VISA AMONGST MRSA ISOLATES IN A TERTIARY CARE HOSPITAL IN MANIPUR

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ABSTRACT
BACKGROUND
Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital acquired and community-acquired infections. MRSA isolates are important for their resistance to many commonly used antibiotics. Vancomycin, a glycopeptide, was considered to be the best alternative for the treatment of MRSA. However, there are increasing number of reports indicating the emergence of vancomycin-resistant *S. aureus* (VRSA). The prevalence of MRSA in a similar setup in the state showed a prevalence of MRSA to be 84% in 2008. Therefore, the objective of this study was to find prevalence of the VISA amongst the MRSA isolates in our Hospital.

MATERIALS AND METHODS
A cross-sectional study was conducted in a tertiary care teaching hospital of Imphal, Manipur, India during the period from December 2015 to May 2016. A total of 126 *Staphylococcus aureus* isolates from different clinical specimens like wound swabs, pus and urine received from inpatients and outpatients of this referral tertiary care hospital were included in the study. Staphylococci were obtained either as pure culture or as an isolate of a polymicrobial infection. *S. aureus* was identified conventionally. MRSA were identified using cefoxitin disc (30 μg) by Kirby-Bauer disc diffusion technique according Clinical and Laboratory Standards Institute (CLSI) guidelines 2014. All MRSA isolates were subjected to susceptibility testing by the Kirby–Bauer's disc diffusion method using different antimicrobial agents. All the MRSA isolates irrespective of their susceptibility pattern to Vancomycin by disc diffusion were resorted to E-test (bioMerieux) to determine the minimum inhibitory concentration (MIC). MIC ≤2 μg/mL were identified as sensitive, 4 to 8 mg/L were identified as vancomycin-intermediate and isolates with a vancomycin MIC >16 mg/L were identified as vancomycin-resistant *S. aureus* according to CLSI, 2014. Quality control was performed using the *S. aureus* ATCC 25213 strain. Antibiotic susceptibility testing of VISA isolates was done by Kirby-Bauer's Disc diffusion method using the same drugs used for MRSA.

RESULTS
Prevalence of MRSA was (100/126) 79.36%. MRSA isolates were 100% resistant to Penicillin followed by Ampicillin (90%), Cotrimoxazole (76%). 100% sensitivity was seen with Linezolid followed by Ciprofloxacin (75%), Ofloxacin (79%), Nitrofurantoin (76%), Amikacin (62%), Gentamycin (70%) and Tetracycline (66%). Prevalence of VISA isolates was 3% (3/100) in this study. Amongst the VISA isolates, linezolid was the most sensitive for all the three isolates and nitrofurantoin was sensitive for both the urine isolates and clindamycin was also sensitive for the only pus isolate.

CONCLUSION
This study emphasises the need to study the prevalence and antimicrobial susceptibility pattern of MRSA isolates area-wise in order to guide policy on the appropriate use of antibiotics which would minimise the irrational use of vancomycin and so the emergence of resistance to vancomycin.

KEYWORDS
Reduced Vancomycin Susceptibility, E-strip, Antibiogram.

BACKGROUND
Methicillin-resistant *Staphylococcus aureus* (MRSA) is among a major cause of hospital acquired and community-acquired infections. The methicillin resistance in MRSA is due to the acquisition of mecA gene, which encodes the low affinity penicillin-binding protein (PBP2a), a cell wall transpeptidase, which, in conjunction with native PBP2, allows continued cell

wall synthesis in the presence of β-lactams.[1] MRSA isolates are important for their resistance to many commonly used antibiotics. They exhibit remarkable versatility in their behaviour towards antibiotics which poses a serious therapeutic problem. Thus, MRSA remains as a major cause of morbidity and mortality among hospitalised patients despite the availability of numerous effective anti-Staphylococcal antibiotics.[2] Vancomycin, a glycopeptide, was considered to be the best alternative for the treatment of MRSA. However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant *S. aureus* (VRSA). Initially vancomycin-intermediate *S. aureus* (VISA) noted in Japan in 1996 and subsequently in United States in 1997.[3] Sequential mutations in Vancomycin Susceptible *Staphylococcus aureus* (VSSA) led to the emergence of vancomycin heteroresistant-intermediate
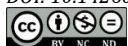
Staphylococcus aureus (hVISA) and ultimately Vancomycin intermediate resistance *Staphylococcus aureus* (VISA).

