

## Original Article

# Antimicrobial Susceptibility and Coagulase Typing of MRSA Strains at Mymensingh Medical College

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### Abstract

Antimicrobial susceptibility and coagulase typing of *Staphylococcus aureus* isolates, with particular emphasis to Methicillin Resistant *S. aureus* (MRSA), were studied among strains isolated from various types of specimens collected at Mymensingh. A newly developed panel of anti-sera against different coagulase enzymes was used for coagulase typing. The study included 79 strains of *S. aureus* and of those, 40 were identified as MRSA on the basis of resistance to oxacillin (1 gm) discs. The rate of resistance of *S. aureus* to different antibiotics observed: penicillin (88.61%), oxacillin (48.10%), cephadrine (44.30%), gentamicin (27.85%), erythromycin (34.18%), fusidic acid (25.0%) and rifampicin (20.0%). None of the isolates showed vancomycin resistance. Both MRSA and non-MRSA strains were found belonging to Coagulase type VI. Drug resistance pattern and coagulase type of MRSA strains indicated a homogenous epidemiologic origin that needs further exploration including strains from different geographic locations.

**Key words:** MRSA, Coagulase type, Antimicrobial resistance

### Introduction

*Staphylococcus aureus* is well-known as one of the principal human pathogen, though 30% healthy individual carries it as normal flora of skin and nasal mucosa. It is responsible for various infections either in hospital or in community. Almost all strains of *S. aureus* were sensitive to penicillin in the year 1940 but currently, >95% of the strains were reported penicillin resistant.<sup>1</sup> The beta-lactamase enzyme that degrades beta-lactam ring as well as mutation in penicillin binding proteins (PBP) are known to be responsible for penicillin resistance. The *S. aureus* normally produces coagulase that clots plasma by converting fibrinogen to fibrin and is necessary for pathogenesis. Two types of coagulase are

produced: free and bound. Free coagulase converts fibrinogen to fibrin by activating a coagulase reacting factor (CRF) present in plasma, whereas bound coagulase (clumping factor) converts fibrinogen to fibrin without requiring a CRF.<sup>2</sup> Free coagulase has at least 8 antigenic types (I-VIII) which are determined by clot inhibition reaction of rabbit plasma in presence of specific antiserum.<sup>3</sup> Isolates of *S. aureus* showing resistance to penicillinase-resistant penicillinase, exemplified by methicillin, are known as methicilline-resistant *S. aureus* (MRSA). One study in Okinawa, Japan, found high rates (54.3%) of MRSA among isolates of *S. aureus* with majority (65.2%) coagulase type II, followed by Coagulase type III (12.7%). This study indicated an increase of about 17% in the MRSA isolation rate with a concomitant increase of 11% in the coagulase type II and a decrease of 27% of coagulase type III, considering a similar study previously carried out in 1989.<sup>4</sup>

Infections due to *S. aureus*, including the MRSA strains, has long been common in Bangladesh. Because, indiscriminate

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use of antibiotics being a common practice, hospital environments are not adequately hygienic as well as over crowding of patients and attendants favour spread of the infectious agents including *S. aureus*. Hospital acquired infections, either in surgery or in medical wards, prolongs the stay of the patients, keeping under antibiotic therapy in the hospital. All of the factors found involved in healthcare facilities have been proved enough for acquisition of resistance among *S. aureus* strains. Current status of the drug-resistance among *S. aureus* strains in the local settings needed to be explored. Coagulase type of the Staphylococci strains is related with drug sensitivity pattern, geographical distribution, risk factors and role in pathogenesis of the organism. But no such study, particularly on coagulase typing of MRSA, was as yet undertaken or reported in our country. Having the described background, the study was carried out to see current resistance pattern of the MRSA strains with antigenic type of coagulase, to have an assumption on epidemiologic origin.

**Methods**

This observational study was done in the department of Microbiology, Mymensingh Medical College, during the period from March, 2004 to February, 2005. Cases were selected from Inpatient Departments of Mymensingh Medical College Hospital (MMCH) and Community Based Medical College Hospital (CBMCH), Mymensingh, having history and evidence of long-term infections, showing clinically no response to multiple courses of antibiotic.

A total of 105 pathogenic strains of bacteria were isolated from 550 various clinical specimens like pus, wound swabs, exudates of burn ulcer, using appropriate culture media. The isolates of *S. aureus* were identified by standard techniques (colony morphology, Gram's stain, biochemical test and antibiogram) and by subculture in Mannitol salt agar.<sup>5</sup> Isolates of *S. aureus* were put into antibiotic susceptibility test by Kirby-Bauer disk diffusion technique, using panel of antibiotics as per recommendation of National Committee for Clinical Laboratory Standards, USA.<sup>6</sup>

Coagulase typing of *S. aureus* strains was done using "Coagulase typing kit" (neutralization test with type-specific antisera). The procedure described in the kit manual was strictly followed for the purpose.

