

## A study on determination of Minimum Inhibitory Concentration (MIC) of Vancomycin of MRSA Isolates and their impact in treatment of MRSA Isolates

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### Abstract

**Introduction:** *Staphylococcus aureus* is one of the most common causes of nosocomial infections. Methicillin-resistant *S. aureus* (MRSA) is among the top three clinically important pathogens. The glycopeptide vancomycin is considered to be the best alternative for the treatment of MRSA. MRSA usually exhibit vancomycin-susceptible phenotype (VSSA) but some strains exhibit reduced susceptibility to vancomycin which can be heterogeneous-intermediate (hVISA), intermediate (VISA) or fully resistant (VRSA) phenotypes which results in treatment failure. More recently, poor clinical outcome is observed in infections with MRSA strains with an elevated levels of vancomycin MIC within the susceptible range.

**Aim:** This study was done to know the prevalence of MRSA and to determine the vancomycin MIC.

**Materials & Methods:** *S. aureus* isolated from clinical samples were screened for methicillin resistance using cefoxitin discs (30 µg). The vancomycin MIC of these MRSA isolates was determined using E-strips.

**Results & Discussion:** A total of 102 isolates of *S. aureus* were subjected to study. Among these, 42 isolates were MRSA (41.2%). The different MIC values are as follows: 0.38 µg/mL (2 isolate), 0.75 µg/mL (1 isolate), 1 µg/mL (3 isolates), 1.5 µg/mL (32 isolates) & 2 µg/mL (4 isolates). Although all the MRSA strains were within the susceptible range of vancomycin MIC, their increased MIC values (>1 µg/mL) can lead to treatment failures.

**Conclusion:** Increased risks of treatment failure has been observed in infections caused by MRSA isolates with vancomycin MIC in the upper end of susceptible range (MIC > 1µg/ml), emphasising the need for determination of vancomycin MIC to assess the treatment outcome.

**Keywords:** S.aureus, Methicillin, Vancomycin, Minimum Inhibitory Concentration (MIC), treatment failure

### Introduction

*Staphylococcus aureus* is one of the commonest cause of nosocomial infections and also continues to be the major cause of community-acquired infections.<sup>(1)</sup> Methicillin-resistant *S. aureus* (MRSA) is one among the top three clinically significant pathogens.<sup>(2,3)</sup> Serious infections such as endocarditis, sepsis, pneumonia and osteomyelitis due to MRSA are frequently reported in the hospital settings.<sup>(4)</sup>

MRSA strains are usually multi-drug resistant (to macrolides, tetracycline and aminoglycosides) making the treatment options limited.<sup>(1)</sup> The antibiotic vancomycin belonging to glycopeptide group emerged as the best alternative for the treatment of multidrug resistant MRSA isolates.<sup>(5)</sup> However, there are increasing reports of emergence of MRSA strains with decreased susceptibility or resistance to vancomycin.<sup>(6)</sup> Three different phenotypes of vancomycin resistance reported are Vancomycin Sensitive *S. aureus* (VSSA), Vancomycin Resistant *S. aureus* (VRSA) and Vancomycin Intermediate *S. aureus* (VISA). *S. aureus* exhibiting heteroresistance to vancomycin (hVISA) is also more common. hVISA strains contain subpopulation which exhibit intermediate susceptibility to vancomycin but are phenotypically susceptible to vancomycin in sensitivity tests.<sup>(6,7)</sup>

VRSA is due to acquisition of vanA gene from enterococci.<sup>(1,7)</sup> VISA and hVISA is due to alterations

in the bacterial cell wall with cell wall thickening which prevents vancomycin from reaching the target site.<sup>(7-11)</sup> These changes are more common when there is previous exposure to vancomycin.<sup>(12,13)</sup>

According to CLSI, the modified vancomycin MIC breakpoint for different phenotypes of *S. aureus* is as follows: VSSA ≤ 2µg/ml, VISA 4-8 µg/ml and VRSA ≥ 16 µg/ml.<sup>(14)</sup> Many recent studies have demonstrated an association between poor clinical outcome in infections with *S. aureus* strains with an elevated vancomycin MIC values (>1µg/ml) within the susceptible range in which hVISA has been ruled out.<sup>(15,16,17,18)</sup> Hence it is crucial to determine the vancomycin MIC to prevent treatment failures.

Laboratory detection methods such as Disc diffusion and automated methods are not reliable in detecting these vancomycin resistant phenotypes especially hVISA.<sup>(8,11)</sup> The gold standard method for detecting these phenotypes is Population Analysis Profile (PAP), but not commonly employed because it is time consuming, labour intensive and test results are also delayed. E-test were evaluated against the gold standard PAP and shows good sensitivity & specificity.<sup>(5,19,20)</sup> Moreover these E-test can be done easily in all laboratories for determining the vancomycin MIC which helps in rapid identification of these resistant phenotypes.

