Emergence of Vancomycin Resistant Staphylococcus aureus during Hospital Admission at a Tertiary Care Hospital in Bangladesh

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Abstract

Background: Glycopeptides such as vancomycin are frequently the choice of antibiotics for the treatment of infections caused by methicillin resistant Staphylococcus aureus (MRSA). For the last 7 years incidence of vancomycin intermediate S. aureus and vancomycin resistant S. aureus (VISA and VRSA respectively) has been increasing in various parts of the world. Objective: The present study was carried out to find out the presence of VISA and VRSA among isolated MRSA strains. Methodology: This cross sectional study was carried out in the Department of Microbiology in Dhaka medical college during period of January 2010 to December 2011. All S. aureus isolates were screened to detect methicillin resistance and then all MRSA isolates were subjected for MIC testing against vancomycin and oxacillin by agar dilution method, disc diffusion testing and PCR for meca and pvl genes detection. Result: A total 112 S. aureus were isolated from 500 nasal swab sample collected from adult patients who were admitted in various departments and wards in Dhaka Medical College Hospital. Among 38 MRSA strains out of 112 Staph aureus isolates 3(7.89%) strains were resistance to vancomycin of which 2(5.26%) strains had MIC > 256 μg/mL and one strain had MIC 256μg/mL. All vancomycin resistance strains had MIC of oxacillin > 256 μg/mL. All isolates possess mec-A gene. Conclusion: The present study reveals that emergence of VRSA upon admission at a tertiary care hospital in Bangladesh. Continuous efforts should be made to prevent the spread and the emergence of VRSA by early detection of the resistant strains and using the proper infection control measures in the hospital setting. [Bangladesh Journal of Infectious Diseases 2016;3(1):11-16]

Keywords: Vancomycin intermediate; vancomycin resistant; Staphylococcus aureus; VRSA; PCR

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Contributions to authors: SK conceived and designed the work, sample collection and performed strain isolation and identification, antibiotic susceptibility testing, PCR tests and prepared the manuscript. JAH has contribution on study proposal and scientific advisor. MMR has contribution on patients data collection. KZM and SMS have prepared and have revised the manuscript. Mamun KZ—Guide, Shamsuzzama SM—co-guide.
Introduction

Staphylococcus aureus (Staph aureus) is one of the most common causes of both endemic and epidemic infections acquired in hospitals, which results in substantial morbidity and mortality. Colonization with Staph aureus has been identified as an important risk factor for the development of Staph aureus infections in both community and hospital settings. Multidrug-resistant strains of Staphylococci have been reported with increasing frequency worldwide, including isolates that are resistant to methicillin, lincosamides, macrolides, aminoglycosides, fluoroquinolones, or combinations of these antibiotics. Recommended treatments for multidrug resistant MRSA are glycopeptides, particularly vancomycin.

In the 1980s, due to the widespread occurrence of methicillin-resistant Staphylococcus aureus (MRSA), empiric therapy for Staphylococcal infections particularly nosocomial sepsis was changed to vancomycin in many health-care institutions. Vancomycin use in many countries also increased during this period because of the growing numbers of infections with Clostridium difficile and coagulase-negative Staphylococci in health-care facilities. The use of the glycopeptides antibiotic, vancomycin is increasing day by day. As a consequence, selective pressure is established that eventually lead to the emergence of strains of Staph aureus with decrease susceptibility to vancomycin and other glycopeptides. Staph aureus strains whose minimum inhibitory concentration (MICs) of vancomycin are 4 to 8 µg/mL are classified as vancomycin-intermediate (VISA) and the strains whose vancomycin MICs are ≥16 µg/mL are classified as vancomycin-resistant. Resistance to vancomycin seems to develop from pre-existing strains of MRSA in the presence of vancomycin. VRSA strains are characterized by expression of vanA gene residing on Tn1546-like element which was acquired from an Enterococcus species. Therefore, this resistance is potentially transferable to susceptible strains or other organisms. In 1997, the first strain of vancomycin intermediate Staph aureus (VISA) is reported from Japan. First clinical isolate of vancomycin-resistant Staph aureus (VRSA) is reported from the United States in 2002. Subsequent isolation of VISA and VRSA isolates from other countries including Brazil, France, United Kingdom, Germany, India and Belgium has confirmed that emergence of these strains is a global issue.

