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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 of 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[1]. 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[2-3]. 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[4]. Recommended treatments for multidrug resistant MRSA are glycopeptides, particularly vancomycin[5].

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[6-7]. The use of the glycopeptides antibiotic, vancomycin is increasing day by day[8]. 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[9]. *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[10]. Resistance to vancomycin seems to develop from pre-existing strains of MRSA in the presence of vancomycin[11-12]. VRSA strains are characterized by expression of *vanA* gene residing on Tn*1546*-like element which was acquired from an *Enterococcus* species [9, 13]. Therefore, this resistance is potentially transferable to susceptible strains or other organisms[9]. In 1997, the first strain of vancomycin intermediate *Staph aureus* (VISA) is reported from Japan[14]. First clinical isolate of vancomycin-resistant *Staph aureus* (VRSA) is reported from the United States in 2002[8]. Subsequent isolation of VISA and VRSA isolates from other countries including Brazil[15], France[16], United Kingdom[17], Germany[18], India[19] and Belgium[20] 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[21-22]. In 2004, 83.3% MRSA were isolated from wound infection in an orthopedic hospital[2]. In 2005, 70.2% MRSA were isolated from wound infection[24]. In 2007, 50.63% MRSA were isolated from different samples[25]. 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-morbid 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 37^0c. Isolates were identified as *Staph aureus* by colony morphology, Gram staining and standard biochemical tests like catalase, coagulase and mannitol fermentation test[26]. *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[10]. The discrepancies of the result were confirmed by PCR assay which is gold standard method for detection of *mecA* gene by using specific primers[27,28]. 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[10]. 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

VISA. MIC of vancomycin <4µg/ml were considered as VSSA[10]. Minimal inhibitory concentration (MIC) of oxacillin, vancomycin were determined by agar dilution method using CLSI guidelines[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[10]. *S. aureus* ATCC 25923 were used as vancomycin susceptible controls.

Results

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

Table 1: Isolation Rate of *Staphylococcus* from Nasal Swab Sample (n=500)

Staphylococci	**Frequency**	**Percentage**
Staph. aureus	112	22.4
Coagulase -ve *Staph*	143	28.6
Total	**255**	100.0

Staph. aureus =Staphylococcus aureus

Isolation rate of MRSA and MSSA among *Staphylococcus aureus was recorded.* Out of 112 *Staph 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 *mec-A* gene by PCR (Table 2).

Table 2: Isolation Rate of MRSA and MSSA among *Staphylococcus aureus*

Staph. aureus	**Frequency**	**Percentage**
MRSA	38	33.9
MSSA	74	66.1
Total	**112**	**22.4**

Staph. aureus =Staphylococcus aureus

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

Vancomycin disc method (30 µg)	**MIC (µg/ml) of Vancomycin**								**Total**
	<4	**8**	**16**	**32**	**64**	**128**	**256**	**>256**	
VRSA	0	0	0	0	0	0	1	2	3 (7.89%)
VSSA	35	0	0	0	0	0	0	0	35(92.1%)

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 (66.66%) strains had MIC of vancomycin >256 µg/ml and one (33.33%) 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 (66.66%) strains were resistant to rifampicin and fusidic acid, and one (33.34%) 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 4: Antimicrobial Susceptibility Pattern of Isolated VRSA (n=3)

Antimicrobial agents	**Resistant**	**Sensitive**
Rifampicin (5µg)	2 (66.7%)	1(33.3%)
Fusidic acid (10µg)	2(66.7%)	1(33.3%)
Linezolid (30µg)	1 (33.3%)	2(66.7%)
Erythromycin (15µg)	3(100.0%)	1(33.3%)
Gentamycin (10µg)	1(33.3%)	2(66.7%)
Doxycycline (30µg)	1(33.3%)	2(66.7%)
Ciprofloxacin (5 µg)	2 (66.7%)	1(33.3%)

