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# MINIMUM INHIBITORY CONCENTRATION OF VANCOMYCIN AMONG METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES FROM A TERTIARY CARE CENTRE

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**ABSTRACT: Introduction:** Infections due to methicillin resistant *Staphylococcus aureus* (MRSA) are increasing worldwide. Vancomycin is considered as the drug of choice to treat serious infections caused by MRSA strains. The emergence of *S. aureus* isolates with intermediate and complete resistance to vancomycin in the past two decades is a major public health concern. **Aim and Objectives**: To determine the MIC of vancomycin in MRSA isolates from various clinical specimens and to observe the occurrence of VRSA and VISA. **Materials and Methods:** This cross-sectional study was conducted between November 2020 to October 2021 in the department of Microbiology, RMCH, Bareilly. *S. aureus* isolates were identified using routine identification methods. MRSA isolates were subjected to E-test for detection of MIC of vancomycin through vancomycin Ezy MIC Strips (Hi Media Range .016-256µg/ml). **Results:** A total of 180 *S. aureus* strains were isolated during the study period. Out of these strains 100 (55.55%) MRSA and 80 (44.45%) MSSA were isolated. Out of 100 MRSA strains 8 strains showed vancomycin MIC of 0.75 µg/ml, 50 strains showed vancomycin MIC of 1 µg/ml, 36 strains showed vancomycin MIC of 1.5 µg/ml and 6 strains of MRSA showed vancomycin MIC of 2µg/ml. **Conclusion:** Among MRSA isolates, 6 strains with high vancomycin MIC (2µg/ml) were detected along with multidrug resistance to aminoglycosides, fluoroquinolones and macrolides. The study indicates a rising trend in MIC of vancomycin in MRSA isolates which has become a serious public health concern. Routine testing and reporting of vancomycin MIC is crucial so that effective treatment regimen can be initiated for patients and the development of resistance of *S. aureus* to this reserve drug can be prevented.

**INTRODUCTION:** *Staphylococcus aureus* is a prominent pathogen which causes a variety of infections in humans ranging from superficial skin and soft tissue infections to invasive systemic diseases.

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The emergence of methicillin resistant *Staphylococcus aureus* (MRSA) in 1960s and its widespread dissemination has made it one of the most challenging organisms to treat [1].

Methicillin resistance in *S. aureus* is mediated through an altered protein called low-affinity penicillin binding protein (PBP2a). PBP2a is encoded by *mecA* gene which is present in chromosomal mobile genetic element called Staphylococcal cassette chromosome mec (*SCCmec*) [2].

MRSA strains are noted to be resistant to multiple antibiotic groups that include aminoglycosides, cephalosporins, quinolones and Others [3]. Latest reports suggest that India has the highest prevalence of MRSA in the world, ranging from 16.5- 23.5 % in community acquired-MRSA and 29- 46% in hospital acquired MRSA [4]. Vancomycin, a glycopeptide antibiotic, is considered as the drug of choice to treat serious infections caused by MRSA strains [5]. However, in 1997, the first case of vancomycin intermediate *Staphylococcus aureus* (VISA) (VISA; MIC, 4-8ug/ml) was reported which has now become a growing concern [6].

Various studies suggest that prolonged vancomycin exposure to MRSA strains produce a thicker cell wall thereby limiting drug entry. Moreover, conjugative transfer of vanA gene from Vancomycin resistant enterococci (VRE) to *Staphylococcus aureus* can be a probable mechanism for development of resistance to vancomycin among MRSA [7]. Over the past two decades there have been a number of reports indicating a shift in the vancomycin MIC values among MRSA strains. These small increases in MIC, in spite of being within susceptible limits, can affect treatment efficacy [8].

Traditionally used disc diffusion method is not recommended by the CLSI for determination of vancomycin breakpoints in *S. aureus* isolates. Broth microdilution (BMD) is the method recommended by CLSI for vancomycin susceptibility testing which determines vancomycin MIC in two fold dilutions [9]. E-test is a relatively convenient test to use instead of BMD. The E-test strip has a predefined gradient of vancomycin which is used to determine the MIC (ug/ml) on MHA[8]. Aljohani *et al.*[10] reported a higher treatment failure rate among infections caused by MRSA isolates that had vancomycin MIC of >1.5 ug/ml. Proper history of prior vancomycin therapy in patients of MRSA infections along with accurate vancomycin MIC value report can help clinicians to initiate effective treatment regimen [10]. Therefore, it is important to determine the MIC of vancomycin in MRSA isolates from clinical samples for better clinical outcome of patients. The present study is conducted to determine the MIC of vancomycin among MRSA isolates in this region [11].

