 clinical isolates 28% tested positive for inducible clindamycin resistance, while rest of the 72% isolates were negative for D test.

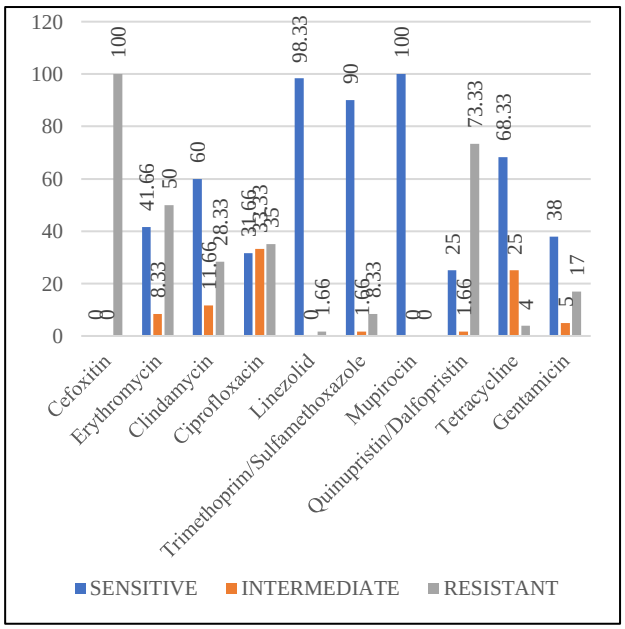

Figure 1: Antibiotic susceptibility pattern of MRSA isolates.

This study was approved by the institutional ethical committee (IEC) at the school of medical education. The data was analysed using Microsoft excel 2019 and statistical package for the social sciences (SPSS) 16.

RESULTS

Out of 60 MRSA isolates, MIC value of 1 isolate were 0.50 µg/ml, MIC value of 2 isolates were 0.75 µg/ml, MIC value of 22 isolates were 1 µg/ml, MIC value of 32 isolates were 1.5 µg/ml, MIC value of 3 isolates was 2 µg/ml for vancomycin Figure 2. MIC value of 10 isolates were 0.047 µg/ml, MIC value of 22 isolates were 0.064 µg/ml, MIC value of 28 isolates were 0.094 µg/ml for tigecycline. MIC value of 6 isolates were 0.125 µg/ml, MIC value of 6 isolates were 0.19 µg/ml, MIC value of 10 isolates were 0.25 µg/ml, MIC value of 6 isolates were 0.38 µg/ml, MIC value of 6 isolates were 0.50 µg/ml, MIC value of 4 isolates were 0.75 µg/ml and MIC value of 22 isolates were 1.0 µg/ml for daptomycin. MIC value of 23 isolates were 0.38 µg/ml, MIC value of 19 isolates were 0.25 µg/ml, MIC value of 11 isolates were 0.50 µg/ml, MIC value of 5 isolates were 0.19 µg/ml, among 2 isolates MIC value of one isolate was 0.125 µg/ml and other was 0.75 µg/ml for ceftaroline. MIC value of 22 isolates were 0.064 µg/ml, MIC value of 6 isolates were 0.094 µg/ml, MIC value of 26 isolates were 0.125 µg/ml, MIC value of 4 isolates were 0.19 µg/ml and MIC value of 2 isolates were 2.0 µg/ml for mupirocin. MIC values of 4 isolates were 0.75 µg/ml. MIC value of 24 isolates were 1.0 µg/ml. MIC values of 28 isolates were 1.5 µg/ml. MIC value of 4 isolates were 2.0 µg /ml for linezolid Figure 3.

Figure 2: Determination of minimum inhibitory concentration by antibiotic gradient strips.

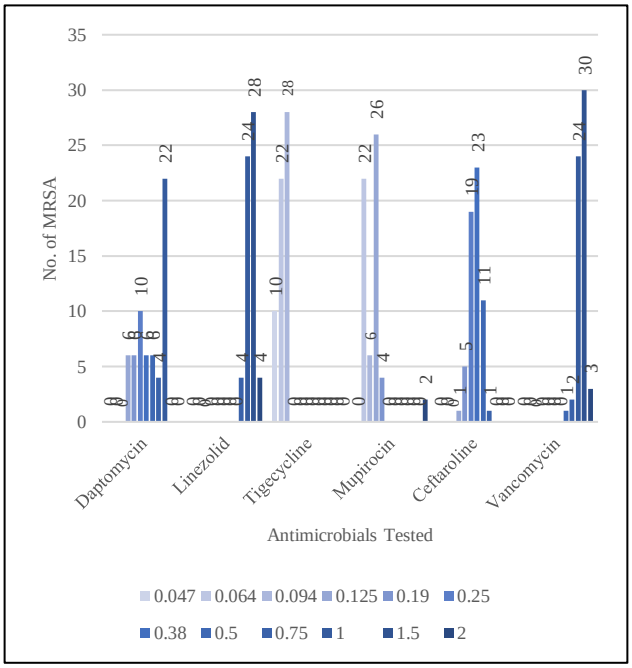

Figure 3: MIC distribution in MRSA isolates.