The hallmark changes are alterations in the bacterial cell wall resulting in reduced autolytic activity and wall thickening. Multiple genetic mutations have been implicated in the pathogenesis of these cell wall modifications. While the genetic determinants of hVISA and VISA are partially understood, the relative contributions of these mutations—and of altered cellular processes that contribute to hVISA and VISA—in determining the vancomycin MIC are unknown.[4] The prevalence of MRSA in a similar set up in the state showed a prevalence of MRSA to be 84% in 2008.[5] Therefore, the objective of this study was to find incidence of the VISA amongst the MRSA isolates in our Hospital.

MATERIALS AND METHODS

A cross-sectional study was conducted in a tertiary care teaching hospital of Imphal, Manipur, India during the period from December 2015 to May 2016. A total of 126 *Staphylococcus aureus* isolates from different clinical specimens like wound swabs, pus and urine received from inpatients and outpatients of this referral tertiary care hospital were included in the study. Staphylococci were obtained either as pure culture or as an isolate of a polymicrobial infection. These were incubated at 37°C for 24 hours. *S. aureus* was identified conventionally based on Gram's stain morphology, colony characteristics, and positive catalase and coagulase tests. The *Staphylococcus* species were identified on the basis of both slide and tube coagulase test.[6] 100 MRSA strains amongst the total 126 *Staphylococcus aureus* isolates were identified using cefoxitin disc (30 μg) by Kirby-Bauer disc diffusion technique according Clinical and Laboratory Standards Institute (CLSI) guidelines 2014.[7]

All MRSA isolates were subjected to susceptibility testing by the Kirby–Bauer's disc diffusion method using different antimicrobial agents e.g., penicillin (10 units), clindamycin (2 μg), ciprofloxacin (5 μg), co-trimoxazole (25 μg), azithromycin (15 μg), gentamicin (10 μg), linezolid (30 μg), ofloxacin (5 μg), tetracycline, amikacin, ampicillin, vancomycin (30 μg) (Hi-media). Zone diameter of the test strain was measured in millimetre with a template. All the MRSA isolates irrespective of their susceptibility pattern to Vancomycin by disc diffusion were resorted to E-test (bioMerieux) to determine the minimum inhibitory concentration (MIC) and confirmation of their resistance status. 0.5 McFarland equivalent inocula, prepared by using 18-24 hours old culture was inoculated on the Mueller-Hinton agar plates on which E-test strip was implanted according to the user guideline. Plates were incubated at 35°C for 24 hours before assessing the visible growth. The MIC is the value at which the elliptical zone of inhibition converges on the strips. Isolates with a vancomycin MIC ≤2 μg/mL were identified as Sensitive, 4 to 8 mg/L were identified as vancomycin-intermediate and isolates with a vancomycin MIC >16 mg/L were identified as vancomycin-resistant *S. aureus* (CLSI, 2014).[7] Vancomycin breakpoints, according to which the current definition for VISA is an *S. aureus* isolate with a vancomycin broth MIC of 4-8 μg/mL since they use doubling dilutions of antibiotic (otherwise all isolates with MIC between 2 and 16 μg/mL are called VISA strains; strains with MIC ≤ 2 μg/mL are sensitive and ≥ 16 μg/mL are

resistant). MIC >8 must be referred to a reference laboratory according to the CLSI guidelines.[8] Quality control was performed using the *S. aureus* ATCC 25923 strain.

Statistical Analysis

The data collected were put into MS-Excel sheets for analysis. Percentages were used for interpretation of the data in this study.

