Phenotypic and Genotypic Isolation of MRSA from Burn Wound Infected Patients at a Tertiary Care Hospital in Bangladesh

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Abstract

Background: Patients of burn wound are generally more susceptible to infection due to concurrent status of malnutrition and immunosuppression. Among many bacteria, MRSA has appeared as a challenging pathogen irrespective of effective antibiotic therapy. **Objective:** The purpose of the present study was to detect the prevalence and antibiogram of MRSA in burn wound infected patients. **Methodology:** The present cross sectional study was conducted at Department of Microbiology in Rajshahi Medical College, Rajshahi, Bangladesh. The samples were collected from Burn and Plastic Surgery Unit of Rajshahi Medical College Hospital, for a period of one year. Phenotypic detection of MRSA were done by cefoxitin disc diffusion method and genotype (*mecA* gene) was detected by PCR which was conducted at Dhaka Medical College, Dhaka among all the *S. aureus* isolates. **Result:** A total of 212 wound swabs were subjected to bacteriological culture media for isolation of MRSA and other organisms. As a whole 89.62% samples yielded growth in culture of which *Pseudomonas aeruginosa* was the predominant pathogen (34.18%). Out of 21 *S.aureus* isolates, 07(33.33%) were detected as MRSA by cefoxitin resistance and presence of *mecA* gene. Although over 85% MRSA were resistance to ciprofloxacin and ceftriaxone, but the present study could not detect any vancomycin resistant MRSA. **Conclusion:** The high prevalence and decreased sensitivity of MRSA to commonly used antibiotics has been observed in Burn Unit of RMCH. Therefore it is necessary to establish an antimicrobial surveillance system in hospital settings to prevent the spread of MRSA. [*Bangladesh Journal of Infectious Diseases, June 2020;7(1):12-17*]

Key words: MRSA; *Staphylococcus aureus*; *mecA* gene; cefoxitin; antibiotic susceptibility

Introduction

Infections caused by *Staphylococcus aureus*, especially methicillin-resistant *Staphylococcus aureus* (MRSA), are emerging as a major public health problem in hospital and community settings. The emergence and spread of both health care and community-associated MRSA has made infection control intervention and treatment challenging[1]. According to WHO, among 12 deadliest drug resistant bacteria MRSA is within the high priority group and is the commonest pathogen in the department of resuscitation, burn and traumatology[2]. Burn wards within hospital have become a significant reservoir for MRSA that have special characteristic of spreading quickly in hospital environment and causing outbreaks of infection[3].

In Bangladesh, the isolation rates of methicillin-resistant *Staphylococcus aureus* in hospitals at different cities were reported as 32-62%, which is a high incidence comparable to the United States and European countries[4]. Different studies showed that MRSA was 56.7% in India[5], 36.1% in Pakistan[6], and 34.1% & 22.5% in Bangladesh[7,8]. In 2004, Centers for Disease Control and prevention (CDC) reported that MRSA was responsible for more than 50% of all health care associated *Staphylococcus aureus* infections within the United States[8].

Methicillin-resistant *Staphylococcus aureus* has evolved after acquiring the *mecA* gene that encodes the penicillin-binding protein 2a which confers resistance to methicillin and other b-lactam antibiotics. 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 b-lactam resistance. Detection of the *mecA* gene by PCR is the gold standard for identifying MRSA but this test is not available in many clinical laboratories in developing countries and is relatively expensive[7]. Apart from the molecular methods, cefoxitin disk diffusion test is the most accurate phenotypic test[9]. It is better inducer of the *mecA* gene and provide more reproducible and accurate results than tests with oxacillin[10].

Due to arbitrary use of antibiotics, common pathogen develops resistance against frequently used drugs. Early detection of methicillin-resistant *Staphylococcus aureus* and formulation of effective antibiotic policy in tertiary care hospitals is of paramount importance from the epidemiological point. Therefore the present study was designed to provide an insight into the prevalence of MRSA and its antimicrobial susceptibility pattern in burn wound infection.