**MATERIAL & METHODS:** This cross-sectional study was conducted between November 2020 to October 2021 in the Department of Microbiology, Rohilkhand Medical College and Hospital, Bareilly, after approval from institutional ethics committee. A total of 180 *S. aureus* strains were isolated from various clinical specimens like urine, blood, body fluids, respiratory (Sputum, ET aspirate and bronchoalveolar lavage) and swabs from wound, ear and throat that were received during the study period. *S. aureus* isolates with zone of inhibition around cefoxitin ≤ 21mm (resistant) were included in the study (screening test for MRSA) [9].

All the specimens received in the bacteriology lab were inoculated on blood agar and MacConkey agar and incubated at 37°C for 24-48 hours. Gram staining was performed from growth on the agar plate and identification of *S. aureus* isolates was done as per standard operating procedures[12]. Isolated organisms were subjected to antibiotic susceptibility testing through Kirby Bauer Disc Diffusion method according to CLSI guidelines (2020) [9].

The strains of *S. aureus* which showed zone diameter of cefoxitin ≤21mm were considered to be resistant to cefoxitin and screened as MRSA. These strains were subjected to E-test for determination of MIC of vancomycin through vancomycin Ezy MIC Strips (Hi Media Range. 016-256µg/ml)[13]. The MIC testing was performed according to the manufacturer's guidelines. Quality control of the strip was performed with *S. aureus* ATCC 25923 as recommended by CLSI (2020) [9].

**RESULTS:** A total of 180 *S. aureus* strains were isolated during the study period. Out of them 100 (55.55%) MRSA and 80 (44.45%) MSSA were isolated from various clinical specimens. All the 100 MRSA strains were distributed according to age groups and gender.

It was observed that the majority of MRSA strains were isolated from patients of 31-40 years age group (n= 19) followed by 17 MRSA strains from 11-20 years and 21-30 years. Among the 100 MRSA isolates, 47 (47%) were from male patients and 53 (53%) were from females **Table 1.**

**TABLE 1: DISTRIBUTION OF MRSA ISOLATES ACCORDING TO AGE AND GENDER (N=100)**

| Age group | Male | Female |
|---|---|---|
| < 10 years | 05 | 09 |
| 11-20 years | 07 | 10 |
| 21-30 years | 11 | 06 |
| 31-40 years | 08 | 11 |
| 41-50 years | 05 | 08 |
| 51-60 years | 08 | 04 |
| 61-70 years | 01 | 03 |
| >70 years | 02 | 02 |
| Total | 47 | 53 |

Maximum MRSA isolates were found in pus samples (38), 28 from blood, 28 from urine, 1 from ET aspirate, 1 from throat swab, 1 from tissue, 2 from semen and 1 from vaginal swab **Table 2.**

**TABLE 2: TYPES OF SAMPLES RECEIVED FROM DIFFERENT DEPARTMENTS (N=100)**

| Department | Samples | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pus | Blood | Urine | E.T. aspirate | Throat swab | Tissue | Semen | Vaginal swab | Total |
| Medicine | 2 | 12 | 9 | 1 | 0 | 0 | 2 | 0 | 26 |
| Surgery | 22 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 23 |
| Pediatrics | 0 | 9 | 2 | 0 | 1 | 0 | 0 | 0 | 12 |
| Orthopedics | 9 | 1 | 5 | 0 | 0 | 1 | 0 | 0 | 16 |
| Gynaecology | 3 | 1 | 10 | 0 | 0 | 0 | 0 | 1 | 15 |
| Casualty | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| TB & Chest | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 4 |
| Dental | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| NICU | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| ENT | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Total | 38 | 28 | 28 | 1 | 1 | 1 | 2 | 1 | 100 |

Amongst a total of 72 non urine MRSA isolates, all 72 strains were sensitive to linezolid while all 72 strains were resistant to ampicillin. Tetracycline sensitivity was found in 42 strains (58.33%) and 67 strains (93.05%) were resistant to ciprofloxacin. **Fig. 1.**

