VANCOMYCIN SUSCEPTIBILITY STATUS AMONG CLINICAL MRSA ISOLATES AND DETECTION OF VISA, hVISA IN A TERTIARY CARE HOSPITAL
Sanjay Kumar¹, Anil Kumar Singh², Narendra Pal Singh³

¹Assistant Professor, Department of General Medicine, Late Baliram Kashyap Memorial Government Medical College, Jagdalpur, Chhattisgarh.
²Assistant Professor, Department of Microbiology, Late Baliram Kashyap Memorial Government Medical College, Jagdalpur, Chhattisgarh.
³Professor, Department of Microbiology, University College of Medical Sciences, New Dehli.

ABSTRACT
BACKGROUND
In the present scenario due to indiscriminate use of many antibiotics including vancomycin Staphylococcus aureus (S. aureus) demonstrates a reduced susceptibility to it. Reduced susceptibility to vancomycin is one of the bigger problems in the treatment of infections caused by S. aureus.

METHODS
To detect reduced susceptibility for vancomycin, vancomycin disk diffusion by Kirby-Bauer disk diffusion method, vancomycin agar screen by Hiramatsu et al (4 µg/ml of vancomycin) and CDC/CLSI (6 µg/ml of vancomycin) method and vancomycin minimum inhibitory concentration (MIC) were performed. MIC’s were determined by broth microdilution as well as ETEST. Simplified Population Analysis Profile (SPAP) was also performed for confirmation of h-VISA isolates.

RESULTS
MIC’s of 165 isolates was ≤2µg/ml by ETEST while 35 isolates had MIC’s >2µg/ml. Among these 35 isolates, two isolates had MIC of 4µg/ml and one had MIC of 6µg/ml and therefore, these three isolates may have been VISA as per CLSI guidelines. Using broth microdilution, all the isolates had MIC’s of ≤2µg/ml. Vancomycin agar screen was performed by cut using method followed by Hiramatsu et al (4 µg/ml of vancomycin) and CDC/CLSI (6µg/ml of vancomycin) method. By the former method, four isolates demonstrated growth at 4 µg/ml and 196 isolates had no growth. These four isolates were further confirmed by Simplified Population Analysis Profile (SPAP) and were confirmed as h-VISA (Heterogeneous – Vancomycin Intermediate Staphylococcus aureus) isolates with MIC’s 8 µg/ml. Using CDC/CLSI method, all 200 isolates did not grow at 6 µg/ml of vancomycin.

CONCLUSIONS
Detection of hVISA hetero-resistance phenotype is very difficult. The best method for detecting hVISA is controversial. Population Analysis Profile (PAP) is considered as the gold standard, but this method is time consuming, expensive and labour intensive. So, it can’t be routinely used in a large busy setup.

HOW TO CITE THIS ARTICLE: Kumar S, Singh AK, Singh NP. Vancomycin susceptibility status among clinical MRSA isolates and detection of VISA, hVISA in a tertiary care hospital. J. Evid. Based Med. Healthc. 2019; 6(14), 1150-1153. DOI: 10.18410/jebmh/2019/240

BACKGROUND
S. aureus is one of the most common causes of nosocomial and community acquired infections. It is also the most common cause of surgical wound infections and nosocomial bloodstream infection.¹ S. aureus causes infections of the skin and soft tissue, musculoskeletal, respiratory, central nervous system, endovascular, urinary tract and toxin mediated syndromes like food poisoning, staphylococcal scalded skin syndrome, and toxic shock syndromes.²

1. Beta lactam antibiotic was the drugs of choice for the treatment of infections caused by this organism. Later in the mid 1940s, penicillinase producing S. aureus were detected and by 1948 majority of S. aureus were already resistant to penicillin. In 1960 after resistant to penicillin there are development of penicillinase resistant penicillins like methicillin, oxacillin and nafillin. Within a year methicillin resistant S. aureus (MRSA) was reported from Europe. Over next 10 years increasing numbers of isolates and outbreaks were reported from many European countries and some parts of Asia. Most of the MRSA isolates are multi drug resistant (MDR) and are susceptible to glycopeptide antibiotics only.³

2. Vancomycin is the treatment of choice for serious infections caused by MRSA, but due to increase in MRSA infections and widespread use of vancomycin, strains with reduced susceptibility to this drug have been emerged.⁴ According to Clinical and Laboratory Standards Institute (CLSI) defined breakpoints, most of these strains have
vancomycin MIC within the susceptible range but some studies have reported a generalised increase in vancomycin MIC over a time period, this is known as "MIC creep".5

Since 1997, MRSA strains with intermediate susceptibility to vancomycin (VISA) have been reported from Japan, France, United States, Korea and Germany. These strains were recovered from patients who failed therapy with vancomycin for prolonged periods of time. Other strains, named hetero-VISA, are borderline susceptible to vancomycin but exhibit low-level subpopulations (10^6 cells) able to grow at vancomycin concentrations of 4-8 μg/ml. These strains have been described in Europe, Asia and Brazil. Hetero-VISA strains may be first-step mutants that are precursors of VISA strains in a patient receiving prolonged courses of vancomycin treatment.6

