MODIFIED PAP METHOD TO DETECT HETERORESONANCE TO VANCOMYCIN AMONG METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES AT A TERTIARY CARE HOSPITAL

*RN Iyer, V Hittinahalli

Abstract

This study was an attempt at developing, establishing, validating and comparing the modified PAP method for detection of hetero-vancomycin resistant Staphylococcus aureus (h-VRSA) with the routine antimicrobial susceptibility testing (using the BSAC standardized disc diffusion method), minimum inhibitory concentrations of vancomycin using standard E-test methodology and the Hiramatsu’s screening method. A total of 50 methicillin resistant Staphylococcus aureus obtained from various clinical specimens, along with the Mu 3 and Mu 50 strains as controls, were studied. No VRSA isolates were obtained. However, four of the test strains were positive by the Hiramatsu’s screening method, of which only one isolate could be confirmed by the modified PAP analysis method. This isolate was a coloniser from the drain fluid of a liver transplant recipient. The sensitivity, specificity, positive predictive value and the overall efficiency of the Hiramatsu’s screening method with the modified PAP analysis as the gold standard were found to be 100, 93.8, 25 and 94%, respectively. It is very essential for clinical laboratories to screen for h-VRSA, given the increasing use of glycopeptide antibiotics in therapy and the potential for failed therapy in patients infected with these strains.

Key words: h-VRSA, Hiramatsu’s method, modified PAP, VRSA, Methicillin resistant staphylococcus aureus

Methicillin resistant Staphylococcus aureus (MRSA) is a well-recognized problem pathogen all over the world, both in the nosocomial as well as in the community setting. Recognition of MRSA isolates assumed importance as glycopeptides were the mainstay of treatment of infections caused by these organisms. Predictably, resistance to vancomycin emerged in MRSA in 1996[1] and in coagulase negative Staphylococci in 1987. The subsequent years have seen more reports of heteroresistance to vancomycin among S. aureus isolates and reports from around the world indicate that 0.5-20% of the MRSA isolates may be heteroresistant to vancomycin.[2]

Vancomycin resistance in S. aureus may be of two types; namely, Vancomycin resistant S. aureus (VRSA) and Vancomycin heteroresistant S. aureus (h-VRSA). Mu 50 and Mu 3 were the two isolates reported from Japan[3] with vancomycin MICs of ≥8 mg/L and 2-4 mg/L respectively. Discrepancies in MIC breakpoints to vancomycin for S. aureus in the UK and the USA[4] have left the definition and classification of h-VRSA in different parts of the world unclear. Nevertheless, it is important to detect VRSA and h-VRSA in the clinical laboratory as both the aforementioned phenotypes have been associated with treatment failure with glycopeptides in deep seated infections.[5]

The BSAC (British Society for Antimicrobial Chemotherapy) standardized method for disc diffusion testing[6] has been used in our laboratory to evaluate vancomycin susceptibility in our MRSA isolates. This is supplemented with the MIC testing using the E-test methodology. It is a known fact that the VRSA strain Mu 50 reported from Japan exhibits susceptible results on disc diffusion testing. A screening method developed by Hiramatsu et al.[7] appeared to show promise a few years ago, but was found to yield both false positives and false negative results.[4,8] There is a need for the modern day clinical laboratory to develop a definitive method to confirm heteroresistance to vancomycin among MRSA isolates as this could direct antibiotic therapy. The chief options in this direction are modified PAP method, gradient plates and the addition of Mu 3 cell wall material to media. Hence this study is an attempt at developing, standardizing and comparing modified PAP with the existing methodologies in our laboratory to detect heteroresistance to vancomycin in MRSA isolates.

Materials and Methods

Fifty isolates of MRSA obtained from various clinical specimens in our laboratory over a period from 1st January 2003-1st January 2004 were used in the study. Mu 50 and Mu 3 were obtained from Dr. Hiramatsu’s laboratory (Juntendo University, Japan) and were used as positive control strains.
isolates of S. aureus were identified as MRSA using standard biochemical reactions and the antimicrobial susceptibility testing was performed using the BSAC standardized disc diffusion methodology. MIC determinations were performed using standard E-test procedures. Isosensi test agar plates (ISA Oxoid Basingstoke, UK) were inoculated with a 0.5-ml McFarland standard suspension of the test organisms and the vancomycin E-test strips were placed on the inoculated plates according to the manufacturer’s instructions. Plates were incubated at 37°C for 24 hours before a reading was taken.

A screen for h-VRSA was performed according to the method described by Hiramatsu et al. Briefly, this was performed by inoculating a 10 μL of a 0.5 McFarland standard broth on BHI agar plates with 6 mg/L of vancomycin (Sigma Aldrich Chemicals). The plates were incubated at 37°C for 24-48 hours. Growth at the end of 24 hours denoted a VRSA strain while growth at the end of 48 hours denoted a h-RSA strain.

The 50 test strains were inoculated in batches of 10 isolates in each with Mu 50, Mu 3 strains and the Oxford S. aureus NCTC 6571 was included as a negative control in each batch.