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

Sample No.	MICs μg/mL		Antimicrobial susceptibility pattern						Patients Profile			
	OX	VAN	FD	RD	LZ	CI	DO	G	Age (Yrs)	Sex	Comorb	H/O Hospi
176	>256	>256	R	R	R	R	S	S	70	M	CVD	Yes
180	>256	256	R	R	S	R	S	S	70	M	CVD,DM	Yes
401	>256	>256	S	S	S	S	R	R	36	F	DM	Yes

Note: OX - Oxacillin, VAN = vancomycin, FD = Fusidic acid, RD = Rifampicin, LZ = Linezolid, CI= Ciprofloxacin, DO = Doxycycline, G = Gentamycin; Hospi= 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 may be due to indiscriminate and incomplete uses of antibiotics[21-22]. In Bangladesh, the rate of MRSA infection ranges from 32% to 63% in different hospitals[29]. 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[29]. 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 methicillin-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[14]. 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 male 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[22].

In Bangladesh, a study conducted at Dhaka Medical College reported that all MRSA isolates were susceptible to vancomycin[24]. 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. Nancye 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 methicillin-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

resistance was first reported in 2001 in clinical isolates of *Staph aureus*[31]. The study of Ruiz et al[32] from the USA reported two linezolid resistant MRSA strains; patients were above 70 years old and associated with multiple co-morbidities. Peeters and Sarria[33] 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[34] 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

1. Von Eiff C, Becker K, Machka K. Nasal carriage as a source of *Staph aureu* bacteremia. N Engl J Med 2001;344:11-6
2. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staph aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997;10:505-20
3. Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK, Foshei GE. Changes in the prevalence of nasal colonization with *Staph aureus* in the United States. J Infect Dis 2008; 197: 1226–34
4. Lowy FD. *Staph aureus* infections. N Engl J Med 1998;339:520-32
5. Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP. A modified population analysis (PAP) method to detect hetero-resistance to vancomycin in *Staph aureus* in a UK hospital. J Antimicrob Chemother 2001;47:399-403
6. Ena J, Dick RW, Jones RN, Wenzel RP: The epidemiology of intravenous vacomycin usage in a university hospital: a 10 year study**.** JAMA 1993;269**:**598-602
7. Cunha BA: Vancomycin. Med Clin North Am 1995;79**:**817-831
8. Centers for Disease Control and Prevention (CDC). *Staph aureus* resistant to vancomycin- United States. MMWR 2002;51: 565-7
9. Tenover CF, Biddle JW, Lancaster MV. Increasing resistance to vancomycin and other glycopeptides in *Staph aureus*. Emerg Infect Dis 2001;**7**:327-32
10. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Seventeenth informational Supplement. Document M100-S17. Wayne, PA. CLSI 2007
11. Olivier D, Nonhoff C, Baudouin B, Knoop C, Bobin-Dubreux S, Struelens MJ. Emergence of vancomycin-intermediate *Staph aureus* in a Belgian hospital: microbiological and clinical features. J Antimicrob Chemother 2002;50:383-91
12. Saderi H, Owlia P, Maleki Z, Habibi M, Rahmati N. Susceptibility to vancomycin in *Staph aureus* isolated from patients of four University-affiliated Hospitals in Tehran. Iran J Path 2008;3:161-6
13. Nancye CC, Weigel LM, Patel JB, Tenover FC. Comparison of Tn*1546*-Like elements in Vancomycin-Resistant *Staph aureus* isolates from Michigan and Pennsylvania. Antimicrob Agents Chemother 2005;49:470–2
14. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staph aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 1997;40:135-6
15. Oliveira GA, Dell'Aquila AM, Masiero RL, Levy CE, Gomes MS, Cui L, et al. Isolation in Brazil of nosocomial Staphylococcus aureus with reduced susceptibility to vancomycin. Infect Control Hosp Epidemiol 2001;22:443–8
16. Poly MC, Grelaud C, Martin C, de Lumley L, Denis F. First clinical isolate of vancomycin-intermediate *Staphylococcus aureus* in a French hospital. Lancet 1998;351:1212
17. Howe RA, Bowker KE, Walsh TR, Feest TG, Mac-Gowan AP. Vancomycin-resistant Staphylococcus aureus. Lancet 1998;351:602
18. Bierbaum G, Fuchs K, Lenz W, Szekat C, Sahl HG. Presence of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Germany. Eur J Clin Microbiol Infect Dis 1999;18:691-6
19. Assadullah S, Kakru DK, Thoker MA, Bhat FA, Hussain N, Shah A. Emergence of low level vancomycin resistance in MRSA. Indian J Med Microbiol. 2003;21:196-8
20. Pierard D, Vandenbussche H, Verschraegen I, Lauwers S. Screening for *Staphylococcus aureus* with a reduced susceptibility to vancomycin in a Belgian hospital. Pathologie Biologie 2004;52:486-8
21. Khan MA, Mourshed MG, Khan WA, Aziz KMS. The emergence of methicillin resistant *Staph aureus* isolated from skin lesion. Bangladesh J Microbiol 1991;8:21-5
22. Mamun KZ, Shears P, Tabassum S, Hart CA. Antimicrobial use and antimicrobial resistance in rural Bangladesh. Trans Roy Soc Trop Med Hygn 1996;90:213
23. Jahan Y, Jahan F, Mamun KZ, Hossain MA, Shirin T, Rahman S, et al. Emergence of methicillin resistant *Staph aureus* (MRSA) associated with wound infections. Mymensingh Med J 2004;13: 76-81
24. Afroz S. Detection of MRSA in patients and carriers by evaluating different methods of identification, its typing and susceptibility to vancomycin. [MPhil thesis] Dhaka, Bangladesh: Department of Microbiology, DMCH; 2005