METHODS
A total of 200 MRSA strains were used in this study. These strains were collected in November 2011-December 2012 from patients admitted to Guru Teg Bahadur Hospital, New Delhi. Clinical specimens includes blood (n=70, 35%), pus (n=16, 8%), sputum (n=10, 5%), swab (n=44, 22%), and urine (n=60, 30%). All the specimens were inoculated on blood agar and MacConkey agar plates and incubated at 37°C for 18-24 hours and identified as S. aureus by colony characteristics, Gram’s staining, catalase test, slide coagulase test and Voges Proskauer test and confirmed by tube coagulase test, Growth on Mannitol Salt Agar and Modified Hugh and Leifson Oxidative/Fermentative Test. Antibiotic sensitivity and resistance pattern of S. aureus were performed by Kirby Bauer disk diffusion method. Screening of methicillin resistance was done by cefoxitin 30 μg disk. Screening of vancomycin resistance was done by vancomycin 30μg disk on Mueller-Hinton Agar (MHA) plate by Kirby- Bauer disk diffusion method and by inoculating isolated S. aureus strains over brain heart infusion agar screen containing 4 μg/ml (Hiramatsu Method) and 6 μg/ml (CDC/CLSI Method) of vancomycin (Figure 1). hVISA were detected by Simplified Population Analysis Profile, (SPAP). Growth after 48 hours from brain heart infusion agar screen containing 4μg/ml of vancomycin was sub-cultured, and from sub-clones, MIC’s were determined again by broth microdilution and if it is ≥8μg/ml then it is considered as a confirmed hVISA.7 VISA were detected by determining MIC’s by ETEST (Fig.2) and if it is 4-8μg/ml then it is considered as VISA according to CLSI.8

RESULTS
Definition of VISA, VRSA and hVISA
Vancomycin Resistance According to CLSI
By disk diffusion vancomycin 30 μg disk will show no zone of inhibition around the disk (zone = 6 mm). But it is not reliable, and it should be confirmed by MIC determination according to CLSI guidelines.

MIC’s of Vancomycin
According to CLSI guidelines 2011 the staphylococci with MIC of vancomycin ≤ 2 μg/ml is susceptible, 4-8 μg/ml is intermediate and ≥ 16 μg/ml is resistant.

hVISA
The hVISA are the subpopulations of vancomycin intermediate S. aureus (VISA) at a rate of 1 organism per 10^6-10^7 that can grow in the presence of ≥ 4 μg/ml of vancomycin.

All the isolates (100%) were resistant to cefoxitin and were considered MRSA. Vancomycin disk diffusion though not validated by CLSI, was used to detect VRSA only all the isolates were sensitive to it. Four isolates demonstrated growth at 4μg/ml vancomycin agar screen by Hiramatsu method and 196 isolates did not grow. By CDC/CLSI, with 6μg/ml vancomycin, no growth was observed for any of the isolates (Table 1).

<table>
<thead>
<tr>
<th>No. of Isolates</th>
<th>Hiramatsu Method 4 μg/ml Vancomycin</th>
<th>CDC/CLSI Method 6 μg/ml Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Isolates n (200)</td>
<td>+ve (4)</td>
<td>+ve (0)</td>
</tr>
<tr>
<td>-ve (196)</td>
<td>-ve (200)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Number of Isolates with Vancomycin Agar Screen by Two Different Methods

By simplified population analysis profile the sub-clones from these four isolates were grown on MHA. Five random colonies each from them were selected and MIC’s were determined by broth microdilution method. All the 4 isolates had sub-clones with MIC of vancomycin at 8 μg/ml (Table
DISCUSSION
Vancomycin has been the most reliable therapeutic agent against MRSA for the past three decades. Widespread empirical use of vancomycin to cover Gram-positive organisms, including MRSA, has contributed to the increasing burden of less susceptible strains, and many health care facilities have reported an upward trend of vancomycin MICs for MRSA isolates over the last 5 years.10 Vancomycin resistance can be difficult to detect in clinical microbiology laboratory. Disk diffusion sensitivity testing by standard 30µg vancomycin frequently misclassifies immediately susceptible isolates as fully susceptible. Presently MIC determinations by broth or agar dilution or by ETEST are the gold standard for determining vancomycin susceptibility11 but financial logistics do not allow routine use of these methods in clinical diagnostic laboratories. Detection of VISA is possible with standard laboratory methods but the detection of hVISA remains difficult. Currently, no standardized method exists for identifying hVISA. Population analysis profiling (PAP) has been proposed as the most precise method of determining heteroresistance, but this method is laborious, time-consuming and impractical for use in routine laboratories. It requires at least 10^6 CFU/well for detection of hVISA whereas 10^4 CFU/well is the required inoculum for the standard MIC method by broth microdilution.

