Heterogeneous Vancomycin Intermediate Resistance within Methicillin-Resistant Staphylococcus Aureus Clinical Isolates in Alexandria Province

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Glycopeptides such as vancomycin are frequently the antibiotics of choice for the treatment of infections caused by methicillin resistant Staphylococcus aureus (MRSA). Since vancomycin-intermediate Staphylococcus aureus (VISA) was first reported in Japan in 1997, there has been great concern that heterogeneous vancomycin-intermediate Staphylococcus aureus (hVISA) is the putative precursor of VISA. The aim of this study was to explore the prevalence of VISA and hVISA among MRSA strains isolated from hospitalized patients in Alexandria University Hospital over a 2 years period, and to investigate their clinical significance and mechanism of vancomycin resistance. Sixty two MRSA isolates were screened by using brain heart infusion agar supplemented with 4 µg/ml vancomycin (BHI-V4) and macro E test. Minimum inhibitory concentrations (MICs) of vancomycin were determined by broth microdilution and standard E test. Population analysis profile (PAP) was performed for detecting the frequency of heterogeneous resistance for subpopulations with MIC of vancomycin of > 4 µg/ml at frequency of 1 in 10⁶ CFU/ml or higher. Vancomycin resistant subpopulations of hVISA were viewed with scanning electron microscopy, and tested for the presence of van A gene by PCR. Twenty one (33.87 %) MRSA isolates grew on BHI-V4, 7 (11.29 %) isolates were suspected of having reduced susceptibility to vancomycin by macro E test. The PAP confirmed 6 (9.68 %) isolates as hVISA since they produced heterogeneous vancomycin MIC values for all isolates were ≤ 4 µg/ml. The clinical outcome of hVISA infection was variable as the hemodialysis patient improved, while death occurred in the two patients from the ICU, and the three diabetic patients underwent variable degrees of amputations. Scanning electron micrographs of vancomycin intermediate resistant subpopulations of hVISA showed enhanced cell wall thickness with evidence of increased extra-cellular material and irregular shape compared to vancomycin susceptible cells. All hVISA isolates were van A gene negative by PCR. It was concluded that this study is an early warning that MRSA strains with full resistance to vancomycin might emerge in Egypt in the future. Due to the increased use of vancomycin for treatment of MRSA infections, screening for hVISA in MRSA strains should be considered as a necessary part of infection control practice emphasizing the importance of a laboratory capability of its identification.

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) has emerged as a hospital-acquired pathogen of major world importance (¹), and is an increasingly frequent cause of community-acquired infections. (²) Because vancomycin is first-line therapy for serious MRSA infections, an increase in the incidence of both hospital-acquired and community-acquired infections may lead to a substantial increase in the rate of vancomycin usage. As a consequence of the selective pressure of vancomycin use, the first strain of MRSA with reduced susceptibility to vancomycin, strain Mu50 with a minimum inhibitory concentration (MIC) = 8 µg/ml, was reported from Japan in 1997. (³)

The Clinical Laboratory Standard Institute (CLSI, formerly NCCLS) guidelines define Staphylococcus aureus (S. aureus) for which the MIC of vancomycin is ≤ 4 µg/ml to be susceptible (VSSA), while isolates for which the MIC is 8 to 16 µg/ml are intermediate (VISA), and those for which the MIC is ≥ 32 µg/ml are resistant (VRSA). (⁴, ⁵) VISA isolates have been reported from the United States, Europe, and the Far East. (⁶, ⁷) Moreover, to date, four VRSA isolates have been identified in the USA. (⁸ - ¹¹)

Although the incidence of VISA or VRSA remains low, there is an increasing number of reports from many countries of strains of S. aureus showing heterogeneous-intermediate resistance to vancomycin (hVISA) (¹² - ¹⁵) since the first report of the prototype strain Mu3 by Hiramatsu et al 1997. (¹⁶) Generally, hVISA strains are defined as strains of S. aureus that contain subpopulations at a frequency of 10⁻⁶ or higher of vancomycin-intermediate daughter cells but for which the MICs of vancomycin for the parent strain are ≤ 4 µg/ml. (¹⁶, ¹⁷) Although the criteria for identifying hVISA strains have not been standardized, complicating any determination of their clinical significance and role in treatment failure, many reports suggest that hVISA is responsible for the failure of vancomycin therapy (¹², ¹⁸, ¹⁹), and a potential source of emerging VISA subclones in patients given vancomycin for a prolonged period. (¹⁷, ²⁰)

