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Original Article

Prevalence and antimicrobial susceptibility pattern of methicillin-resistant, vancomycin-resistant, and Panton-Valentine leukocidin positive *Staphylococcus aureus* in a tertiary care hospital Dhaka, Bangladesh

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ABSTRACT

Objectives: To observe the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA), and Panton-Valentine leukocidin (PVL)-positive *S. aureus,* this study was carried out in a tertiary care hospital in Dhaka, Bangladesh.

Materials and methods: S. aureus strains were recovered from 200 postoperative wound swab samples from patients hospitalized in Dhaka Medical College Hospital between July 2011 and June 2012. Methicillin resistance was determined by the oxacillin and cefoxitin disc diffusion method, the minimum inhibitory concentration (MIC) of oxacillin, and *mecA* gene detection. VRSA resistance was determined by the disc diffusion method, the MIC of vancomycin, and screening for the *vanA* and *vanB* genes. The PVL gene was also detected in MRSA strains.

Results: Fifteen of the 44 isolated strains of *S. aureus* were MRSA (2 of them were VRSA) and 29 were methicillin-sensitive *S. aureus.* All MRSA isolates were highly resistant to oxacillin (MIC $\geq$ 256 μg/mL). When compared with polymerase chain reaction (PCR), the sensitivity and specificity of the oxacillin disc diffusion method were 93.33% and 100% respectively; for the cefoxitin disc diffusion method and MIC of oxacillin both the sensitivity and specificity were 100%. Four (26.67%) MRSA isolates were positive for PVL genes which were also *mecA* positive. The MRSA strains were highly resistant to ciprofloxacin (93.33%), ceftriaxone (86.63%), azithromycin (73.33%), gentamycin (73.33%), and amoxiclav (66.67%). All (100%) MRSA strains were sensitive to linezolid and 86.67% were sensitive to vancomycin. The VRSA strains had an MIC $\geq$256 μg/mL for vancomycin and were positive for the *vanB* gene but negative for the *vanA* gene.

Conclusion: The results of this study provide insight into the high proportion of MRSA and presence of VRSA in Bangladesh.

1. Introduction

Infections caused by *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), are emerging as a major public health problem in hospital and community settings, causing a wide range of diseases. The emergence and spread of both health care and community-associated MRSA has made infection control intervention and treatment challenging [1].

MRSA has evolved after acquiring the *mecA* gene that encodes the penicillin-binding protein 2a which confers resistance to methicillin and other β-lactam antibiotics [2]. The *mecA* is a highly conserved gene found only in methicillin-resistant strains with no allelic equivalent in methicillin-susceptible *Staphylococci*, making it a useful marker for β-lactam resistance [3]. Detection of the *mecA* gene is the gold standard for identifying MRSA [4] but this test is not available in many clinical laboratories in developing countries and is relatively expensive. Different methods have been developed for the detection

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of MRSA including minimum inhibitory concentrations (MIC), the oxacillin screening agar method, and disc diffusion testing. The major problem in routine screening is that MRSA strains are heterogeneous in the expression of resistance to β-lactam agents [5].

S. aureus produces numerous virulence factors, including Panton-Valentine Leukocidin (PVL), which is a pore forming cytotoxin more often identified in community-associated MRSA strains than hospital-associated strains [6]. PVL-positive Staphylococcal infection typically presents as a life-threatening infection of the skin and soft tissues, bone, or joints, although it can also lead to necrotizing pneumonia, a severe and often fatal condition involving primarily young, healthy patients [3,6].

In 1996, VISA (vancomycin-intermediate *S. aureus*) was first reported in Japan [7], and then in 2002 vancomycin-resistant *S. aureus* (VRSA) was first reported in the USA [8]. Subsequent isolation of VRSA from different countries has confirmed that the emergence of these strains is a global issue [9–12].

The aim of this study was to determine the prevalence of MRSA and VRSA in postoperative wound swabs using antimicrobial susceptibility profiles and to detect the PVL and *mecA* genes in MRSA as well as the *vanA* and *vanB* genes in VRSA.

2. Materials and methods

2.1. Samples

Two hundred swab samples were collected from infected wounds from postoperative patients admitted to Dhaka Medical College Hospital (DMCH) in Bangladesh over a 12-month period from July 2011 to June 2012. The age and sex of patients were recorded.

2.2. Inclusion criteria

Wound swabs collected from patients admitted to DMCH irrespective of age, sex, and antibiotic intake. Urine samples received in the Department of Microbiology of DMCH for culture and sensitivity irrespective of age, sex, and history of antibiotic intake were also included.

2.3. Ethical issues

Informed written consents were taken from each patient prior to collecting samples. Approval was obtained from the Research Review Committee and Ethical Review Committee of Dhaka Medical College according to the Declaration of Helsinki and national and institutional standards.