Briefly the procedure of coagulase typing followed was: (i) Preparation of coagulase antigen solution: One colony of *S. aureus* isolate was inoculated in Brain Heart Infusion broth (5 ml in a test tube). The inoculated isolate was incubated at 37°C aerobically. Finally, the tube was centrifuged at 2000-3000 rpm for more than 5 minutes. Supernatant was used as the test specimen of "Coagulase antigen solution". (ii) Coagulase typing: 9 test tubes were arranged in tube rack per specimen. An amount of 50µl of "coagulase antigen solution" was taken into each of the 9 tubes. Into the 1st tube, 50µl of anti-type I immune serum was added. Similarly, 50µl of anti-coagulase type II-VIII immune sera were put into the 2nd to 8th tubes. In the final (9th) tube, 50µl of diluted normal rabbit sera (negative control) was added. The test tubes were stirred with the mixer (two to three seconds) and then all tubes were incubated at 37°C for 1 hour. An amount of 100µl of diluted normal rabbit plasma was added into all the tubes. After stirring all the tubes with tube mixer, the tubes were kept at 37°C. Judgment of coagulase type was done after 1 hour. In undetermined case, judgment was done 2, 4, 24 or 48 hours after addition of the rabbit plasma. Test tubes were declined and clotting was observed in each test tube, including the negative control (the 9th tube, normal rabbit serum).

**Result**

This study included 550 various clinical specimens for aerobic culture and 105 (19.1%) strains of different pathogenic bacteria were isolated which included 79 *Staphylococcus aureus*. Majority of the specimens comprised of wound swab (459, 83.45%) showing culture positive results in 74 (16.1%). (Table I)

Table I: Culture results of the clinical specimens

Specimens □	Culture positive □	Culture negative
Wound swab (n=459) □	74 (16.1 %) □	385 (83.9 %)
Pus (n=65) □	20 (30.8 %) □	45 (69.2 %)
Aural swab (n=10) □	02 (20.0 %) □	08 (80.0 %)
Exudates from □ Diabetic ulcer (n=8)	05 (62.5 %) □	03 (37.5 %)
Exudates from □ burn ulcer (n=8)	04 (50.0 %) □	04 (50.0 %)
Total (550) □	105 (19.1%) □	445 (80.9 %)

Distribution of 105 bacterial isolates among the specimens shows that *S. aureus* remains at the top of the list (75.24%),

followed by *Escherichia coli* (14.28%) and *Pseudomonas aeruginosa* (10.48%). (Table II)

Table II: Distribution of bacterial isolates

Sl No	Type of organisms	Number	Percentage
1	<i>Staphylococcus aureus</i>	79	75.24
2	<i>Escherichia coli</i>	15	14.28
3	<i>Pseudomonas aeruginosa</i>	11	10.48
4	Total	105	

Out of 79 *S. aureus*, 40 (50.63%) were MRSA, majority (62.5%) of which were isolated from wound swabs, followed by exudates of diabetic (33.3%) and burn (33.3%) ulcers, and pus (20.0%). (Table III)

Table III: Specimen-wise distribution of *Staphylococcus aureus* and the MRSA strains

Type of specimen	No. of <i>S. aureus</i>	No (%) of MRSA
Wound swab	56	35 (62.50)
Pus	15	03 (20.00)
Aural swab	02	00 (00.00)
Exudates from diabetic ulcer	03	01 (33.33)
Exudates from burn ulcer	03	01 (33.33)
Total	79	40 (50.63)

Antibiotic resistance pattern of 79 *S. aureus* isolates show that the highest number of isolates was resistant to penicillin (88.61%), followed by oxacillin (50.63%), co-trimoxazole (45.57%), cephadrine (44.30%), erythromycin (34.18%) and gentamicin (27.85%). (Table IV)

Table IV: Antibigram of *Staphylococcus aureus* (n=79)

Antibiotics	Sensitive	Resistant
Penicillin	09 (11.39%)	70 (88.61%)
Oxacillin	39 (49.37%)	40 (50.63%)
Cephadrine	44 (55.70%)	35 (44.30%)
Ceftriaxone	62 (78.48%)	17 (21.52%)
Ciprofloxacin	54 (68.35%)	25 (31.65%)
Gentamicin	57 (72.15%)	22 (27.85%)
Cotrimoxazole	43 (54.43%)	36 (45.57%)
Tetracycline	47 (59.49%)	32 (40.51%)
Erythromycin	52 (65.82%)	27 (34.18%)
Azithromycin	68 (86.08%)	11 (13.92%)

Antibiotic resistance pattern of MRSA by disk diffusion method shows that none of 40 MRSA strains was resistant to vancomycin and imipenem, whereas 100% of the strains were resistant to penicillin and oxacillin. (Table V)

Table V: Rate of resistance of MRSA strains (n=40).

