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ORIGINAL ARTICLE

Prevalence of Methicillin-Resistant *Staphylococcus aureus* in Diabetic Foot Ulcers at Dhaka Medical College Hospital

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ABSTRACT

Background: *Diabetic foot ulcer is a significant global health burden. These ulcers are more common in patients with long-standing or poorly controlled diabetes. A major concern in the management of these ulcers is the emergence of antibiotic-resistant pathogens, notably Methicillin-Resistant Staphylococcus aureus (MRSA). MRSA colonization in these ulcers has been linked to delayed healing, increased healthcare costs, and other complications. This study aimed to determine the prevalence of Methicillin-Resistant* Staphylococcus aureus *(MRSA) among patients with diabetic foot ulcers.* ***Methods & Materials:*** *This cross-sectional study was conducted in the Microbiology Department of Dhaka Medical College, Bangladesh, from January 2024 to December 2024. Staphylococcus aureus was isolated by culture, microscopy and different biochemical tests. MRSA was isolated by cefoxitin disc diffusion method using Kirby-Bauer technique and MIC of oxacillin by agar dilution method. PCR for mecA gene was done.* ***Results:*** *Out of 35 Staphylococcus aureus, 34.29% were identified as Methicillin-Resistant Staphylococcus aureus by cefoxitin disc diffusion method and oxacillin agar dilution method. All MRSA isolates were confirmed by detection of mecA gene.* ***Conclusion:*** *The study shows a high prevalence of MRSA in diabetic foot ulcers; therefore, early diagnostic facilities should be made available in both government and non-government hospitals, and appropriate treatment protocols must be followed.*

Keywords: *MRSA; mecA gene; Staphylococcus aureus; Diabetic Foot Ulcer*

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INTRODUCTION

Diabetes mellitus is a major global health concern. A serious & common complication of diabetes is diabetic foot ulcers, which cause significant morbidity, mortality. A study found that 44.5% of diabetic people in Bangladesh were at risk of developing diabetic foot ulcers[1].

Diabetic foot Infections caused by multidrug-resistant organisms, particularly Methicillin-Resistant *Staphylococcus aureus* (MRSA), is one of the greatest challenges in managing diabetic foot ulcers. MRSA leads to adverse clinical outcomes due to its resistance to multiple antibiotics, especially beta-lactams[2].

MRSA carries the *mecA* gene which encodes an alternative penicillin-binding protein, PBP2a, which has a low affinity for β-lactam antibiotics. The result is that bacterial cell-wall synthesis can continue despite the presence of these antibiotics, which would typically inhibit this process by binding to the native penicillin-binding protein involved in peptidoglycan synthesis[3].

Glycopeptides particularly vancomycin are active against MRSA. They inhibit bacterial cell wall synthesis using a method that differs from that of β-lactams[3].

The global prevalence of MRSA in diabetic foot ulcers varies from 11% to 61%, depending on the region. In South America, it was 61%, in North America 20%, in Europe 19%, in Africa 13% and in Asia 11%. MRSA not only increases the duration of hospital stay but also raises the risk of treatment failure, sepsis and amputation[4].

MATERIALS & METHODS

This was a cross-sectional type of study conducted in the Department of Microbiology of Dhaka Medical College, Dhaka, Bangladesh from January 2024 to December 2024. A total of 334 samples were collected after taking consent. Samples were collected from patients who had diabetic foot ulcers attending Dhaka Medical College Hospital, irrespective of age and sex.

Samples were collected by rubbing the deepest area of the ulcer with a sterile cotton wool tipped swab. *Staphylococcus*

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aureus was identified by Gram staining, catalase test, coagulase test, colony morphology, hemolytic property, pigment production and mannitol fermentation test in mannitol salt agar media as per standard procedures[5].

Antimicrobial drugs	Resistant ≤	Intermediate	Sensitive ≥
Azithromycin (15)	13	14-17	18
Cefoxitin (30)	21	-	22
Ciprofloxacin (5)	15	16-20	21
Co-trimoxazole (25)	10	11-15	16
Clindamycin (2)	14	15-20	21
Clarithromycin (15)	13	14-17	18
Doxycycline (30)	12	13-15	16
Gentamicin (10)	12	13-14	15
Linezolid (30)	22	23-25	26
Teicoplanin (30)	10	11-13	14
Vancomycin (30)	14	15-16	17

Figure 1: Growth of *Staphylococcus aureus* in blood agar media

All *Staphylococcus aureus* isolates were tested for antimicrobial susceptibility testing by modified Kirby-Bauer disk-diffusion technique using Mueller-Hinton agar plates and the zone of inhibition were interpreted according to CLSI guidelines 2024[6].