In Bangladesh, the frequency of MRSA is alarming may be due to indiscriminate and incomplete uses of antibiotics. In 2004, 83.3% MRSA were isolated from wound infection in an orthopedic hospital. In 2005, 70.2% MRSA were isolated from wound infection. In 2007, 50.63% MRSA were isolated from different samples. In Bangladesh, though MRSA infection is more frequent, but there is no adequate information on VRSA and VISA strains and their resistance pattern. Therefore the present study was carried out to find out any VRSA and VISA among isolated MRSA strains.

Methodology

This cross-sectional study was carried out in the Department of Microbiology in Dhaka Medical college during the period of January 2010 to December 2011 for a duration of two(02) years. Five hundred adult patients were screened within 24 hours of their admission in different wards in Dhaka Medical college Hospital by taking nasal swab from both anterior nares and were analyzed. Data regarded by age, sex, previous h/o hospitalization (within past 12 month) and their co-morb sibling conditions such as DM, COPD, CVD, CKD were collected from hospital records or directly from patients using predesigned data collection form. Nasal swab samples were plated on blood agar media and incubated at 37°C. Isolates were identified as Staph aureus by colony morphology, Gram staining and standard biochemical tests like catalase, coagulase and mannitol fermentation test. Staph aureus isolates were screened for methicillin resistance by disc diffusion method using oxacillin (1µg) and cefoxitin (30µg) disc and by determination of minimum inhibitory concentration (MIC) of oxacillin by agar dilution method per recommendation of CLSI method. The discrepancies of the result were confirmed by PCR assay which is gold standard method for detection of mecA gene by using specific primers. All MRSA isolates were tested for susceptibility against ceftriaxone (30µg), ciprofloxacin (5µg), doxycycline (30µg), erythromycin (15µg), gentamycin (10µg), rifampicin (5µg), vancomycin (30µg), fusidic acid (10µg) and linezolid (30µg) by disc diffusion method as recommended by CLSI. The discs from each batch were standardized by testing against reference stain of Staph aureus ATCC-25923. VRSA were detected by disc diffusion method and by determination of MIC of vancomycin by agar dilution method. If inhibition zone diameter around vancomycin (30µg) disc was ≤14 mm and MIC of vancomycin was ≥ 16 µg/ml than it was considered as VRSA. MIC of vancomycin was 4 to 8 µg/ml, was considered as
Emergence of VRSA
Khanam et al
Bangladesh J Infect Dis
13
June 2016 │ Volume 3 │ Number 1

VISA. MIC of vancomycin <4µg/ml were considered as VSSA\textsuperscript{10}. Minimal inhibitory concentration (MIC) of oxacillin, vancomycin were determined by agar dilution method using CLSI guidelines\textsuperscript{10}. Briefly, gradient plates of Mueller-Hinton agar were prepared with oxacillin (0.5–256 μg/ml) (with 2% NaCl), vancomycin (0.5–256 μg/ml). By direct colony suspension method 0.5 McFarland equivalent inoculums were prepared in normal saline from culture plate. The suspension was further diluted to achieve desired inoculum concentration of 10\(^5\) CFU/ml. All strains were spotted onto gradient plates. Plates were incubated overnight at 37°C for any visible growth. Readings were taken according to CLSI guidelines\textsuperscript{10}. S.\textit{aureus} ATCC 25923 were used as vancomycin susceptible controls.

Results

Isolation rate of Staph\textit{aureus} among admitted patients from nasal swab sample were recorded. After screening 500 nasal swabs, 255 (51\%) isolates were culture positive for Staphylococcus. Out of 255 Staph\textit{aureus}, 112 (22.4\%) were Staph\textit{aureus} and 143 (28.6\%) were coagulase negative Staph\textit{aureus} (Table 1).

Table 1: Isolation Rate of \textit{Staphylococcus} from Nasal Swab Sample (n=500)

<table>
<thead>
<tr>
<th>Staphylococci</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph\textit{aureus}</td>
<td>112</td>
<td>22.4</td>
</tr>
<tr>
<td>Coagulate -ve Staph</td>
<td>143</td>
<td>28.6</td>
</tr>
<tr>
<td>Total</td>
<td>255</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Isolation rate of MRSA and MSSA among \textit{Staphylococcus aureus} was recorded. Out of 112 Staph\textit{aureus}, 38 (33.9\%) strains were detected as MRSA and 74 (66.1\%) strains were detected as MSSA by different phenotypic method and by detection of \textit{mec-A} gene by PCR (Table 2).