In this study 24 (12%) isolates were recovered from ICUs and their history suggested that all of them had prolonged stay in the hospital and were on broad spectrum antibiotics. In the present study the results of vancomycin MICs by broth microdilution method suggest 100% sensitivity to vancomycin. Two studies from South India have also reported 100% sensitivity to vancomycin. However, they have used either disk diffusion or agar dilution methods.12,13

Another study from India also shows 100% sensitivity to vancomycin by disk diffusion method but by the agar dilution method they had detected 3 VISA isolates with MIC of 8µg/ml.14 So for vancomycin susceptibility testing we can’t rely totally on disk diffusion method. MIC determination by dilution or ETEST is more important and reliable for vancomycin susceptibility testing.

By the ETEST MIC determination method 33 isolates (16.5%) had vancomycin MIC of < 2 µg/ml (range 0.5-1.5µg/ml), 132 isolates (66%) had MIC of 2µg/ml, and 35 isolates (17.5%) had MIC of >2 µg/ml (Table 5). Out of 35 isolates which had MICs of >2µg/ml 31 had MIC 3µg/ml, two had MIC 4µg/ml and one each isolate had MICs of 2.5µg/ml and 6µg/ml respectively. Since ETEST method detects the serial dilution of MICs, so there is less chance of missing the intervening MICs. Using ETEST method three isolates may be labelled as VISA as among these two isolates had MICs 4µg/ml and one isolate had MIC 6µg/ml. However it was presumed in the study that other thirty two strains having MICs between 2 and 3µg/ml may be potential hVISA isolates.

It was observed that out of 200 isolates 169 had MICs of 2µg/ml by broth microdilution; however, by ETEST only 132 isolates had MIC’s of 2µg/ml. There were 35 isolates with MICs >2 µg/ml by ETEST (Table 5).

In this study 4 strains were grown on BHIA screen by method by Hiramatsu et al. at 4µg/ml vancomycin at 48 hours and no growth was observed by the CDC/CLSI method that contained 6µg/ml of vancomycin at 24–48 hours. These four strains had sub-clones with MICs for vancomycin 8µg/ml and were designated as “confirmed hVISA”.

The prevalence of hVISA has been reported worldwide. In France (0.76%), Australia (9.4%), United States (0.3-2.3%), and several Asian countries including Japan (1.3-20%), India (6.3%), South Korea (6.1%), and Singapore (2.3%). In recent study in China the prevalence of hVISA for fourteen cities was 13-16%.15

---

### Table 2. Vancomycin MICs (SPAP) Subclone Analysis

<table>
<thead>
<tr>
<th>No. of Isolates</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

---

### Table 3. Vancomycin MICs by ETEST Method

<table>
<thead>
<tr>
<th>Antimicrobial Concentration (µg/ml)</th>
<th>&lt;2 (Range 0.5-1.5)</th>
<th>2</th>
<th>&gt;2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Isolates in Vancomycin</td>
<td>33</td>
<td>132</td>
<td>35</td>
<td>200</td>
</tr>
</tbody>
</table>

---

### Table 4. No. of Isolates with Vancomycin MICs <2 µg/ml and >2 µg/ml by ETEST

<table>
<thead>
<tr>
<th>Antimicrobial Concentration (µg/ml)</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>0.75</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>1.5</td>
<td>20</td>
</tr>
<tr>
<td>2.5</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

---

### Table 5. Comparison of The Two Different Methods of MICs Determination for Vancomycin

<table>
<thead>
<tr>
<th>MICs µg/ml</th>
<th>&lt;2</th>
<th>&gt;2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Isolates by ETEST</td>
<td>33</td>
<td>132</td>
</tr>
<tr>
<td>No. of Isolates by Broth Microdilution</td>
<td>31</td>
<td>169</td>
</tr>
</tbody>
</table>
In our study four (2\%) isolates were confirmed hvISA. From above mentioned studies it is evident that prevalence of hvISA varies from place to place. In a study by Bhateja et al. using the method of Hiramatsu as many as 23 strains grew on 4μg/ml vancomycin plates and seven amongst them were positive after 48 hours of incubation. These strains would be considered as hetero VISA (hvISA).\textsuperscript{16} However they had not further validated these strains as “confirmed hvISA” as there is no mention of presence of sub-clones with vancomycin MICs of ≥ 8μg/ml.

**CONCLUSIONS**

Although all the isolates were sensitive to vancomycin by disk diffusion, disk diffusion can’t detect the intervening sensitivity. Vancomycin intermediates were detected by ETESQ and by agar screen methods by determining MICs. Detection of VISA is possible with standard methods, but the detection of hvISA is very difficult. Population analysis profile was used to detect the hvISA, but it is very time consuming and labour intensive. So, it can’t be routinely used in a large busy hospital setup. By molecular and genetic testing, the hvISA can be detected more accurately and easily.

**REFERENCES**