Staphylococcus aureus ATCC 25923 was used as control strain to assess the performance of the method.

Interpretative zone diameter (in mm) of *Staphylococcus aureus* (CLSI, 2024)

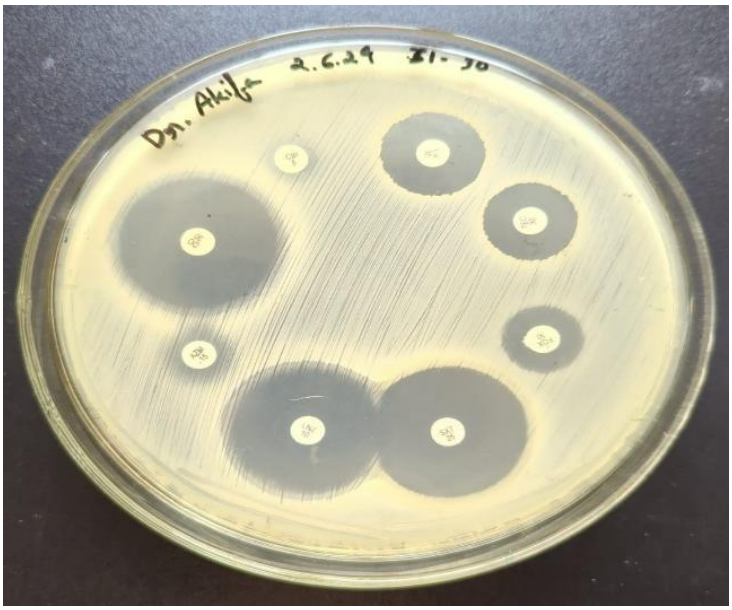

Figure 2: Antibiotic susceptibility pattern of MRSA

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For Minimum Inhibitory Concentration (MIC), each plate was prepared with 50 ml of Mueller-Hinton agar media (MHA)and 1µl, 2µl, 4µl, 8µl, 16µl, 32 µl, 64 µl and 128 µl of oxacillin stock solution was added to 50 ml sterile Mueller Hinton agar to achieve concentration of then 0.5 µg/ ml, 1µg/ ml, 2µg/ ml, 4µg/ ml, 8µg/ ml, 16µg/ ml, 32µg/ ml and 64µg/ ml per plate respectively[8].

For standardization of inoculum, a 0.5 McFarland turbidity standard was prepared (A 1% (v/v) H_2SO_4 solution was prepared by adding 1 ml concentrated sulphuric acid to 99 ml water, and a 1.175% (w/v) $BaCl_2$ solution was made by dissolving 2.35 g barium chloride in 200 ml distilled water. Then 0.05 ml $BaCl_2$ was mixed with 9.95 ml H_2SO_4, transferred to a screw-cap tube, and stored as a McFarland 0.5 turbidity standard corresponding to 1.5 × $10^{[8]}$ organisms/ml). The turbidity of bacterial suspension in normal saline was compared with 0.5 McFarland turbidity standards. One µl of 10 times diluted test inoculum was placed on MHA plate and incubated aerobically at 37°C overnight. The lowest concentration of antibiotic impregnated into MHA showing no visible growth on agar medium was considered as MIC of that strain of bacteria[8].