Table 2: Isolation Rate of MRSA and MSSA among \textit{Staphylococcus aureus}

<table>
<thead>
<tr>
<th>Staph. aureus</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>38</td>
<td>33.9</td>
</tr>
<tr>
<td>MSSA</td>
<td>74</td>
<td>66.1</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>22.4</td>
</tr>
</tbody>
</table>

Out of 38 MRSA, 3 (7.89\%) were VRSA and 35 (92.1\%) were VSSA detected by Disc diffusion method and MIC (agar dilution method). Among 3 VRSA strains, 2 (66.6\%) strains had MIC of vancomycin >256 µg/ml and one (33.3\%) strain had MIC of 256µg/ml. Thirty five (92.1\%) VSSA strains had MIC< 4µg/ml (Table 3). Antimicrobial resistance pattern of VRSA was varied. Out of 3 VRSA isolates two (66.6\%) strains were resistant to rifampicin and fusidic acid, and one (33.3\%) VRSA strain was also resistant to linezolid but sensitive to doxycycline and gentamycin. All (100\%) were resistant to erythromycin (Table 4). Antimicrobial susceptibility pattern and patients profile among the VRSA cases were recorded. Among 3 VRSA cases, 2 VRSA colonized male patients were above 70 years of age and one female was 35 years of age. All had history of previous hospitalization and all had multiple co-morbidities (Table 5).

Table 3: Distribution of MIC of Vancomycin of VRSA and VSSA among MRSA Isolates Detected By Disc Diffusion Method

<table>
<thead>
<tr>
<th>Vancomycin disc method (30 µg)</th>
<th>MIC (µg/ml) of Vancomycin</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;4</td>
<td>8</td>
</tr>
<tr>
<td>VRSA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VSSA</td>
<td>35</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4: Antimicrobial Susceptibility Pattern of Isolated VRSA (n=3)

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Resistant</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin (5µg)</td>
<td>2 (66.7%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Fusidic acid (10µg)</td>
<td>2 (66.7%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Linezolid (30µg)</td>
<td>1 (33.3%)</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>Erythromycin (15µg)</td>
<td>3 (100.0%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Gentamycin (10µg)</td>
<td>1 (33.3%)</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>Doxycycline (30µg)</td>
<td>1 (33.3%)</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>Ciprofloxacin (5µg)</td>
<td>2 (66.7%)</td>
<td>1 (33.3%)</td>
</tr>
</tbody>
</table>
Emergence of VRSA

Khanam et al

Table 5: Antimicrobial Susceptibility Pattern and Patients Profile among the VRSA cases

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>MICs µg/mL</th>
<th>Antimicrobial susceptibility pattern</th>
<th>Patients Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OX</td>
<td>VAN</td>
<td>FD</td>
</tr>
<tr>
<td>176</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>R</td>
</tr>
<tr>
<td>180</td>
<td>&gt;256</td>
<td>256</td>
<td>R</td>
</tr>
<tr>
<td>401</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>S</td>
</tr>
</tbody>
</table>

Note: OX = Oxacillin, VAN = Vancomycin, FD = Fusidic acid, RD = Rifampicin, LZ = Linezolid, CI = Ciprofloxacin, DO = Doxycycline, G = Gentamycin; H/O = Hospitalization; Comorb = Comorbidity; M = Male, F = Female, DM = Diabetes mellitus, CVD = Cerebro vascular disease