Methodology

This cross sectional study was conducted among 212 burn wound infected patients admitted in Burn and Plastic Surgery Unit of RMCH from January 2016 to December 2016. Patients who have history of burn >3 days irrespective of age, sex and duration of hospital stay were included in the present study. All relevant information's were recorded into a predesigned data sheet. Microbiological and molecular methods were performed in Microbiology Department of Rajshahi Medical College and Microbiology Department of Dhaka Medical College respectively. The collected samples were inoculated in nutrient agar media and blood agar media at 37°C for 24 hours.

Isolation of *Staphylococcus aureus:* Suspected colonies of *Staphylococcus aureus* were sub-cultured on mannitol salt agar at 35°C for 24 hours. *Staphylococcus aureus* was identified by observing hemolysis on blood agar media, golden yellow pigment on nutrient agar media, Gram's staining, positive catalase and coagulase tests, and mannitol fermentation on mannitol salt agar media[11].

Detection of MRSA by cefoxitin disc diffusion method: Screening for methicillin-resistance was determined using the Kirby-Bauer disc diffusion method[10] with 30 μg cefoxitin discs. According to the Clinical and Laboratory Standards Institute (CLSI) – 2015[12], a zone of growth inhibition around the cefoxitin disk of ≥ 22 mm ruled out MRSA; a zone size ≤ 21 mm indicated that the *mecA* gene is present and the isolate was reported as MRSA. Cefoxitin was used in place of oxacillin to detect MRSA as it is better inducer of the *mecA* gene, and test using cefoxitin give more reproducible and accurate results than tests with oxacillin[10].

Detection of *mecA* gene by PCR

DNA Extraction: DNA was extracted using the boiling method. Bacterial colonies were suspended in 300 mL of distilled water and heated for 10 minutes in a heat block, then immediately placed on ice for 5 minutes. After centrifugation at 14,000 rmp at 4°C for 5 minutes, the supernatant was placed in eppendorf tube and preserved at -12°C until used as a DNA template[13].

Amplification of *mecA* gene: The mecA gene was amplified using the following primers (Table 1).

Table 1: PCR Primer Sequence, Amplification Size and PCR condition

Gene	Primer Sequence	Size of amplified product (base pair)	Annealing temperature	Cycle
mecA	5´-AAAATCGATGGTAAAGGTTGGC-3´ 5´-AGTTCTGCAGTACCGGATTTTGC-3´	533 bp	55^{0}C	32

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 of mixture that was prepared with 12.5 µl of master mix-a mixture of dNTP, taq polymerase, Mgcl$_2$, and PCR buffer (Promega corporation, USA), 2 µl of forward primer, 2 µl of reverse primer, 2 µl of extracted DNA template and 6.5 µl of nuclease free water in a PCR tube. After a brief vortex, the tube was centrifuged in a micro centrifuge machine for few seconds. DNA amplification was carried out using the following thermal cycling profile: initial denaturation at 95˚C for 10 minutes, 32 cycles of amplification (denaturation at 95˚C for 1 minute, 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 as the molecular size marker. The gels were stained with 1% ethidium bromide and visualized under UV light[7].

Antimicrobial Susceptibility Testing: Standard disc diffusion techniques as recommended by the Clinical Laboratory Standards Institute (CLSI), were performed for susceptibility testing of amikacin, gentamycin, ciprofloxacin, ceftriaxone, ceftazidime, amoxiclav, cloxacillin, meropenem and vancomycin (oxoid, UK) for all *S. aureus* and MRSA.

Results

A total of 212 wound swabs were collected among which 89.62% were culture positive and 10.38% were sterile (Table 2).

Table 2: Culture positivity of isolated organisms.

Culture	Single isolated cases	Multiple isolated case
Positive	184 (86.79%)	06(2.83%)
Negative	0(0.0%)	0(0.0%)

Among the culture positive isolates *Pseudomonas aeruginosa* was predominant (34.18%) followed by

Proteus spp. (23.47%), *Klebsiella* spp. (14.80%), *Escherichia coli* (11.73%), *Staphylococcus aureus* (10.731%), *Acinetobacter* spp. (2.04%), CoNS (2.04%) and *Enterobacter* spp. (1.03%) (Table 3).