Vancomycin exerts its antimicrobial effects by inhibiting the synthesis of the S. aureus cell wall through irreversible binding to the terminal D-alanyl-D-alanine of bacterial cell wall precursors. (¹⁷) Two mechanisms of S. aureus resistance to vancomycin have now been identified. The first mechanism suggests that VISA strains synthesize additional quantities of peptidoglycan with an increased number of D-
alanyl-D-alanine residues that bind vancomycin molecules and effectively sequester them, thereby prevent them from reaching their bacterial target. (7, 21) The second mechanism supports the exchange of genetic material among VRSA strains. Acquisition of van A gene from vancomycin-resistant Enterococcus faecalis allows the synthesis of a cell wall precursor that ends in D-alanyl-D-lactate which has dramatically reduced affinity for vancomycin. (21 - 23)

In this study, we screened all isolates of MRSA from clinical specimens recovered from hospitalized patients in Alexandria, Egypt for the detection of VISA and hVISA to investigate their prevalence, clinical significance and mechanism of vancomycin resistance.

MATERIALS AND METHODS

Sixty two isolates of MRSA recovered from a total of 156 (39.74 %) S. aureus isolates collected from hemodialysis unit, intensive care units (ICUs), and diabetic foot clinic in the Main Alexandria University Hospital during the period from January 2004 to December 2005 were screened for VISA and hVISA. Only single isolate was taken from each patient.

In the hemodialysis unit, polyurethane, uncoated subclavian or internal jugular double-lumen catheters for chronic intermittent hemodialysis in chronic renal failure patients were interviewed. All catheters were withdrawn at least 2 weeks after insertion. At catheter withdrawal, catheter tip and peripheral blood cultures were performed and the colonization and catheter-related bloodstream infection were assessed. In the diabetic foot clinic, consecutively attending diabetic patients with diabetic foot ulceration and / or gangrene were recruited. Immediately after hospitalization, specimens like pus and wound exudate were cultured. Also, in one medical and three surgical ICUs, patients were followed for the detection of MRSA colonization using samples from wounds after recent surgical intervention, from sputum, respiratory aspirate, or blood from patients with pneumonia whether ventilator-associated or not, and from insertion site or blood stream in catheter-related infections patients. The distribution of isolates according to their source was shown in table I.

Identification of MRSA isolates:

Staphylococcus aureus isolates were identified by the conventional bacteriological techniques including colony morphology, Gram staining films, catalase and tube coagulase production, and mannitol fermentation. (24)

All S. aureus isolates were screened for oxacillin resistance by oxacillin agar screen method. Mueller-Hinton (MH) agar (Oxoid, Unipath LTD, Hampshire, England) plates supplemented with 4 % NaCl and oxacillin 6 µg/ml were inoculated with 0.5 McFarland broth cultures of the organisms. Any growth after incubation for 24 hours at 35 ºC was interpreted as a positive oxacillin agar screen result for MRSA. (25)

Vancomycin susceptibility tests:

1- Vancomycin agar screening method:

All isolates were screened by in-house prepared brain heart infusion (BHI) agar (BBL, Becton Dickinson, USA) containing 4 µg/ml vancomycin (BHI-V4). Briefly, overnight cultures in BHI broth were adjusted to 0.5 McFarland turbidity (about 10⁸ CFU/ml) and 10 µl of cell suspension was inoculated onto BHI-V4 plate. The plates were incubated at 37 ºC for 48 hours. If cell growth was not apparent within 48 hours, the isolate was considered susceptible to vancomycin. If confluent growth was seen within 48 hours, the isolate was considered potentially VISA. If a countable number (1 – 30) of colonies were apparent within 48 hours, the isolate was designated as possibly hVISA. (16)

All isolates that showed growth on BHI-V4 were selected for vancomycin MIC determination by broth microdilution method and E test. Population analysis profile (PAP) was performed for detecting the frequency of heterogeneous resistance.