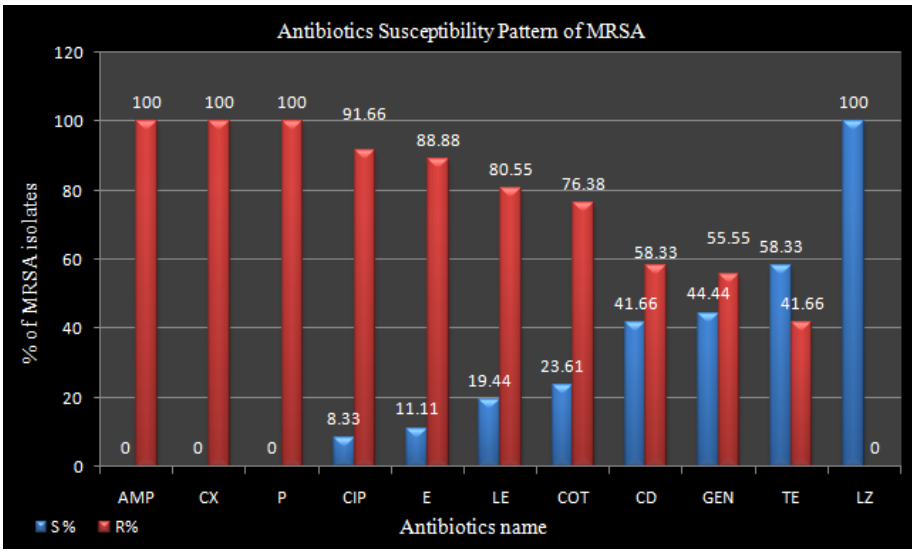


**FIG. 1: ANTIBIOTICS SUSCEPTIBILITY PATTERN OF MRSA ISOLATES IN 72 NON URINE SAMPLE**

All 28 MRSA strains isolated from urine samples were sensitive to linezolid, 25 MRSA strains (89.28%) were sensitive to nitrofurantoin, 23 MRSA strains (82.14%) were sensitive to fosfomycin. Twenty seven MRSA strains (96.42%) were resistant to norfloxacin and 25 MRSA strains (89.28%) were resistant to ciprofloxacin **Fig. 2.**

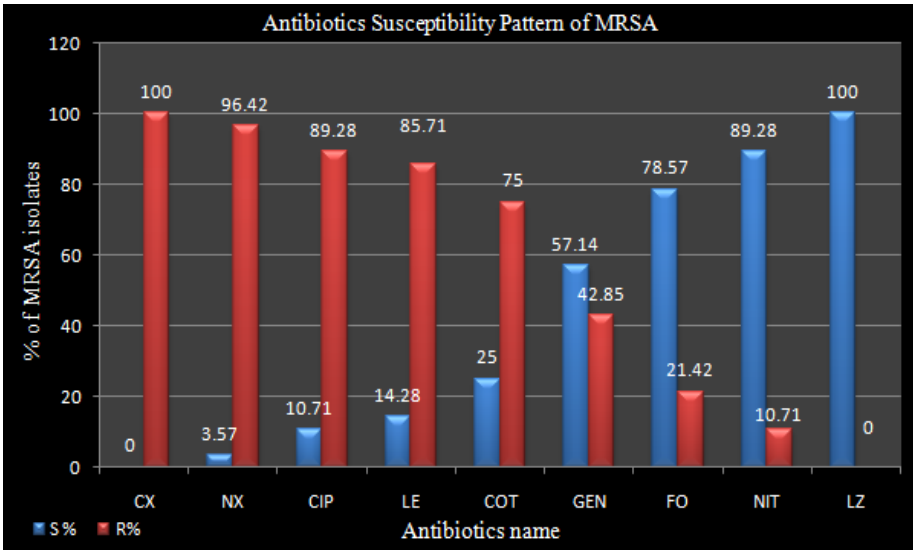


**FIG. 2: ANTIBIOTICS SUSCEPTIBILITY PATTERN OF MRSA ISOLATES IN 28 URINE SAMPLES**

In the present study, the MIC of vancomycin was determined using epsilometer test (E-test) method. Out of 100 MRSA isolates 50 isolates showed MIC of 1 µg/ml, 36 isolates showed MIC of 1.5 µg/ml, 8 isolates showed MIC of 0.75 µg/ml and 6 isolates showed vancomycin MIC of 2 µg/ml **Table 3.**

**TABLE 3: VANCOMYCIN MIC VALUES IN MRSA ISOLATES**

| MIC value (ug/ml) | No. of MRSA isolates (%) |
|---|---|
| 0.75 | 08 |
| 1 | 50 |
| 1.5 | 36 |
| 2 | 06 |
| Total | 100 |