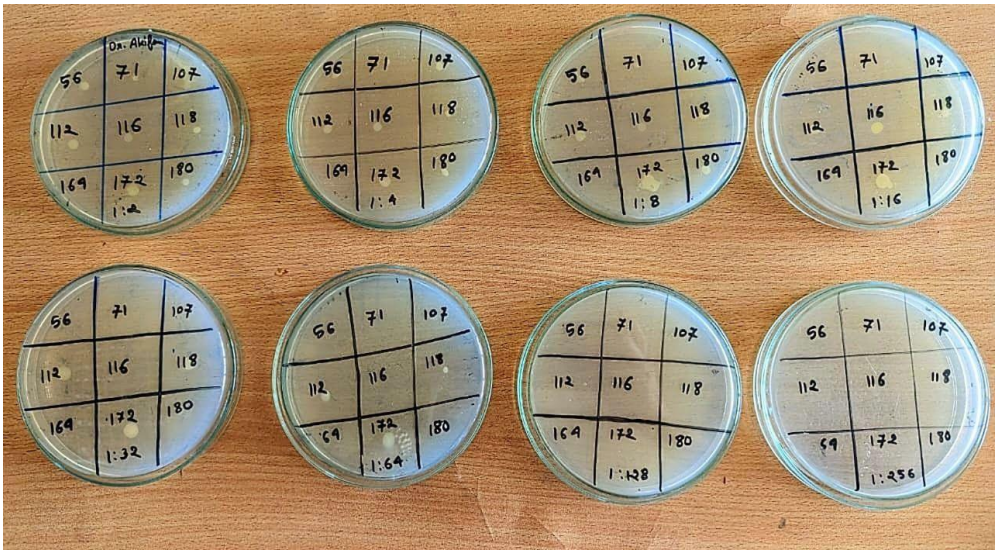

Figure 3: Minimum inhibitory concentration of oxacillin of *Staphylococcus aureus* by agar dilution method.

Procedure of polymerase chain reaction (PCR)

Conventional PCR was performed to confirm MRSA by detecting the *mecA* gene. The following steps were performed for PCR:

a. Bacterial pellet formation
b. DNA extraction
c. Mixing of mastermix and primer with DNA template
d. Amplification through thermal cycler
e. Agarose gel electrophoresis
f. Gel staining and de-staining
g. Visualization and interpretation of results

a. Bacterial pellet formation

Approximately 5 to 6 colonies of specific bacteria were subcultured on Mueller-Hinton agar and incubated at 37°C for 24 hours. A loopful of bacterial colonies was then transferred into a microcentrifuge tube containing trypticase soy broth. After overnight incubation, the tubes were centrifuged at 4,000 rpm for 10 minutes and the supernatant was poured off. The bacterial pellets in the microcentrifuge tubes were stored at -20°C until DNA extraction[9].

b. DNA extraction

For DNA extraction three hundred microliters of distilled water were added to the bacterial pellet and for EDIN genes, Triton X-100 buffer was added to the bacterial pellet then vortexed thoroughly to ensure proper mixing. The mixture was then kept in heat block at 100°C for 10 minutes (DAIHA Scientific, Seoul, Korea). After heating, the tube was immediately placed on ice for 5 minutes and then centrifuged at 14,000 rpm at 4°C for 6 minutes. The supernatant was transferred to a new microcentrifuge tube and used as template DNA for PCR[9].

c. Mixing of mastermix and primer with DNA template

Primers were diluted with Tris-EDTA (TE) buffer as per the manufacturer's instructions. A 25 µl reaction mixture for each sample was prepared by mixing 12.5 µl of master mix (which contained dNTPs, Taq polymerase, $MgCl_2$ and PCR buffer), 1 µl of forward primer, 1 µl of reverse primer (Promega Corporation, USA), 2 µl of DNA template and 8.5 µl of nuclease free water in a PCR tube. The tubes were centrifuged in a mini spin for a few seconds[9].

d. Amplification through thermal cycler

A DNA thermal cycler was utilized for PCR. Initial denaturation at 94°C for 5 minutes, followed by 32 cycles of amplification, including denaturation at 94°C for 50 seconds, annealing at 56°C for 50 seconds and extension at 72°C for 50 seconds. A final extension was performed at 72°C for 10 minutes[10].

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Selected primers [10]:

Primer sequence (5′- 3′)	Target gene	Size (bp)
Forward: AAAATCGATGGTAAAGGTTGGC	mecA	533
Reverse: AGTTCTGCAGTACCGGATTTGC		

e. Agarose gel electrophoresis

PCR products were detected by electrophoresis using 1.5% agarose gel prepared with 1× TBE buffer (Tris-Borate-EDTA). Agarose was dissolved by heating, cooled to 60–70°C, poured into a gel tray with a comb, and allowed to solidify. After removing the comb, amplicons and a 5 µL DNA ladder were loaded into the wells, along with a negative control. Electrophoresis was carried out at 130 V for 35 minutes[9].

f. Gel staining and de-staining

After electrophoresis, the gel was stained with ethidium bromide for 30 minutes. De-staining was done with distilled water for 15 minutes.

g. Visualization and interpretation of results

Under UV trans-illuminator (Gel doc, Major science, Taiwan), the gel was observed for DNA bands. The DNA bands were detected based on molecular size using molecular weight marker (100 bp DNA ladder) loaded in a separate lane. Samples showing the corresponding bp band were regarded as positive for that organism[9].