RESULTS

The prevalence of MRSA isolates was (100/126) 79.36%. Among 100 MRSA isolates, 5 strains were found to be resistant to vancomycin by disc diffusion test. Of these strains, only one isolate was found to be VISA showing an MIC range between 4-6 mg/L, when tested by E-test (bioMerieux). And, amongst the 95 isolates which were sensitive by disc diffusion two more isolates were found to be Vancomycin Intermediate *Staphylococcus aureus* by E-strip method. Therefore, VISA isolates were observed in only 3% of isolates in our study.
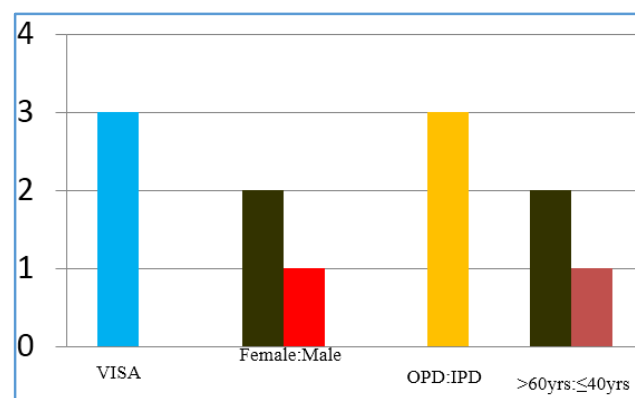
Figure 1. VISA According to Gender, IPD, OPD & Age Groups

Figure 1 shows Vancomycin intermediate resistant Staphylococcus aureus according to gender, inpatient/outpatient department and age groups. In our study, the incidence of VISA was 3%, out of which two were female and one was male. And all the VISA isolates were from inpatient department. Of the three VISA isolates, one was pus sample of a 40-year-old male patient from male surgery ward. And the other two VISA isolates were from urine samples, one from a 75-year-old female patient and another one from a 64-year-old female patient from female medicine ward.

No Vancomycin resistant *Staphylococcus aureus* was detected.
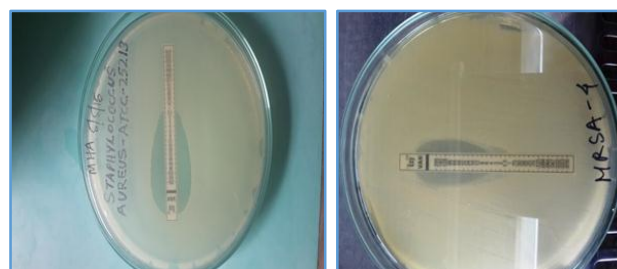
Figure 2. Showing MIC by E-strip Technique for (left) Staphylococcus Aureus 25213 and (right) MRSA Isolate.

Figure 2 (left) shows that the MIC of the control strain is about 1.5 mg/L which is < 2 mg/L and the picture on the right

is of an MRSA isolate of a clinical sample which shows an MIC of 4 mg/L.

Antibiotic	Sensitivity (%)
Linezolid	100
Vancomycin	95
Ofloxacin	79
*Nitrofurantoin	76
Ciprofloxacin	75
Gentamycin	70
Tetracycline	66
Amikacin	62
Azithromycin	60
**Clindamycin	55
Cotrimoxazole	24
Ampicillin	10
Penicillin	0
Table 1. Showing Antibiotic Sensitivity Pattern amongst the MRSA isolates by Kirby Bauer's Disc Diffusion Method	

*Nitrofurantoin only for urine isolates. **Clindamycin not for urine samples.

The antibiotic sensitivity pattern of MRSA isolates as shown in Table 1: linezolid (100%), vancomycin (95%), ofloxacin (79%), nitrofurantoin (76%), ciprofloxacin (75%), gentamycin (70%), tetracycline (66%), amikacin (62%), azithromycin (60%), clindamycin (55%), cotrimoxazole (24%), ampicillin (10%) and penicillin (0%).

Antibiotic	Sensitivity number (%)
Linezolid	3 (100)
Vancomycin	2 (66.66)
Ofloxacin	(0)
*Nitrofurantoin	2 (100)
Ciprofloxacin	0 (0)
Gentamycin	0 (0)
Tetracycline	0 (0)
Amikacin	0 (0)
Azithromycin	0 (0)
**Clindamycin	1 (100)
Cotrimoxazole	1 (33.33)
Ampicillin	0 (0)
Penicillin	0 (0)
Table 2. Showing Antibiotic Sensitivity Pattern amongst the VISA Isolates by Kirby-Bauer's disc Diffusion Method	

*Nitrofurantoin only for urine isolates.
**Clindamycin not for urine samples.