**DISCUSSION:** Emergence and spread of MRSA, both in community and hospital settings have been associated with high morbidity and mortality as it is getting increasingly challenging to treat these multidrug resistant infections with few effective therapeutic drugs available. Vancomycin is the drug of choice to treat invasive MRSA infections despite limitations like renal toxicity associated with high trough level [14]. But the emergence of *S. aureus* strains with reduced vancomycin susceptibility; MIC 4-8µg/ml (VISA) and resistant strains with MIC ≥16 µg/ml (VRSA) has led to great concern. Additionally, over time, reports have shown rising vancomycin MICs for *S. aureus* which are well within the current susceptible range (MIC ≤2µg/ml) but often cause failure of vancomycin therapy. MRSA screening is being performed routinely in all laboratories but very few are testing vancomycin MIC in routine susceptibility testing which is recommended by CLSI (2020). In the present study 180 *S. aureus* strains were isolated from various clinical samples in our institute during the study period. The prevalence of MRSA was 55.6%. Our results were similar to the findings of Ferrer-Burgua LG *et al.*[15] who reported MRSA prevalence of 52% from Spain. Other researchers like Khalili H *et al.*[16] found MRSA prevalence of 56.1% from Tehran. However, in another study by Lohan K *et al.*[17] prevalence of MRSA was found to be 33.7%.

The antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method in our study. Approximately 90% of MRSA isolates were resistant to fluoroquinolones and erythromycin. Almost half of the isolates were sensitive to gentamycin, clindamycin and tetracycline. Moderately high resistance was observed against cotrimoxazole (76%). Ferrer-Burger LG *et al.*[15] reported the antimicrobial susceptibility pattern similar to our result. All isolates were sensitive to linezolid. Our results were in concordance to the finding of Goswami NN *et al.*[18] who reported 100% isolates to be sensitive to linezolid. Varying trends in antimicrobial susceptibility patterns have been observed across different geographical locations depending on many factors like local antibiotic prescribing policies, genotypic characteristics of strains, hospital infection prevention and control programs and patient population demographics. As there are more reports on increasing multidrug resistance in MRSA infections to macrolides, quinolones and aminoglycosides, vancomycin is used as first line

drug to treat them. Additionally, recent reports indicate that there is a reversal of susceptibility to older antistaphylococcal agents thus routine testing of older antibiotics should also be done [19].

VISA was first reported by Hiramatsu K *et al.*[20] from Japan in a four-month-old infant in 1997 while VRSA was first reported in 2002 from Michigan [21]. VISA isolates are being frequently isolated in India but there are very few reports of VRSA. CLSI recommends broth microdilution and agar dilution methods for MIC testing of vancomycin. Dilution methods are tedious and time taking so in the present study we used convenient E-test method by using Hi Media Ezy MIC strips. Song KH *et al*. [22] compared vancomycin MIC by BMD and E-test and found that higher number of isolates with increased MIC were detected by E strip as compared to BMD (19.5% vs 8.5%). Additional advantage of E-test over BMD is that even minor changes in MIC values can be determined by it as the predefined gradient incorporates traditional as well as intermediate (1.5 ug/ml) values of MIC [8]. Out of 100 MRSA isolates, half of them showed MIC of 1 µg/ml while 6 isolates showed MIC of 2 µg/ml **(Table 3)**. No VISA or VRSA isolates were detected in our study. Similar findings were also reported by Anitha TK *et al.* [23] from Mysore who found MIC of vancomycin in the range of 0.5-2 µg/ml by the E-test. Our results also very well correlated to studies conducted by Moses VK *et al.* [3] and Maharjan M *et al*. [5] Meanwhile, studies reported by Sreenivasulu Reddy P *et al.* [24] from Andhra Pradesh found MIC value of 100 isolates varying from 0.5-16 µg/ml. Kaur K *et al.* [25] from Punjab reported MIC value in 162 isolates, and found 97.8% isolates sensitive to vancomycin with MIC of ≤ 2µg/ml, 11.7% as VISA with MIC 4-8 µg/ml, whereas 2.46% as VRSA with MIC ≥16 µg/ml.

MRSA isolates with reduced vancomycin susceptibility have subpopulations of vancomycin resistant *S. aureus* which would ultimately lead to lesser clinical efficacy if dose of vancomycin is not titrated or if another antibiotic is not started. It could also give rise to true VRSA with repeated exposure to vancomycin due to selection pressure. This makes detection and reporting of vancomycin MIC important in clinical microbiology laboratories.

**CONCLUSION:** The occurrence of MRSA was found to be 55.6% in our study. Most common MRSA strains were isolated from pus samples. MRSA isolates showed high resistance to aminoglycosides, fluoroquinolones and macrolides. All MRSA isolates had MIC of vancomycin in susceptible range. Six isolates showed vancomycin MIC value at the higher end *i.e.* 2 ug/ml. It is crucial to detect vancomycin MIC in MRSA isolates in laboratories to provide much needed knowledge about increasing MIC value trend thereby helping clinicians in choosing alternative antibiotics in cases of treatment failure by vancomycin.

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**CONFLICTS OF INTEREST:** The authors declare they have no conflict of interest.

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