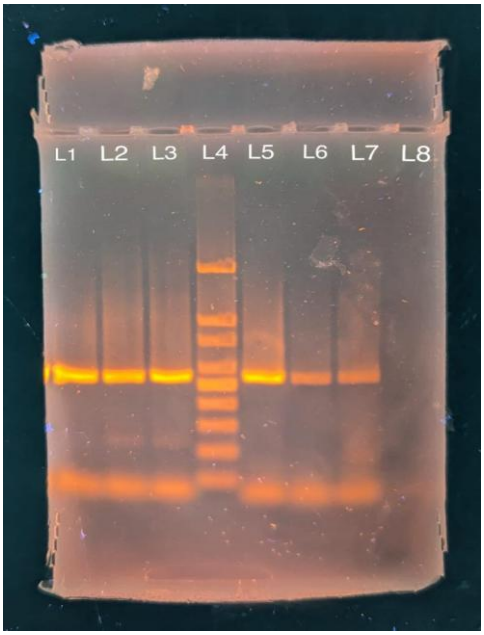

Figure 4: Photograph of amplified DNA of 533 bp for *mecA* gene (Lane 1,2,3,5,6 and 7). *Staphylococcus aureus* ATCC 25293 (negative control) (Lane 8). Hundred bp DNA Ladder (Lane 4)

RESULTS

Out of 334 samples, 318 bacteria were isolated. *Staphylococcus aureus* was found in 35 samples. Isolated *Staphylococcus aureus* showed 65.71% sensitive to cefoxitin. About 94.29% sensitive to vancomycin and 100% sensitive to Linezolid (Table I). Among 35 isolated *Staphylococcus aureus,* 12 (34.29%) were resistant to cefoxitin and 23 (65.71%) were sensitive to cefoxitin by disc diffusion method (Fig.5). MIC of oxacillin among *Staphylococcus aureus*, 12 were detected as MRSA where 3 (8.57%) had MIC of 128 µg/ml, 2 (5.72%) had MIC of 64 µg/ml, 3 (8.57%) had MIC of 24 µg/ml, 4 (11.43%) had MIC of 16 µg/ml (Table II). Among 12 MRSA isolates, *mecA* gene was found in 100% cases (*Table III*).

Table I: Antibiotic susceptibility pattern of isolated *Staphylococcus aureus* by disc diffusion method (*n*=35)

Antibiotics	Sensitive	Resistant
Azithromycin	8 (22.86%)	27 (77.14%)
Ciprofloxacin	9 (25.72%)	26 (74.28%)
Clindamycin	15 (42.86%)	20 (57.14%)
Cotrimoxazole	17 (48.58%)	18 (51.42%)
Clarithromycin	17 (48.58%)	18 (51.42%)
Cefoxitin	23 (65.71%)	12 (34.29%)
Doxycycline	26 (74.29%)	9 (25.71%)
Gentamycin	26 (74.29%)	9 (25.71%)
Teicoplanin	32 (91.43%)	3 (8.57%)
Vancomycin	33 (94.29%)	2 (5.71%)
Linezolid	35 (100.00%)	0 (0.00%)