Discussion

Infections caused by methicillin-resistant S. aureus have been associated with high morbidity and mortality rates. In Bangladesh, the frequency of MRSA is alarming due to indiscriminate and incomplete uses of antibiotics 20, 21. In Bangladesh, the rate of MRSA infection ranges from 32% to 63% in different hospitals 22. The present study showed 33.9% out of 112 Staphylococcus aureus were MRSA. Vancomycin is the main antimicrobial agent available to treat serious infections with MRSA but unfortunately, decrease in vancomycin susceptibility of S. aureus and isolation of vancomycin-intermediate and resistant S. aureus have recently been reported from many countries 23. In the present study, 3(7.89%) isolates were resistant to vancomycin among 38 MRSA strains. MIC of vancomycin was determined by agar dilution method. In this study, of the three VRSA strains, 2 VRSA strains had MIC > 256 µg/ml and one strain had MIC 256µg/ml. In this study VRSA isolates were also meticillin-resistant had MIC of oxacillin >256µg/ml and contained mec-A gene. The first strain of S. aureus with reduced susceptibility to vancomycin was found in 1997 named MU50 showed MIC of 8µg/ml 24. Vancomycin resistance Staphylococcus aureus (VRSA) strain was first reported in United States in 2002 which had MIC 32 µg/ml 8. Saderi et al. 12 from Iran reported that out of 139 Staphylococcus aureus strains 5 VRSA isolates had MIC >128 µg/mL. That study also reported that one VRSA strain had MIC more than 256 µg/mL. In the present study, out of 3 VRSA- patients, 2 were above 70 years old and one was 36 years old female. All the three patients were associated with multiple co-morbidities, had history of previous hospital admission and received multiple antibiotics. The only female patient had diabetes mellitus and had wound infection. Misuse of antimicrobial agents and the spread of multidrug-resistant strains are facilitated by poor hygiene and are related with multidrug resistance strains of MRSA. A study in Bangladesh reported that widespread and suboptimal use of antimicrobial agents was an important factor for high prevalence of resistant strains 25.

In Bangladesh, a study conducted at Dhaka Medical College reported that all MRSA isolates were susceptible to vancomycin 26. In comparison with previous study it reflected that the sensitivity of vancomycin was reduced over the past years. However, it might be possible that irrational and over use of antibiotic is increasing day by day which may contribute to the development of multidrug resistance. Another possible mechanism of VRSA is presence of van-A gene which was not studied in this study. Nancey et al. 13 and Tenover et al. 9 reported that vancomycin-resistant enterococci containing van-A were isolated from patients with MRSA strain. The van-A gene is usually found in enterococci which confers high-level vancomycin resistance (MICs=512-1024µg/ml) and van-A determinant is transferred via plasmids from enterococci to a resident MRSA strain, resulting in the VRSA 30. In this study VRSA isolates were also meticillin-resistant and contained mec-A gene. If prompt measures are not taken to prevent indiscriminate use of antibiotics and prevent hospital spread of VRSA then it may be a serious health problem in Bangladesh.

In the present study, all the three isolated VRSA strains were also resistant to a wide range of different antimicrobial agents. Resistance to both rifampicin and fusidic acid was 66.7% in each antibiotic. All (100%) were resistant to erythromycin. But these were sensitive (66.7%) to linezolid, gentamycin and doxycycline. In the present study one VRSA isolate which was also resistant to linezolid was found in a patient aged 70 years, suffering from multiple co-morbidities with history of previous hospitalization. Linezolid
Emergence of VRSA

Khanam et al.

resistance was first reported in 2001 in clinical isolates of Staphylococcus aureus. The study of Ruiz et al. from the USA reported two linezolid resistant MRSA strains; patients were above 70 years old and associated with multiple co-morbidities. Peeters and Sarria reported that linezolid resistant strains were associated with deep organ involvement, presence of foreign device and/or prolong therapy with linezolid (>3wks). Torre et al. reported 12 cases of first outbreak of linezolid resistance in ICU ward in Spain occurred and half of the patients died. Eleven cases were full-blown infection; including five ventilator associated pneumonia, five bacteremia and one was catheter related sepsis.

Conclusion

In conclusion a significant number of VRSA has been found in the admitted patients in a tertiary care hospital in Bangladesh. This study also showed that VRSA colonized patients had history of previous hospitalization and were associated with multiple co-morbidities. In this study all VRSA strains were resistant to wide range of different antimicrobial agents. Although this study was confined within one tertiary care hospital in Bangladesh, the emergence of VRSA/VISA might also be prevalent in other hospitals as antibiotic misuse is equally common there. Hence, there should be an immediate response from the concerned authorities to check further emergence and spreading of these notorious VRSA strains. A strict regulation on irrational antibiotic usages might be an appropriate and effective approach in this direction. Moreover, nationwide surveillance program should be carried out to map the vancomycin susceptibility pattern in this country.

References