2.4. Isolation of S. aureus

S. aureus was identified by observing opaque colonies on blood agar media, Gram's staining, positive catalase and coagulase tests, and mannitol fermentation on mannitol salt agar [13].

2.5. Antimicrobial susceptibility testing

Standard disc diffusion techniques as recommended by the Clinical Laboratory Standards Institute (CLSI) were performed for susceptibility testing of oxacillin, cefoxitin, gentamycin, cotrimoxazole, ciprofloxacin, ceftriaxone, amoxiclav, vancomycin, and linezolid (oxoid, UK) for all *S. aureus*. [14].

2.6. Detection of MRSA and VRSA by the disc diffusion technique

Screening for methicillin-resistance was determined using the Kirby-Bauer disc diffusion method with 1 μg oxacillin and 30 μg cefoxitin discs. VRSA was detected by a 30 μg vancomycin disc. Three to five were emulsified into 3 mL of sterile normal saline. The turbidity of the suspension was compared with the 0.5 McFarland turbidity standard and the suspension was incubated on Mueller–Hinton agar plates at 37°C for 24 hours. An inhibition zone diameter of $\leq$ 10 mm around the oxacillin disc was considered resistance; 11–12 mm indicated intermediate, and $\geq$ 13 mm was considered sensitive. For the cefoxitin disc, an inhibition zone diameter of $\leq$ 21 mm was considered resistance and $\geq$ 22 mm was considered sensitive [14].

2.7. Detection of MRSA by the MIC of oxacillin and VRSA by MIC of vancomycin

The MIC of oxacillin by the agar dilution method was determined according to CLSI guidelines. An MIC of oxacillin $\geq$ 4 μg/mL was considered MRSA and $\leq$ 2 μg/mL was considered methicillin-sensitive *S. aureus* (MSSA) [14]. For preparation of the oxacillin stock solution, a 500-mg base of oxacillin was added to 50 mL distilled water to a concentration of 10 mg/mL. For each plate, 50 mL Mueller–Hinton medium was prepared and impregnated with 10 μL, 20 μL, 40 μL, 80 μL, 160 μL, 320 μL, 640 μL, or 1280 μL of the oxacillin stock solution to achieve concentrations of 2 μg/mL, 4 μg/mL, 8 μg/mL, 16 μg/mL, 32 μg/mL, 64 μg/mL, 128 μg/mL, or 256 μg/mL per plate, respectively.

For detection of VRSA, an MIC of vancomycin $\geq$ 16 μg/mL was considered resistant, $\leq$ 2 μg/mL was sensitive, and 4–8 μg/mL was VISA [14]. A vial of a 500 mg base of commercially available vancomycin injection was added to 50 mL distilled water to a concentration of 10 mg/mL. For each plate, 50 mL sterile Mueller–Hinton agar was prepared and impregnated with 10 μL, 20 μL, 40 μL, 80 μL, 160 μL, 320 μL, 640 μL, or 1280 μL of vancomycin stock solution to achieve a concentration of 2 μg/mL, 4 μg/mL, 8 μg/mL, 16 μg/mL, 32 μg/mL, 64 μg/mL, 128 μg/mL, or 256 μg/mL per plate, respectively.

2.8. DNA extraction

DNA was extracted using the boiling method. Bacterial colonies were suspended in 300 μL of distilled water and boiled for 10 minutes in a heat block, then placed on ice for 5 minutes. After centrifugation at 13,000 × *g* at 4°C for 5 minutes, the supernatant was placed in a microtube and kept at 4°C until used as a DNA template [15].

2.9. Amplification of mecA and PVL genes

The *mecA* gene was amplified as described previously [16], using primers *mecA*-F: 5′-AAAATCGATGGTAAAGGTTGGC-3′ and *mecA*-R: 5′-AGTTCTGCAGTACCGGATTTTGC-3′. The DNA of the *S. aureus* ATCC 43300 and ATCC 25923 strains was used as positive and negative controls, respectively, for this polymerase chain reaction (PCR) assay of *mecA*. The PCR assay was performed in a total volume of 25 μL containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.25 mM each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), and 1 U of *Taq* DNA polymerase (Promega Corporation, USA). DNA amplification was carried out using the following thermal cycling profile: initial denaturation at 95°C for 10 minutes, 35 cycles of amplification (denaturation at 95°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute), and a final extension at 72°C for 10 minutes in a thermal cycler (Mastercycler gradient, Eppendorf AG, Germany). PCR products were analyzed on 1.5% agarose gel with 0.53 Tris-borate-EDTA buffer. A 100-bp DNA ladder (Promega Corporation) was used