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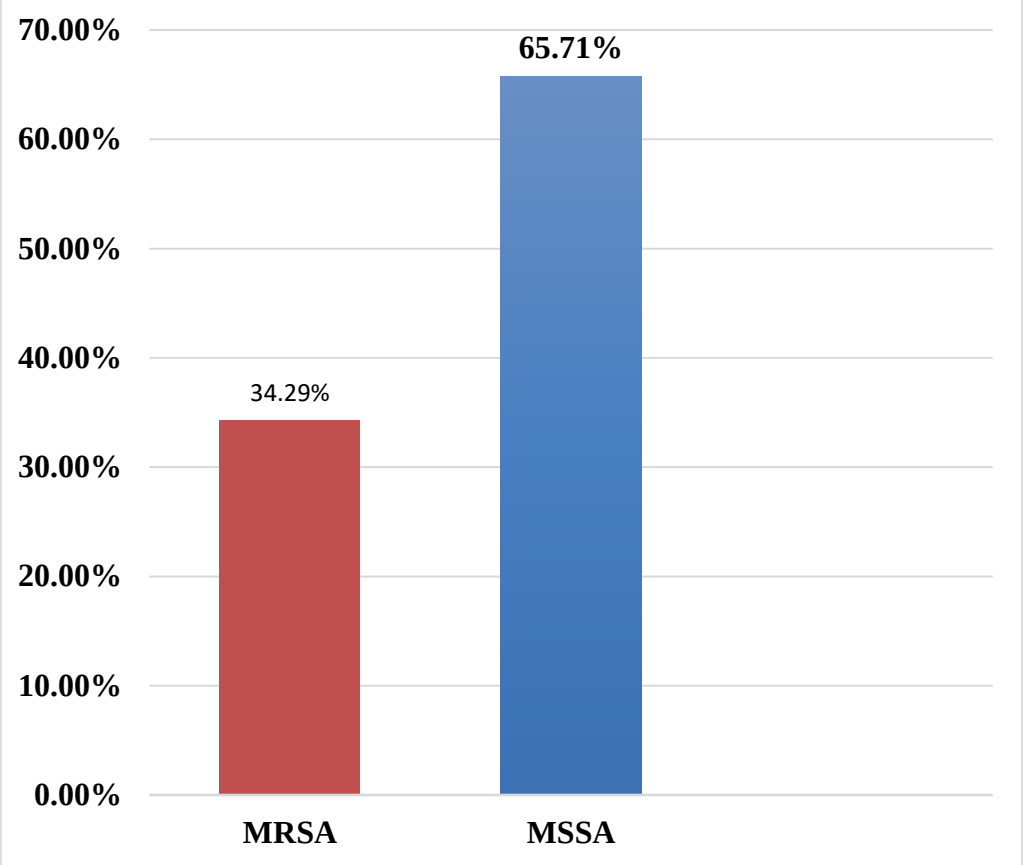

Figure 5: Detection rate of MRSA and MSSA among isolated *Staphylococcus aureus* by cefoxitin disc diffusion method (*n*=35)

Table II: MIC of oxacillin among *Staphylococcus aureus* (*n*=35)

MIC of oxacillin (µg/ml)	Number	Percentage (%)
128	3	8.57%
64	2	5.72%
32	3	8.57%
16	4	11.43%
8	0	0.00%
4	0	0.00%
2	10	28.57%
1	13	37.14%
Total	35	100.00%

CLSI break point of MIC of oxacillin for *Staph. Aureus; Sensitive ≤2µg/ml; Resistant ≥4µg/ml*

Table III: Detection of *mecA* gene by PCR among MRSA detected by cefoxitin disc diffusion and MIC methods (*n*=12)

METHODS of MRSA detection	Number	mecA gene	
		Positive n (%)	Negative n (%)
Cefoxitin disc diffusion	12	12 (100.00%)	0(0.00%)
MIC of oxacillin	12	12 (100.00%)	0(0.00%)

DISCUSSION

MRSA can be detected by various methods. In this study, both the cefoxitin disc diffusion and oxacillin agar dilution methods were used to detect MRSA phenotypically. Detection of *mecA* gene by PCR is considered as a gold standard method[11]. PCR confirmed the presence of the *mecA* gene in these isolates. This study is almost similar to another study that also reported that both the cefoxitin disc diffusion method and oxacillin agar dilution method correlated with the presence of the *mecA* gene[12]. Cefoxitin is a potent inducer of the *mecA* regulatory system and is being widely used as a marker for *mecA* gene-mediated methicillin resistance[13].

In this study, MRSA was identified in 34.29% of cases. Previous study found that MRSA prevalence rates of 27.5% in diabetic foot ulcers which is similar but slightly lower than the present study[14]. In contrast, another study reported 41.6% of MRSA. These variations are due to hospital settings and antibiotic misuse[15].

Early and accurate identification of MRSA is very important to guide appropriate antimicrobial therapy and prevent complications such as delayed wound healing, increased risk

of amputation, and systemic infection. Diabetic patients should be educated on proper foot hygiene to reduce the risk of foot ulcers and infections.

A limitation of this study is that the *mecC* gene of MRSA was not included in the molecular analysis. MRSA strains carrying *mecC*-mediated resistance may have been missed.

CONCLUSION

The study showed a significant prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in diabetic foot ulcers. Phenotypic methods, including cefoxitin disc diffusion and oxacillin agar dilution, were consistent with PCR for detection of *mecA* gene. Proper identification of MRSA and appropriate antibiotic use are essential for management of diabetic foot infection.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

FINANCIAL DISCLOSURE

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