The modified PAP method was performed as described by Wootton et al. Overnight broth cultures of organisms were diluted to obtain 10⁻³ and 10⁻⁶ and this was manually spiral plated on BHI agar plates containing vancomycin at concentrations of 0.25 μg/mL, 0.5 μg/mL, 1 μg/mL, 1.5 μg/mL, 2 μg/mL, 4 μg/mL, 6 μg/mL and 8 μg/mL. Colonies were counted at the end of 48 hours incubation at 37°C and a viable count was plotted against the vancomycin concentration on a logarithmic paper, as depicted in the figure. This was used to calculate the area under the curve (AUC). A ratio of the AUC of the test MRSA strain to that of the Mu 3 strain was obtained to distinguish between VRSA, h-VRSA and vancomycin susceptible MRSA isolates. A ratio of ≥0.9 was used to diagnose a h-VRSA isolate. All isolates were tested in batches of 10, with the Mu 3 strain used as a positive control. An attempt was made to work out the sensitivity, specificity, positive predictive value and the overall efficiency of the Hiramatsu’s screening method using the PAP analysis as the gold standard.

Results

All MRSA isolates were found susceptible to vancomycin by the disc diffusion methodology and the MIC testing using the E-test methodology. Twenty four isolates showed a MIC of 2-4 μg/mL. Twelve isolates showed a MIC of 1.5 μg/mL, three isolates had a MIC of 1 μg/mL while five isolates each had a MIC of 0.38 and 0.75 μg/mL respectively. One isolate had a MIC of 0.016 μg/mL. Four of the 50 isolates showed growth of colonies at the end of 48 hours using the Hiramatsu’s screening method while none of the isolates showed any growth at the end of 24 hours incubation at 37°C. This suggests that there were four possible h-VRSA and no VRSA isolates detected by this method. The Mu 50 strain could be grown on all the tests conducted on the strain at the end of 24 hours, while the Mu 3 strain could be grown in only 90% of the tests conducted. This strain showed a faint growth or no growth in the remaining 10% of the tests conducted. The average AUC value of the Mu 50 strain tested on eight occasions was 22.24 ± 0.60, while the average AUC value of the Mu 3 strain tested on 12 occasions was 21 ± 0.22, suggesting a good reproducibility. One isolate of MRSA showed an AUC ratio of 1.1 (≥0.9) and hence denoted a h-VRSA. This isolate was repeatedly tested on three occasions and was found to be reproducible in its AUC ratio. This isolate was obtained from the drain cultures of a liver transplant surgery.

Table: Isolates identified as VRSA and h-VRSA from MRSA isolates using different methods (n = 50)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Disc diffusion</th>
<th>MIC E-test</th>
<th>Screening (Hiramatsu et al.)</th>
<th>Modified PAP analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRSA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>hVRSA</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure: AUC for the Mu3 strain

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recipient. The distribution of 50 MRSA isolates showing VRSA and h-VRSA is shown in the table. Using the modified PAP analysis as a gold standard, the sensitivity, specificity, positive predictive value and the overall efficiency of the Hiramatsu’s screening method were found to be 100, 93.8, 25 and 94% respectively.

Discussion

It has become necessary for all clinical laboratories to establish and validate methods to detect h-VRSA. Whilst VRSA and h-VRSA have not become a prevalent problem as yet, it is essential to be able to detect this phenotype and institute appropriate infection control measures to prevent the dissemination of such strains in a tertiary care facility. Different methods have been proposed to detect VRSA and h-VRSA with varying degrees of sensitivity and specificity. Disc diffusion methodology as well as the standard MIC methods may be able to detect VRSA phenotypes that express homogenous vancomycin resistance. However, we did not find any VRSA isolates in our study and the same has been the experience in other studies. As is evident from the table, four of the 50 isolates tested positive by the Hiramatsu’s screening method for h-VRSA. However, only one out of four isolates had a AUC ratio of 1:1 and hence was diagnosed to be a h-VRSA. This isolate was clinically found to be a coloniser of the abdominal drain in a liver transplant recipient. Although a coloniser without any blood stream dissemination, this finding could be significant in immunosuppressed patients as these isolates do have a potential for bacteremia and sepsis. The screening method as described by Hiramatsu is not labour intensive, but has been an inappropriate method to detect h-VRSA due to the false positive and false negative results. We also found three isolates to exhibit false positive results in this method. Caution should therefore be exercised before accepting the results of the screening method. It would be a good practice to confirm the results with a gold standard method such as the modified PAP method.

Our laboratory has set up the modified PAP method to confirm h-RSA isolates which are initially suspected to exhibit resistance to vancomycin on the basis of the Hiramatsu’s screening method. The modified PAP method is labour intensive and requires technical expertise and manual dexterity. Hence it may not be suitable as a screening method for all MRSA isolates. However, it is the only reliable method to confirm the heterogeneity of vancomycin susceptibility.

The results of all epidemiological studies on detection of h-VRSA should employ modified PAP method as a confirmatory test for the same, and this has been suggested by other studies too. Population methods in the past have been criticised, both for their labour intensiveness and the possibility that they may select for rather than detect vancomycin resistance. However, the development of PAP - AUC ratios have demonstrated this to be a method to detect and not select for h-VRSA strains, thus circumventing the high number of false positives found with other population methods.

The emergence of h-VRSA is clearly a cause for concern, as dissemination of these strains among hospital patients could lead to an outbreak and pose infection control problems associated with it. Unfortunately, these strains often go undetected as the more conventional methods of antimicrobial susceptibility testing are incapable of detecting them. They are also expressed at a low frequency. Moreover, they are also capable of transformation into the homogenously resistant strains and adhering to artificial surfaces, 20-fold higher than the usually encountered MRSA isolates. This certainly has implications in the management of infections caused by these strains in the nosocomial setting. It is essential for clinical laboratories to screen for and confirm vancomycin resistance in the clinical laboratory.

References


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