Table 3: Identified Aerobic Bacteria from Burn Wound Infection Cases (n=196)

| Isolated Organisms	Single Isolation	Multiple Isolation	Total
P. aeruginosa	63	4	67(34.2%)
Proteus spp.	45	1	46(23.5%)
Klebsiella spp.	26	3	29(14.8%)
Escherichia coli	19	4	23(11.7%)
S. aureus	21	0	21(10.7%)
Acinetobacter spp.	4	0	4 (2.0%)
CoNS	4	0	4 (2.0%)
Enterobacter spp.	2	0	2(1.0%)

S. aureus = Staphylococcus aureus; P. aeruginosa = Pseudomonas aeruginosa

Out of 21 *S. aureus* isolates, 7(33.3%) were detected as MRSA by cefoxitin disc diffusion method and PCR (Table 4).

Figure I: Figure showing growth of *S. aureus* in mannitol salt agar media

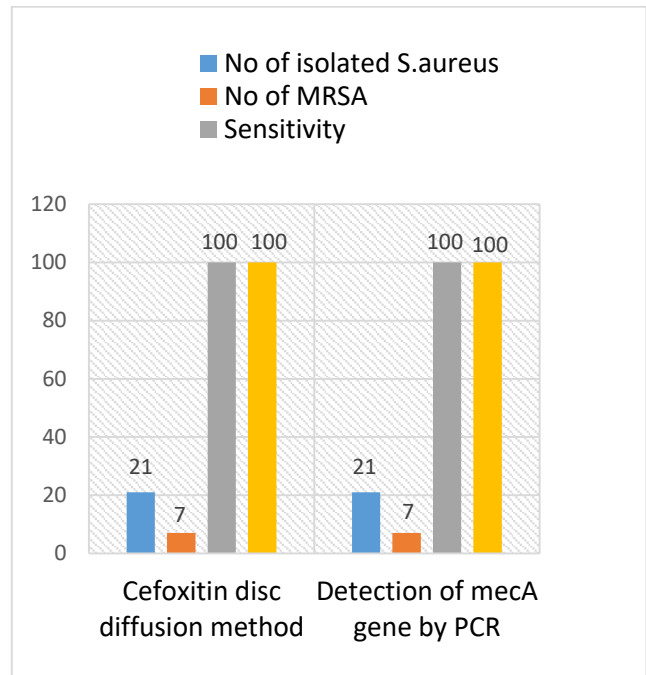**Table 4: Frequency of MRSA in Burn Wound Infection (n=196)**

Identified *S. aureus*	Identified MRSA strains	
	Cefoxitin disk Diffusion test	PCR
21(10.71)	7(33.3%)	7(33.3%)
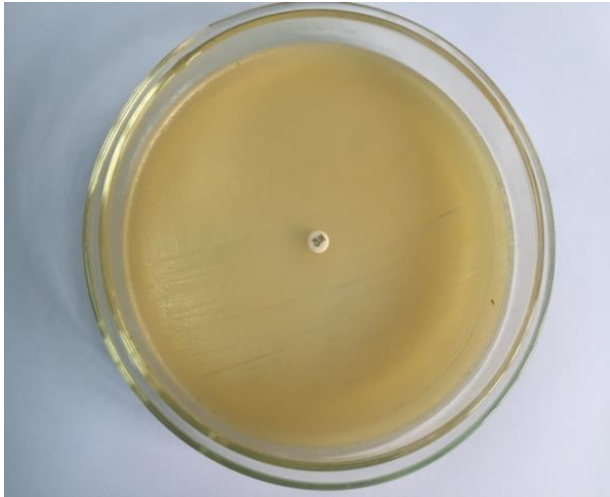
Figure II: MRSA showing cefoxitin resistance in cefoxitin disk diffusion test
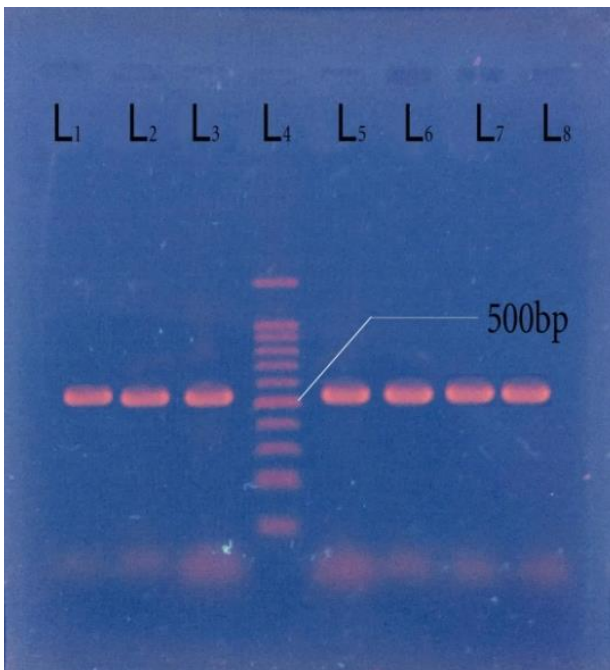
Figure III: Photograph shows bands of amplified DNA of *mecA* gene; Lane 1,2,3: *mecA* gene; Lane 4: 100 bp DNA ladder; Lane 5,6,7,8: *mecA* gene