The antibiotic sensitivity pattern of the VISA isolates as shown in Table 2 linezolid (100%), vancomycin (66.66%), ofloxacin (0%), nitrofurantoin (100%), ciprofloxacin (0%), gentamycin (0%), tetracycline (0%), amikacin (0%), azithromycin (0%), clindamycin (100%), cotrimoxazole (33.33%), ampicillin (0%) and penicillin (0%).

DISCUSSION

In our study, among 100 MRSA, 5 strains were found to be resistant to vancomycin by disc diffusion test. Of these strains, only one was found to be Vancomycin intermediate *Staphylococcus aureus* (VISA) by E-strip method. And, two more isolates amongst the 95 isolates which were sensitive by disc diffusion method, turned out to be VISA by E-strip method. As clearly stated in the CLSI guideline, vancomycin disc diffusion technique does not differentiate between vancomycin susceptible, intermediate and resistant strains.[5] Therefore, a total of 3% VISA was isolated in our study. Moses A et al found a higher incidence of 18.0% of VISA.[9] Tiwari HK et al showed a prevalence of 1.8% VISA which is lesser in comparison to our study.[10] Hu H C et al also found a prevalence of 2.89% of VISA in a study conducted in Taiwan which is a comparable finding with our study.[11]

MRSA isolates were 100% resistant to Penicillin followed by Ampicillin (90%) and Cotrimoxazole (76%). 100% sensitivity was seen with Linezolid followed by Ciprofloxacin (75%), Ofloxacin (79%), Nitrofurantoin (76%), Amikacin (62%), Gentamycin (70%) and Tetracycline (66%). B S Suresh et al also found that linezolid and ofloxacin were amongst the most sensitive drug for MRSA.[11] The antibiotic sensitivity pattern was similar in cases of OPD and IPD isolates.

Amongst the VISA isolates, linezolid was the most sensitive for all the three isolates and nitrofurantoin was sensitive for both the urine isolates and clindamycin was also sensitive for the only pus isolate.[12]

In a study conducted by K Tandel et al, E-test was found to be a feasible alternative method for screening of VRSA as it is easy to perform and its cost effectiveness, but the study also found that E-test provides vancomycin MIC results consistently higher (by 0.5 to 2 log2 dilutions) than those provided by reference agar dilution tests and so the study concluded by stating that Agar dilution test should be done on all those strains showing higher MIC (intermediate or resistant results) by E-test. In our study, we did not confirm the VISA isolates with agar dilution method. Therefore, there is a chance of the incidence of VISA isolates to be less than 3%. But a study conducted by EH Natasha et al mentioned that a number of studies have shown that the E-test, MIC test seems to predict treatment failure better than broth microdilution testing, this may be a convenient and practical MIC determination method.[4]

M Bilal Ahmad et al found that there is a difference between antibiogram of MRSA and MSSA isolates and therefore, routine E-testing of methicillin resistance should be done using cefoxitin disc which at present is the most sensitive method.[13] In our study, we did not compare the antibiotic sensitivity pattern of the two groups MRSA and MSSA, which shall be done in our next study in near future.

In our study, isolation of the Van A/Van B genes by Polymerase Chain Reaction (PCR) was not done. However, in the study conducted by Tiwari and Sen, these genes were absent in the VISA and VRSA strains isolated even in the presence of phenotypic resistance to Vancomycin. Hence, the presence or absence of the resistant Van A/B genes does not necessarily rule out that strains are not VISA/VRSA.[9]

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Limitation
Hetero-resistance vancomycin intermediate *Staphylococcus aureus* (hVISA) cases have been defined as those phenotypic isolates which on in-vitro susceptibility test is vancomycin susceptible (VSSA) but has a minority population of vancomycin intermediate (VISA). hVISA is responsible for vancomycin treatment failure but detection of hVISA was not done in our study.

CONCLUSION
This study emphasises the need to study the prevalence and antimicrobial susceptibility pattern of MRSA isolates area-wise in order to guide policy on the appropriate use of antibiotics which would minimise the irrational use of vancomycin and so the emergence of resistance to vancomycin. So interpretation of susceptibility results should occur in discussion with relevant clinical personnel such as infectious-disease pharmacists and physicians, clinical microbiologists, and hospital antimicrobial-committee members.

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