DISCUSSION

MRSA is an important cause of nosocomial and community acquired infections. It causes a wide range of infections such as abscesses, impetigo, cellulitis, deep seated pyogenic lesions, meningitis, septicaemia and pneumonia. There is a growing concern about MRSA with reduced susceptibility to vancomycin, which is currently the most extensively used antibiotic for its treatment.

In the present study, MIC range of vancomycin was 0.50-2 µg/ml i.e., 100% susceptibility of MRSA isolates to vancomycin. Comparable result was seen in other studies i.e., study by Kulkarni et al, the MIC range was 0.5-2 µg/ml, study by Rani et al, MIC range was 0.5-2 µg/ml.[16,17] But in one study by Anitha et al MIC range of vancomycin

was 0.5-4 µg/ml.[18] A lesser higher MIC value obtained in our study may be due to higher sample size (n=60) in our study, compared to their sample size (n=38). Many reports have stated discrepancies between invitro susceptibility test results for vancomycin and clinical outcomes of MRSA infections treated with it. This has made treatment of MRSA infections difficult due to limited antibiotic choices left. Thus, there is a need for evaluating newer agents as alternatives to vancomycin.

MIC range of tigecycline was 0.047-0.094 µg/ml i.e.,100% susceptibility of MRSA isolates. Almost similar result was seen in other study by Sattar et al in which MIC range was 0.047-0.32 µg/ml. A lesser MIC value obtained in our study may be due to less sample size (n=60) in our study compared to their sample size 100.[19] MIC range of linezolid was 0.75-2.0 µg/ml and demonstrated 100% susceptibility of MRSA isolates. Almost similar result was seen in another study conducted by Aksöz et al in which MIC value of Linezolid range was 0.018-2 µg/ml.[20] Elevated higher MIC value obtained in our study may be due to less sample size (n=60) compared to their sample size of 100. There was another study conducted by Katara et al the MIC value of Linezolid range was 0.25-1 µg/ml.[21] That shows elevated higher MIC value compared to the present study may be due to the higher sample size of 326. MIC range of daptomycin was 0.125-1.0 µg/ml i.e., 100% susceptibility of MRSA isolates to daptomycin. Recently a study from south India by Husain et al documented the MIC range of daptomycin from 0.064-1.5 µg/ml.[22] Lower elevated MIC value obtained in our study may be due to smaller sample size (n=60) compared to their which is 198. Another similar study by Chitnis et al in which MIC range was 0.064-1 µg/ml.[21] That shows elevated lower MIC (0.064 µg/ml) compared to the present study may be due to higher sample size (n=326). MIC range of ceftaroline was 0.125-0.75 µg/ml i.e., 100% susceptibility of MRSA isolates to ceftaroline. Almost similar result was seen in other study by Shivanna et al in which MIC value of ceftaroline range was 0.125-0.38 µg/ml.[23] But in one study MIC range was 0.125-1 µg/ml. Elevated higher MIC value obtained in our study may be due to higher sample size, (n=60) compared to their sample size of 10. There was another study by Mushtaq et al in which MIC range was 0.25-4 µg/ml.[24] That shows elevated higher MIC (4 µg/ml) compared to the present study may be due to the higher sample size, (n=126).

Mupirocin is a commonly used antibiotic for decolonization of MRSA in carriers and for treatment of skin and soft tissue infections caused by MRSA. Emergence of mupirocin resistance due to its irrational use for treatment of skin and soft tissue infections is further worsening the problem of MRSA infections. In the present study, MIC range of mupirocin was 0.064-2.0µg/ml i.e.,100% susceptibility of MRSA isolates to mupirocin. Almost similar result was seen in other study by Rajkumari et al in which MIC range was 0.094-0.75 µg/ml.[25] Compared to the study by Rajkumari et al an elevated MIC value is obtained in the present study.[25]

CONCLUSION

In conclusion, no resistance was detected for vancomycin, tigecycline, linezolid, daptomycin, ceftaroline and mupirocin. In the present study, so these agents may be used alongside vancomycin in the management of infection caused by MRSA. As MRSA is a formidable versatile and unpredictable pathogen as it can, be considered a continuously evolving wonder with constant emergence of new strains often resulting in sustain epidemics, a rational use of the above antimicrobial agents is recommended.

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