25. Khan HA, Shamsuzzaman AKM, Paul SK, Alam MM, Mahmud MC, Musa AKM, *et al*. Antimicrobial susceptibility and coagulase typing of MRSA strains at Mymensingh Medical College. Bangladesh J Microbiol 2007;1: 56-60
26. Cheesbrough M. Microscopic techniques used in microbiology. In: District laboratory practices in tropical countries, Part II. Cambridge University Press; UK: 2000; pp 39-41
27. Rahimi F, Bouzari M, Maleki Z . Antibiotic susceptibility pattern among *Staphylococcus* spp. With emphasis on detection of *mecA* gene in methicillin resistant *Staph aureus* isolates. Iranian J Clin Infect Dis 2009;4:143-150
28. Siripornmongcolchai T, Chomvarin C, Chaicumpar K. Evaluation of different primers for detecting Mec -A gene by PCR in comparison with phenotypic methods for discrimination of Methicillin-resistant *Staph aureus*. Southeast Asian J Trop Med Public Health 2002 ;33 :758-63
29. Haq JA, Rahman MM, Asna SM, Hossain MA, Ahmed I, Haq T, *et al*. Methcillin-resistant *Staph aureus* in Bangladesh: a multicentre study. Int J Antimicrob Agents 2005; 25: 276-7
30. Centers for Disease Control and Prevention (CDC). Investigation and control of vancomycin intermediate and resistant *Staph aureus* (VISA/VRSA). A guide for health departments and infection control personnel. Atlanta GA, 2006. Available at www.CDC.gov/ ncidod/ dhqp/ar/ visavrsa prevention.html. Accessed on 10/08/2011
31. Tsiodras S, Gold HS, Sakoulas G. Linezolid resistance in a clinical isolate of *Staph aureus*. Lancet 2001;358:207- 8
32. Ruiz ME, Guerrero IC, Tuazon CU. Endocarditis caused by methicillin-resistant *Staph aureus*: treatment failure with linezolid. Clin Infect Dis 2002; 35: 1018-20
33. Peeters MJ, Sarria JC. Clinical characteristics of linezolid-resistant *Staph aureus* infections. Am J Med Sci 2005;330:102-4
34. Torre M, Cute S, Nchez M, Morales G. Outbreak of linezolid-resistant *Staph aureus* in intensive care. Abstract from the 48th Inter science conference on antimicrobial agents and chemotherapy (ICAAC), Washington DC: Am Soc Microbiol 2008