2- Broth microdilution method:

Broth microdilution plates were prepared with cation-adjusted MH broth containing serial dilutions of vancomycin. Plates were inoculated with 0.5 McFarland isolate suspension, and incubated for a full 24 hours at 35 ºC. The MICs were read as the lowest concentration at which there was no visible growth. (26)

3- E test:

Vancomycin MICs were determined by two E test protocols (AB Biodisk, Solna, Sweden). In one protocol, the macro E test, the strips for vancomycin (0.016 - 256 µg/ml) were deposited onto BHI agar surface which had been overlaid with isolate suspension grown in BHI broth and calibrated as 2 McFarland. Plates were incubated at 35 ºC for 48 hours.

In the second protocol, standard E test, BHI agar and broth were replaced by MH agar and broth, isolate suspension was adjusted to 0.5 McFarland and results were read after 24 hours incubation at 35 ºC. The MICs were interpreted according to CLSI breakpoints. (27)

4- Disk diffusion method:

Isolates were tested for vancomycin susceptibility using 30 µg vancomycin disks. A zone size ≥ 12 mm indicates susceptibility and a zone size ≤ 9 mm indicates resistance. (28)

Population analysis profile:

This method was used for detecting staphylococci subpopulations intermediate or resistant to vancomycin. For this purpose, the
parents of the selected colonies on BHI-V4 were used. A 100 μl of the starting cell suspension at 0.5 McFarland turbidity and serial 10-fold dilutions of this suspension in BHI broth were spread over BHI agar plates containing vancomycin at concentrations ranging from 1 - 10 μg/ml. After incubation at 37 °C for 48 hours, the numbers of viable cells contained in 100 μl of the starting cell suspension growing on the different concentrations of vancomycin were calculated and plotted on a semi-logarithmic scale. Any screen-positive strain that contained subpopulations with MIC > 4 μg/ml for vancomycin at a frequency of 1 in 10^6 CFU/ml or higher was defined as hVISA.  

**Amplification of van A gene by PCR:**

Bacterial DNA was extracted using Bacterial Genomic DNA Mini-prep Kit (V-Gene Biotechnology Limited) according to the manufacturer instructions. The PCR detection of enterococcal van A gene was carried out with DNA extracted from each strain as a template and with the primers (van A F 5'-CATGAATAGAAATAAGTTGCAATA-3' and van A R 5'-CCCCTTTAACGCTAATACGACGATCAA-3'). The DNA amplification was performed in Hybaid thermal cycler and consisted of the following: one cycle of initial denaturation at 94 °C for 10 minutes; 30 cycles with a 30-s denaturation step at 94°C, a 45-s annealing step at 50 °C and a 30-s extension step at 72°C, with a final extension step at 72 °C for 10 minutes and a holding step at 4 °C until the sample was analyzed. The resulting fragments were electrophoresed in a 1.5 % agarose gel, stained with 10 μM ethidium bromide, and visualized under UV light.  

**Scanning electron microscopy:**

Standard scanning electron microscopical techniques (20) were used to fix, embed, and stain the organisms, which were then observed with a Jeol 100 CX (Tokyo, Japan) scanning electron microscope. Evaluation of cell wall thickness was performed by using photographic images at a final magnification of X 50,000.  

**RESULTS**

In the hemodialysis unit, S. aureus positivity was detected in 31 chronic hemodialysis patients, of whom 19 (61.29 %) were infected with MRSA and 12 (38.71 %) were infected with methicillin-sensitive S. aureus (MSSA) (table I).

In diabetic feet lesions, S. aureus was cultured from 79 patients. The MRSA strains were isolated from 29 patients and represented 36.71 % of all S. aureus isolates (table I). Demographic information revealed that patients were 62.07 % (18/29) males, 86.21 % (25/29) with type II diabetes, diabetes duration was 14.3 ± 3.6 years, and HbA1c 8.5 ± 2.3 %. Ulcers were neuropathic in 55.17 % (16/29), ischaemic in 13.79 % (4/29), and neuroischaemic in 31.04 % (9/29). Wound healing in diabetic feet infection caused by MRSA showed that healing occurred with conservative treatment in 3 patients (10.34 %), while minor- and major-amputation were encountered in 17 (58.62 %) and 7 (24.14 %) patients respectively, whereas deaths occurred in 2 (6.90 %) patients.  