## Aim

The aim of the study is

1. To know the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in a tertiary care hospital in Salem and
2. To determine the vancomycin MIC of these MRSA isolates

## Materials and Methods

This study was done in Department of Microbiology, VMKV Medical College and Hospital, Salem from February- July 2016. A total of 1974 clinical samples received in the microbiology laboratory were subjected to the study and processed according to standard guidelines. The coagulase positive gram-positive cocci isolated from the samples were subjected to further study. The antibiotic susceptibility testing was done by Kirby-Bauer method according to CLSI guidelines and control strains were included.<sup>(21)</sup> Methicillin resistance was identified using cefoxitin discs (30µg). All the MRSA isolates were then subjected to E-test to determine the vancomycin MIC.<sup>(22)</sup>

## Results

Out of 1974 clinical samples, growth was seen in 671 samples (34%). Among these, 102 were *S.aureus* (15.2%) out of which 42 isolates were MRSA (41.2%). Out of 42 MRSA isolates, all the isolates showed vancomycin MIC  $\leq 2$  mg/l indicating all the strains in our study were sensitive to vancomycin. The different MIC values are as follows: 0.38 µg/mL (2 isolate), 0.75 µg/mL (1 isolate), 1 µg/mL (3 isolates), 1.5 µg/mL (32 isolates) and 2 µg/mL (4 isolates).

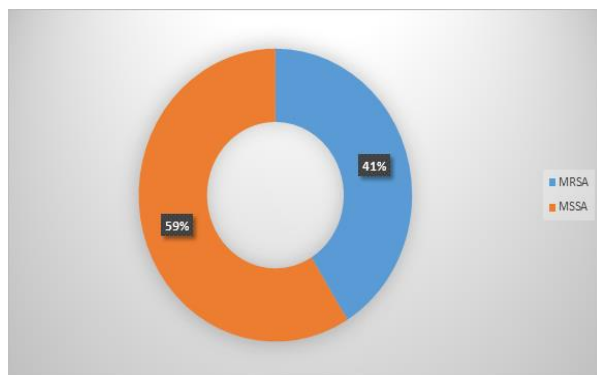


Fig. 1: Percentage of MRSA isolates

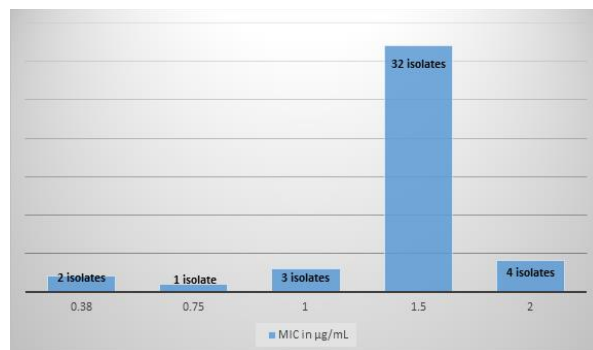


Fig. 2: Vancomycin MIC of MRSA isolates

## Discussion

The prevalence of MRSA in our study is 41%. This is in concordance with the studies done by Tiwari<sup>(23)</sup> and Kandle<sup>(24)</sup> which showed a prevalence of 38.44% and 39.1% respectively. A low prevalence rate of 29.1% is documented in some studies done by Vidya Pai,<sup>(4)</sup> 32.8% by Mehta<sup>(25)</sup> and 26.14% by Kumari.<sup>(26)</sup> On contrary, very high prevalence of 79.6% have been reported in some studies done by Venubabu Thati.<sup>(1)</sup>

The various predisposing factors for MRSA may be patients with compromised immune system, selective pressure by antimicrobials and transmission from colonised or infected patients and health care workers in the hospital setting.<sup>(27)</sup>

In our study, MRSA strains were also resistant to multiple other antibiotics. They were resistant to penicillin, ampicillin, cefatoxime, gentamycin, cotrimoxazole, erythromycin and clindamycin. All the strains were sensitive to vancomycin. They were also sensitive to other antibiotics like Amikacin and Ciprofloxacin making them an alternative treatment options, so that vancomycin can be cautiously used for serious life threatening infections.

At present, vancomycin is the main antimicrobial agent used to treat MRSA infections<sup>(1)</sup> but decrease in vancomycin susceptibility among MRSA isolates are now increasingly being reported.<sup>(28)</sup> This is shown in many recent studies where treatment failures in MRSA infections have been observed when the vancomycin MIC is in the upper end of susceptible range (MIC > 1µg/ml) where hVISA has been excluded.<sup>(7,15)</sup>

In our study, the vancomycin MIC of all the MRSA isolates were within the susceptible range ( $\leq 2$ µg/ml). Among these, 85.7% of isolates had an MIC in the upper end of susceptible range (> 1 µg/ml). This raises the concern over the efficacy of vancomycin in treating these MRSA strains.

One possible explanation for this reduced susceptibility could be the presence of heteroresistance.<sup>(16)</sup> The prevalence of heteroresistance is very low between 0% - 50% as shown in various other studies, hence it is difficult to establish the role of heteroresistance in most of the conditions. Another common risk factor for development of VRSA and VISA is the prior exposure to glycopeptides.<sup>(8,29)</sup> Hence

it is essential to educate the clinicians to use other alternative drugs to prevent the emergence of resistance to vancomycin.

### Conclusion

Infections caused by methicillin-resistant *S. aureus* have been associated with high morbidity and mortality rates. Vancomycin is the most effective antimicrobial agent available to treat serious infections with MRSA, but the emergence of resistant phenotypes raises concern. Determination of vancomycin MIC has a major impact on the clinical outcome in these infections.<sup>(16)</sup> A better knowledge on association between elevated vancomycin MIC values and clinical outcome in treatment of VSSA isolates becomes important for a cautious use of vancomycin. Regular surveillance of hospital infections is important to reduce the burden of MRSA and glycopeptide resistance for better clinical outcome of patients.

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