Antibiotics	Resistant	Antibiotics	Resistant
Penicillin	40 (100%)	Fusidic acid	10 (25%)
Oxacillin	40 (100%)	Rifampicin	08 (20%)
Ciprofloxacin	18 (45%)	Azithromycin	07 (17.5%)
Erythromycin	18 (45%)	Imipenem	00 (00%)
Gentamicin	14 (35%)	Vancomycin	00 (00%)
Ceftriaxone	13 (32%)		

Coagulase typing of the *S. aureus* shows that all of the MRSA (40) and Non-MRSA (39) strains were coagulase type VI. (Table VI)

Table VI: Coagulase typing of MRSA and Non-MRSA strains

Strains	Coagulase type	No. of isolates
MRSA	type VI	40
Non-MRSA	type VI	39

## Discussion

The MRSA has become increasingly prevalent worldwide. Strengthening surveillance and screening of high-risk individuals appears as an important component of effective infection control program to limit the spread of MRSA in hospitals.

In this study, out of 550 clinical specimens from hospitalized patients, wound swabs were >83%. Similarly, wound swabs as predominant samples in bacteriological laboratory were also reported in other studies of the same institute<sup>7</sup> and from abroad.<sup>8</sup> Higher number of culture of wound specimens might be due to higher number of surgical wound infections in hospitalized patients due to substandard hygienic condition, overcrowding of patients and attendants. In addition, clinical experiences suggested that wound infection is usually caused by multi-drug resistant bacteria. So, to limit morbidity of the surgical patients, clinicians send wound specimens for culture and sensitivity to select proper antibiotic with priority. In the present study, among 105 culture-positive samples, >70% were wound swabs. This finding was in accordance with some other studies.<sup>8,9</sup>

Regarding the distribution of organisms in the present study, very high isolation rate of *S. aureus* (75.24%) was found and majority (53.3%) of them were from wound swabs. This finding was in consistent with Mehta *et al* from Bombay,<sup>10</sup> where isolation rate of *S. aureus* was 73%. Higher incidence of *S. aureus* infection in hospitalized patients could be well explained by the fact that *S. aureus* remains as a skin flora, and the bacteria can easily be transmitted to the site of infection, particularly it was found when the wound is present in the exposed surface of the patient.<sup>11</sup>

The resistance pattern of *S. aureus*, isolated in this study, for the commonly used as well as the NCCLS-recommended antibiotics was as follows: penicillin 88.61%, oxacillin 50.63%, cephadrine 44.30%, co-trimoxazole 45.57%, gentamicin 27.85% and erythromycin 34.18%. Previous reports from the same institute<sup>12</sup> and from abroad<sup>13</sup> also documented almost similar pattern of resistance to beta-lactam antibiotic in *S. aureus*. All MRSA isolates of the present study were sensitive to vancomycin and imipenem but many were resistant to rifampicin (20%) and fusidic acid (25%). The Vancomycin intermediate *S. aureus* (VISA) and vancomycin resistant strains of *S. aureus* (VRSA) had already been reported in many countries.<sup>14</sup> No strains of MRSA of the present study was found resistant to vancomycin and imipenem. Since vancomycin is the reserved drug for treating MRSA infections,<sup>15</sup> it carries a good information to the healthcare personnel.

In this study, antigenic typing of coagulase yielded coagulase type VI for all of the 79 *S. aureus* strains. In other study of similar design at Dhaka Medical College Hospital also found coagulase type VI in all of the *S. aureus* isolates. Different coagulase types were reported in other studies from abroad. As for example, studies conducted in Okinawa, Japan, found coagulase type II and coagulase type III as the most prevalent serotypes comprising of 65.2% and 12.7%, respectively.<sup>4</sup> Another study was done in Tokyo, Japan, in 2000 where type VII strain was isolated from *S. aureus* food poisoning outbreaks.<sup>16</sup> Findings of this study indicated the origin of MRSA from homogenous predecessors.

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