In the ICUs, S. aureus was isolated from 46 patients. In 14 (30.43%) patients, infections due to MRSA were diagnosed (table I). Mortality in patients with MRSA infection was 35.71% (5/14) compared with 31.25% (10/32) in patients with MSSA.

Out of the 62 clinical MRSA isolates (from 62 patients) included in this study, 21 (33.87 %) isolates grew countable number (1 – 30) of colonies on BHI-V4 screening plates within 48 hours. Screening of these 21 isolates by heavy inoculum (2 McFarland) macro E test with extended incubation time (48 hours) and using BHI agar, 7 (11.29 %) isolates showed reduced susceptibilities to vancomycin (MIC 6 – 12 μg/ml). Table I shows the distribution of these isolates according to the type of specimen. Two isolates were from blood culture specimens from chronic hemodialysis patients, three isolates were from diabetic feet infection patients, and two isolates were from endotracheal aspirates of patients with ventilator associated pneumonia in the ICU. Meanwhile, by E test with a standard inoculum on MH agar for 24 hours and by broth microdilution method, all the 21 screened positive isolates were susceptible (MIC ≤ 4 μg/ml). By disk diffusion method, the zones of inhibition for all the study isolates were ≥ 12 μg/ml (CLSI interpretation, susceptible) (table II).

The PAP method confirmed 6 (85.71 %) isolates out of the 7 isolates suspected of having reduced susceptibility to vancomycin by macro E test as hVISA, since they produced subpopulations with MICs of vancomycin > 4 μg/ml (5 - 7 μg/ml). Table I shows the distribution of these isolates according to the type of specimen. Two isolates were from blood culture specimens from chronic hemodialysis patients, three isolates were from diabetic feet infection patients, and two isolates were from endotracheal aspirates of patients with ventilator associated pneumonia in the ICU.
material and irregular shape in contrast to the thin and smooth cell wall morphology in vancomycin susceptible cells (figure 2).

**Table I:** Prevalence of hVISA among MRSA isolates from different specimens according to the screening method.

<table>
<thead>
<tr>
<th>Type of specimen</th>
<th>S. aureus isolates</th>
<th>MRSA isolates</th>
<th>hVISA screening test</th>
<th>Population analysis profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Hemodialysis patients</td>
<td>31</td>
<td>19</td>
<td>61.29</td>
<td>6</td>
</tr>
<tr>
<td>Diabetic feet infection</td>
<td>79</td>
<td>29</td>
<td>36.71</td>
<td>10</td>
</tr>
<tr>
<td>ICU</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Pneumonia</td>
<td>46</td>
<td>14</td>
<td>30.43</td>
<td>5</td>
</tr>
<tr>
<td>*Catheter-related</td>
<td>26</td>
<td>10</td>
<td>38.46</td>
<td>4</td>
</tr>
<tr>
<td>*Surgical wound</td>
<td>12</td>
<td>3</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>156</td>
<td>62</td>
<td>39.74</td>
<td>21</td>
</tr>
</tbody>
</table>