Figure IV: Figure showing sensitivity and specificity of disc diffusion method and PCR for detection of MRSA

Regarding antimicrobial susceptibility MRSA were highly resistant to commonly used antibiotics like ciprofloxacin and ceftriaxone (85.711%) but there was no vancomycin resistant MRSA.

Table 4: Antimicrobial Susceptibility Pattern of *S. aureus* and MRSA

Antimicrobial Agents	*Staphylococcus aureus*	MRSA strains
	Resistance	Resistance
Amikacin	42.86 %	57.14 %
Gentamycin	42.86 %	71.43 %
Ciprofloxacin	47.62 %	85.71 %
Ceftriaxone	42.86 %	85.71 %
Ceftazidime	57.14 %	57.14 %
Amoxiclav	33.33 %	42.86 %
Cloxacillin	85.71 %	100 %
Meropenem	4.76 %	14.29 %
Vancomycin	00 %	00 %

Discussion

Methicillin resistant *Staphylococcus aureus* is a very alarming pathogen creating a huge clinical burden in both community and hospital infections worldwide due to its increased virulence and continuous growing antibiotic resistance[6]. Apart from using the molecular method to detect *mecA* gene, which is gold standard, the most accurate phenotypic test is cefoxitin disk diffusion test[14].

In the present study 89.62% samples yielded growth out of 212 wound swabs. Among 21 isolated *S.aureus* 33.33% were identified as MRSA by both phenotypic and molecular method, which correlates with previous reports from other regions of Bangladesh[4,8], Pakistan[6], and USA[15]. However, some studies have reported much higher rates of 56 to 68%[5,16]. Existing literature on MRSA have demonstrated that there is a significant geographical variation in the frequency of the pathogen within and between countries[17].

The increasing prevalence of MRSA is a threat to manage cases all over the world, including Bangladesh. As described in other studies[18,19], MRSA isolates in the present study were resistant to most commonly used antibiotics. More than 85% MRSA isolates were resistant to ciprofloxacin and ceftriaxone which was consistent with studies done in Iran and Pakistan[20,21]. This increased resistance may be due to extensive use of 3rd generation cephalosporin and other β-lactam drugs.

All the MRSA isolates, in this study were sensitive to vancomycin followed by meropenem (85.71%), similar to other studies[5,6]. This suggests that these drugs could be suitable treatment options. As these drugs are expensive and sometimes not available in the local pharmaceutical market, treatment options in Bangladesh are limited for infections caused by MRSA.

In the present study, both the sensitivity and specificity of the cefoxitin disc diffusion method was 100% when compared with PCR and these results are similar to those in other studies[22,23].

Conclusion

The present study revealed huge bacterial proliferation with high frequency of MRSA in burn wound infection focusing the emerging situation in Bangladesh. Multidrug resistant MRSA has also been observed which highlights the need for applying efficient infection control measures and effective antibiotic therapy. Moreover, the results of this study also emphasize the use of cefoxitin disk diffusion test as accurate phenotypic method in clinical laboratories if PCR for *mecA* gene detection is not feasible.

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