**Table II:** Vancomycin minimum inhibitory concentrations (MICs) of the 21 isolates screened by BHI-V4

<table>
<thead>
<tr>
<th>Serial</th>
<th>Strain No</th>
<th>Agar screening method</th>
<th>MICs (µg/ml)</th>
<th>Population analysis profile</th>
<th>Disk Diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Broth micro-dilution</td>
<td>E-test</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Standard McF 0.5</td>
<td>Macro McF 2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>+</td>
<td>1</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>+</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>+</td>
<td>2</td>
<td>1.5</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>+</td>
<td>0.5</td>
<td>0.75</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>+</td>
<td>1</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>+</td>
<td>1</td>
<td>1.5</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td>+</td>
<td>1</td>
<td>0.75</td>
<td>4</td>
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<tr>
<td>8</td>
<td>27</td>
<td>+</td>
<td>2</td>
<td>2</td>
<td>12</td>
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<tr>
<td>9</td>
<td>31</td>
<td>+</td>
<td>1</td>
<td>1</td>
<td>6</td>
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<tr>
<td>10</td>
<td>34</td>
<td>+</td>
<td>0.5</td>
<td>0.38</td>
<td>4</td>
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<tr>
<td>11</td>
<td>40</td>
<td>+</td>
<td>0.5</td>
<td>0.75</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>44</td>
<td>+</td>
<td>0.5</td>
<td>0.25</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>45</td>
<td>+</td>
<td>0.5</td>
<td>0.25</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>46</td>
<td>+</td>
<td>2</td>
<td>3</td>
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<tr>
<td>15</td>
<td>49</td>
<td>+</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>16</td>
<td>50</td>
<td>+</td>
<td>0.25</td>
<td>0.125</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>53</td>
<td>+</td>
<td>1</td>
<td>0.75</td>
<td>4</td>
</tr>
<tr>
<td>18</td>
<td>54</td>
<td>+</td>
<td>1</td>
<td>1.5</td>
<td>4</td>
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<tr>
<td>19</td>
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<td>+</td>
<td>1</td>
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<td>8</td>
</tr>
<tr>
<td>20</td>
<td>58</td>
<td>+</td>
<td>1</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>21</td>
<td>60</td>
<td>+</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

S = sensitive  McF = McFarland
DISCUSSION

In the present study, we recruited MRSA isolates to be screened to detect hVISA, as the majority of hVISA isolates reported in the literature are methicillin resistant. (30, 31) The MRSA isolates are speculated to be more likely to harbor the hVISA phenotype as they represent strains that emerged from heavy antibiotic selection pressure and then were subjected to additional selection pressure through treatment with vancomycin. (32)

However, in the present study, no VISA strains were found and the prevalence of hVISA was found to be 9.68 % (6/62) of the MRSA isolates screened. A comparable prevalence of 9.3 % was reported in a study involving 129 MRSA strains collected from eight university hospitals in Japan, a condition much similar to our study.

Figure 1: Population analysis profile of the 7 isolates suspected of having reduced susceptibility to vancomycin by macro E test.

Figure 2: Scanning electron micrographs of MRSA isolates.
A- Vancomycin intermediate-resistant subpopulations of hVISA showing uneven surfaces with evidence of increased extracellular material.
B- VSSA showing a normal smooth cell wall without increased extracellular material.
population. (20) The prevalence of hVISA reported from different countries varied widely from 0 % (14) to 65 % (12). Also, reports from the same country may show conflicting results as that reported from Germany and France. In the former, the prevalence ranged from 0.54 % (33) to 8.24 % (34), whereas in the latter it ranged from 0 % (35) to 1.75 % (16). Moreover, the prevalence in the same report may vary according to the source of the strains whether hospital or community acquired. (16) The difference in the study populations of the S. aureus strains, screening methods, and including multiple isolates from the same patient may account for the differences in the previously reported prevalence studies. In the current study, the MRSA strains were isolated from hospital units (hemodialysis unit, diabetic foot clinic, and ICU) which are at high risk for the development of vancomycin resistance. This may explain the relatively high prevalence encountered.

Although well standardized microdilution susceptibility testing methods are able to detect S. aureus clinical isolates with reduced susceptibilities to vancomycin (VISA and VRSA), they can not detect the resistant cell population of hVISA isolates which occurs at a frequency of $10^{-6}$ or higher, and can not differentiate these strains from susceptible ones. (37) Similarly, all our isolates positively screened by BHI-V4 agar were interpreted as VSSA since their MIC values ranged from 0.125 µg/ml to 3 µg/ml by both broth microdilution method and standard E test. Also, they produce inhibitory zones ≥ 12 mm by the disk diffusion method. Same results were reported by other investigators. (13, 31, 38)

Currently, no standardized method for identifying hVISA exists. The macrodilution E test with a large inoculum (number 2 McFarland standards), a longer incubation time (48 hour), and rich BHI medium was found to be a reliable and sensitive screening method for detection of hVISA. (26, 39) The PAP has been proposed as the most precise method of determining heteroresistance, and a reliable method for confirmation of the macro E test results, but this method is laborious, time consuming, and impractical for using in routine laboratories. (31, 32)

In the present study, one out of the 62 MRSA isolates (1.6 %) was incorrectly identified as hVISA by the macro E test. This is comparable to the 2.1 % (7/329 MRSA isolates) rate of false positive results described by Walsh et al 2001. (26) Extending the results of this study to a working laboratory, it can be suggested that a macro E test should be performed initially, thus providing a non-labour intensive screening method for many isolates but with few false positives. Alternatively, if the cost of screening all MRSA isolates with macro E test is too high, this could be limited to isolates from patients receiving glycopeptide treatment and growing on BHI-V4 agar. Isolates suspected of showing resistance would then be subjected to analysis using PAP to give a more accurate guide to vancomycin-resistance status.

In the current study, the clinical significance of hVISA is difficult to evaluate because there were very few cases and because not all the cases were treated with vancomycin except lately. Although there was improvement of the hemodialysis patient with vancomycin treatment, the two patients from the ICU died, and the three diabetic patients underwent variable degrees of amputations.

At the present time, the clinical significance of hVISA infections is still controversial as sporadic case reports show contradictory data on their clinical outcomes. Some reports suggest that hVISA was not associated with the failure of vancomycin treatment, (13) while other reports document the failure of vancomycin to treat hVISA infections, sometimes with fatal outcomes.

All our intermediate subpopulations of hVISA were negative for the van A gene. The absence of enterococcal van A gene was consistent with the relatively limited level of vancomycin resistance of these cells (MIC ≤ 12 µg/ml) compared with those of vancomycin-resistant enterococci (MIC ≥ 128 µg/ml) for the van A phenotype. The same was reported in different publications. (38, 40 - 42) At the same time, viewing these subpopulations with electron microscopy showed enhanced cell wall thickness with increased extracellular material and irregular shape, a picture which is absent with VSSA cells. Howden et al 2006 (43) reported increased cell wall thickness among their hVISA / VISA phenotype, but still the genetic changes leading to this have to be clearly determined. Researches had proposed trapping of vancomycin molecules in the cell wall peptidoglycan would be the essential contributor in the mechanism of vancomycin resistance in hVISA / VISA strains. The thicker the cell wall, the more vancomycin molecules would be trapped within it, thus allowing a decreased number of vancomycin molecules to reach the cytoplasmic membrane where the real functional targets of vancomycin are present. (44 - 46)

Cui et al 2006 (47) demonstrated the occurrence of an anomalous diffusion of vancomycin through the VISA cell wall, which is caused by clogging of the cell wall with vancomycin itself. They concluded that a cooperative effect of clogging of the cell wall with vancomycin, and cell wall thickening enables VISA to prevent vancomycin from reaching its true target in the cytoplasmic membrane.
exhibiting a new class of antibiotic resistance in gram-positive bacteria.

In conclusion, this study is an early warning that MRSA strains with full resistance to vancomycin might emerge in the future, emphasizing the importance of a laboratory capability of identifying heterogeneous vancomycin resistance. For screening hVISA, the macro E test is more specific than the BHI-V4 agar screening method. As the E test is an expensive test, therefore, applying the macro E test for isolates that grow on BHI-V4 agar may be the most suitable algorithm for screening hVISA. Also, it may be better to screen only MRSA rather than screening all S. aureus isolates until the clinical significance of hVISA is better understood. Further studies should be carried on to elucidate the genetic mechanism of resistance in hVISA strains which may provide a more definitive means of identification.

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The study was conducted on 120 blood samples collected from patients with suspected fever and viremia admitted to El-Sheikh Zayed, Bassil, and Ramadhan Hospitals in Asyut Governorate.

The blood samples were cultured in BHI-V4 broth, and 30% and 10% BHI-V4 broths were used for the isolation of the isolates. The isolates were identified using the technique of direct microscopy, biochemical tests, and RAPD-PCR.

The results showed that the most common isolate was Staphylococcus aureus, followed by Streptococcus pyogenes and Escherichia coli. The isolates were also subjected to antibiotic sensitivity testing, and the results showed that most of the isolates were resistant to antibiotics such as ampicillin, cotrimoxazole, and ceftriaxone.

The study concluded that the isolation and identification of bacterial isolates from blood samples are crucial for the diagnosis and treatment of infectious diseases.

References:

