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# Comparison of Antibiotic Profiles of Methicillin Sensitive and Methicillin Resistant *Staphylococcus aureus* Isolated from Different Specimens of Hospitalized Patients in Dhaka City

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**Abstract**

**Background:** *Staphylococcus aureus, resistant to methicillin, has appeared as a pathogen connected with diseases acquired in hospitals and in the community globally.*

**Objective:** *The objective of this study was to compare the methicillin-sensitive and methicillin-resistant Staphylococcus aureus isolated from the different samples of hospitalized patients.*

**Methodology:** *This cross-sectional study was carried out in the Department of Microbiology and Immunology at Bangabandhu Sheikh Mujib Medical University, Dhaka from January 2010 to December 2010 for a period of one (01) year. All patients admitted to hospital were chosen as study population with distinct kinds of diseases. These patients gathered different clinical samples depending on the locations of the diseases. Staphylococcus aureus (S. aureus) was isolated and identified through staining, cultivation and biochemical testing. To detect the methicillin-resistant Staphylococcus aureus, an oxacillin disk diffusion test was performed. PCR wa used to detect the MRSA mecA gene.*

**Results:** *Over the period from January 2010 to December 2010, a total of 120 Staphylococcus aureus (S. aureus) isolates were gathered from 266 samples from three hospitals. Patients with age group 61 to 70 years had the largest proportion of incidence of MRSA. The patterns of antimicrobial susceptibility of 19 MRSA strains were reported on disk diffusion. Multi-resistant among them were 14 (73.7 percent) and non-multi-resistant strains were 5 (26.3 percent). Other than the reserve resorts of drugs gentamycin 14(73.7%) were most sensitive followed by cotrimoxazole 11(57.9%), ciprofloxacin 6(31.6%), erythromycin 6(31.6%) and azithromycin 5(26.3%). The site of infection of MRSA was categorized in seven categories depending on the type of specimens. Here skin and soft tissue infection by MRSA strains were predominant and it was 10(14.3%). The incidence of MRSA in three different hospitals was recorded. In BSMMU the incidence was 11 (13.75%), in DMCH 2 (20.0%) and in Popular Diagnostic Center it was 6 (20.0%).*

**Conclusion:** *In conclusion, majority MRSA are multiresistant but sensitive towards vancomycin and linezolid.*

**Keywords:** *Methicillin sensitive;methicillin resistant; Staphylococcus aureus; hospital-acquired.*

## Introduction

*Staphylococcus aureus* is a hospital as well as community-acquired bacteria which causes a broad spectrum of diseases and this is ranging from skin and soft tissue infections to endocarditis and fatal pneumonia[1]. The pathogenicity of these bacteria is associated with different enzymes and toxins which includes enterotoxins, exfoliative toxin, toxic shock syndrome toxin, and Panton-Valentine leucocidin[2]. This bacterium possess the remarkable ability to adapt to different types of antibiotics and now with the emergence of multi-drug resistant (MDR) bacteria, *S. aureus* is a warning for public health[3]. Methicillin-resistant *S. aureus* (MRSA) strains are able to grow in the presence of methicillin, oxacillin and nafcillin[4].

Resistance to methicillin in S. Initially, aureus was identified in Europe in the 1960s, just one year after methicillin was introduced[5]. MRSA isolates are discovered today not only in most countries ' hospitals, but also in societies, and are often resistant to multiple antibiotics[6]. Clinical infections are most common in patients in hospital intensive care units, nursing homes, and other chronic care facilities; however, MRSAs are emerging as an important community acquired pathogen as well. Although there are some reports on the prevalence of vancomycin resistant *S. aureus* (VRSA) and vancomycin intermediate *S. aureus* (VISA), most MRSA isolates are susceptible to vancomycin and teicoplanin; therefore, resistance increase to these antibiotics results in the limitation of treatment options and also the requirement of a new class of antibiotics[7]. The current research was conducted in this context to compare antibiotic profiles of methicillin-sensitive and methicillin-resistant Staphylococcus aureus isolated from separate samples of hospitalized patients.

## Methodology

This cross-sectional study was conducted in the Department of Microbiology and Immunology at Bangabandhu Sheikh Mujib Medical University, Dhaka from January 2010 to December 2010 for a period of twelve (12) months. Different clinical samples were gathered from patients of both sexes at any era, including wound swab, pus, blood, urine, tracheal aspiration, throat swab, sputum, nasal swab, elevated vaginal swab, burn swab, pleural effusion drain fluid and fluid.All samples were aseptically gathered from three hospitals, namely Bangabandhu Sheik Mujib Medical University (BSMMU), Dhaka Medical College Hospital (DMCH), Dhaka and Dhaka Private Diagnostic Center. The samples were inoculated into appropriate media and were incubated aerobically at $37^0$ C for 24 hours. Then colonies were identified for *Staphylococcus* species and were confirmed by Gram staining, colony morphology, haemolytic status, pigment production, mannitol fermentation test, motility test (MIU) and other relevant biochemical tests like catalase test, coagulase test both slide and tube test as per standard procedure[6]. In this study, screening for MRSA was done by oxacillin and oxacillin screening agar. In case of oxacillin the diameter of zone of inhibition ≤10 mm was taken as resistant[7]**.** Conventional PCR was performed to detect *mecA* gene of 22 suspected *S. aureus* strains resistant to oxacillin by disc diffusion method at the Molecular Laboratory in the Department of Microbiology and National Forensic DNA Profiling Laboratory of Dhaka Medical College, Dhaka. Methicillin-resistant *S. aureus* (MRSA) strain [ATCC 43300] were used as positive control. PCR for *mecA* gene detection were performed by formation of bacterial pellet, DNA extraction, preparation of reaction mixture (25µl) and running in thermo cycler. Primers used for detection of the *mecA* gene producing a 309-bp amplicon were as follows:

*mec*A1-F- 5´ TGGCTATCGTGTCACAATCG 3´ (positions 885 to 905) and

*mec*A2-R- 5´ CTGGAACTTGTTGAGCAGAG 3´ (positions 1174 to 1194)

PCR reactions were performed in a thermo cycler under the following conditions: initial denaturation for 10 minutes at 94°C followed by 30 cycles at 94°C for 1 minute, at 54°C for 1

minute, then at 72°C for 1 minute. Final extension was for 7 minutes at 72°C.Mixed the amplicon and ladder with dye (4-5:1 ratio). Then pipetting and dispensing were done onto the wells on gel made by comb. Start the gel electrophoresis at 100 volt for 60 minutes until the end of the reaction indicated by orange color advancement was over. Ethidium bromide (7.5µl) mixed with distilled water (100 ml). Gel was placed in this mixture for 30 minutes staining. Again destaining done in pure water for 20 minutes. The destained gel was placed on UV transilluminator and observed for the presence of DNA bands. Gels were visualized and photographed under ultraviolet illumination. Precautions were taken to prevent the samples from being contaminated by each other or by the skin of laboratory personnel.

## Results

A total of 120 *Staphylococcus aureus (S. aureus)* isolates were collected from 266 specimens from three different hospitals. The highest percentage of MRSA occurrence was in patients having age group 61 to 70, next of which 51 to 60 years (Table 1).

**Table 1:** Age distribution of the patients having *Staphylococcus aureus* infection including MRSA in three different hospitals (n=120)

| Age Groups | MRSA | MSSA | Total |
|---|---|---|---|
| 0 to 20 Years | 0(0.0%) | 28(100.0%) | 28 (23.3%) |
| 21 to 40 Years | 11(40.0%) | 44(80.0%) | 55(45.8%) |
| 41 to 60 Years | 5 (15.4%) | 21 (84.6%) | 26(21.6%) |
| 61 to 80 Years | 3 (30.0%) | 8 (70.0%) | 11 (8.3%) |
| **Total** | **19(15.8%)** | **101(84.2%)** | **120(100.0%)** |

The antimicrobial susceptibility patterns of 19 MRSA strains on disc diffusion were recorded. Among them 14(73.7%) were multi-resistant and 5(26.3%) were non-multi-resistant strains. Other than the reserve resorts of drugs gentamycin 14(73.7%) were most sensitive followed by cotrimoxazole 11(57.9%), ciprofloxacin 6(31.6%), erythromycin 6(31.6%) and azithromycin 5(26.3%) (Table 2).

**Table 2:** Antimicrobial susceptibility pattern of MRSA strains (n=19)

| Antimicrobial agents | Sensitive | Resistant |
|---|---|---|
| **Penicillin** | | |
| Penicillin | 0 (0.0%) | 19 (100.0%) |
| Ampicillin | 0 (0.0%) | 19 (100.0%) |
| Cloxacillin | 2 (10.5%) | 17 (89.5%) |
| Oxacillin | 3 (15.8%) | 16 (84.2%) |
| **Cephalosporin** | | |
| Cefoxitin | 0 (0.0%) | 19 (100.0%) |
| Cephradin | 2 (10.5%) | 17 (89.5%) |
| **Aminoglycoside** | | |
| Gentamycin | 14 (73.7%) | 5 (26.3%) |
| **Quinolone** | | |
| Ciprofloxacin | 6 (31.6%) | 13 (68.4%) |
| **Carbapenem** | | |
| Imipenem | 19(100.0%) | 0 (0.0%) |
| **Macrolid** | | |
| Erythromycin | 6(31.6%) | 13(68.4%) |
| Azithromycin | 5(26.3%) | 14(73.7%) |
| **Lymphosamide** | | |
| Clindamycin | 17(89.4%) | 2(10.6%) |
| **Sulphonamide+Trimeth.** | | |
| Cotrimoxazole | 11(57.9%) | 8(42.1%) |
| **Glycopeptide** | | |
| Vancomycin | 19(100.0%) | 0(0.0%) |
| **Oxazolidinon** | | |
| Linezolid | 19(100.0%) | 0 (0.0%) |

The site of infection of MRSA was categorized in seven categories depending on the type of specimens. Here skin and soft tissue infection by MRSA strains were predominant and it was 10(14.3%) (Table 3).

**Table 3:** Distribution of MRSA isolates according to site of infection (n=120)

| Sites of infection | MRSA | MSSA | Total | P Value |
|---|---|---|---|---|
| Blood stream infection | 3(50.0%) | 3 (50.0%) | 6(100.0%) | 0.001 |
| Respiratory tract infection | 1(100.0%) | 0 (0.0%) | 1(100.0%) | |
| Urinary tract infection | 3(10.0%) | 27 (90.0%) | 30(100.0%) | |
| Skin and soft tissue infection | 10(14.3%) | 60 (85.7%) | 70(100.0%) | |
| Genital tract infection | 1(50.0%) | 1 (50.0%) | 2(100.0%) | |
| ENT infection | 1(11.1%) | 8 (88.9%) | 9(100.0%) | |
| Other body fluid infection | 0(0.0%) | 2 (100.0%) | 2(100.0%) | |
| **Total** | 19(15.8%) | 101(84.2%) | 120(100.0%) | |

The incidence of MRSA in three different hospitals was recorded. In BSMMU the incidence was 11 (13.75%), in DMCH 2 (20.0%) and in Popular Diagnostic Center it was 6 (20.0%) (Table 4).

**Table 4:** Incidence of MRSA and MSSA isolates in three different hospitals (n=120)

| Name of Hospitals | MRSA | MSSA | Total | P value |
|---|---|---|---|---|
| BSMMU | 11 (13.75) | 69 (86.25) | 80(100.0%) | 0.0024 |
| DMCH | 2 (20.0) | 8 (80.0) | 10(100.0%) | |
| Private Diagnostic Centre | 6 (20.0) | 24 (80.0) | 30(100.0%) | |
| **Total** | **19 (15.8)** | **101 (84.2)** | **120**(100.0%) | |

**Discussion**

*S. Aureus* is the leading cause of infection in hospitalized patients and the second leading cause of outpatient infection[8]. Today, *S. aureus* is the leading cause of nosocomial pneumonia and the second leading cause of bloodstream infections in the world[9]. MRSA is also dominant in intensive care unit (ICU) of hospitals in most parts of the world[10].

Around 20.0 percent of healthy individuals are persistent S carriers. Aureus and 60.0 percent are carriers that are intermittent. In patients with haemodialysis, illicit injection of drugs, surgical patients and patients with insulin reliance or poorly monitored diabetes, colonization rates are improved[11]. Healthcare-related infection is the major cause of morbidity and mortality12 and the appearance and fast spread of antimicrobial resistance among the floating organisms in the hospital has critically influenced the management of all these conditions. The significance of MRSA identification, therefore, is particularly important for therapeutic and epidemiological purposes. Hence, the methods used to detect MRSA in clinical samples should have high sensitivity and specificity and most importantly the result should be available within a short time[13].

A total of 120 S*taphylococcus aureus* were isolated from 266 clinical specimens from three hospitals of which 80(66.7%) *S. aureus* were collected after prior confirmation from the department of Microbiology & Immunology laboratory of Bangabandhu Sheikh Mujib Medical University (BSMMU), 10 (8.3%) from Dhaka Medical College Hospital (DMCH) and rest 30(25.0%) were collected from Private Diagnostic Center, Dhakato get a more representative picture of the situation. These isolates were subjected to antimicrobial susceptibility testing by oxacillin and cefoxitin and PCR for detection of the *mecA* gene. The rate of MRSA infection in different age group in comparison to MSSA infection found that, MRSA infection rate increased gradually with age. It was highest in age group 61-70 years (30%) and next of which 51-60 years (23.1%) where as in the age group 0-10 years and 11-20 years the MRSA rate was 0%, This result is in conformity with the reports of Khurram et al[14]from Pakistan, Lepelletier et al[15](2004) from France and Sadoyama et al[16] from Brazil. They revealed that MRSA infection was considerably greater in elderly patients, and this may be due to the reality that elderly individuals are more exposed to antimicrobial agents, which lead to selective antibiotic stress and growth of resistant strain infection and, in relation to decreasing immunity in ancient age, increases the danger of MRSA strain infection, which could be the reason for elevated strain infection.

Therefore, number of methicillin and cefoxitin resistant and sensitive strains isolated by this technique were 17 (14.2%) and 103(85.8%), 19(15.8%) and 101(84.2%) respectively. There is a significant difference in resistance pattern of oxacillin and cefoxitin by disc diffusion method, while vancomycin 117(97.5%) and linezolid showed 120(100%) sensitivity.Bukhari[17] reported 41.9% resistance against oxacillin from 1102 isolates in compare to our 15.8% from 120 isolated *S. aureus*. Other antibiotics like penicillin

1056(95.8%) and cotrimoxazole 699(63.6%) resistance is higher than the present study. Study by Subedi and Brahmadathan[18] reported 18 (15.4%) methicillin-resistance which is in agreement with that of the present study.

The prevalence of MRSA in hospitals varies considerably from one region to another and among hospitals in the same area[17]. This variation ranges from 2-61% as stated by Hafiz et al[9]Prevalence of MRSA as reported by Safdar et al[10] indicates <10% to 65%. In India it ranges from 30 to 70%[11]. According to Sturenburgh et al[19] MRSA accounts for at least 15.0 to 20.0% of clinical isolates of *S. aureus*. Subedi and Brahmadathan[18] published their MRSA percentage 15.4% in their research note. From their experience it can be assume that such variation may also exists between our hospitals and health care facilities. MRSA isolates among the three hospitals is recorded but show little variation regarding MRSA which were 11(13.8%) in BSMMU, 2(20.0%) in DMCH and 2(20.0%) PDC. If larger number of samples could have been included in this study then the picture would have been different.

The prevalence rate of MRSA is 15.8% in the present study and is similar or within the range of almost all above studies. Low prevalence rate can be explained by the fact that they were conductedon isolates of *S. aureus* collected from relatively less ill persons while high prevalence rates were available on studies done on severely ill patients[12]. Infection prevention measures are enforced in BSMMU for prevention of nosocomial infection and in the private hospitals the patients themselves and the management of the hospital both are aware of factors that may be a source of infection to their patients, this may explain low prevalence of MRSA among our cases. Review of numerous other research in Bangladesh shows a growing trend of MRSA across the nation. A multi-center research with four divisional clinics at Ibrahim Medical College (Haq, 2009) revealed an MRSA rate variety of 32-63 percent.

In the current study, among the 19 MRSA strains 14(73.7%) were multi-resistant strains and 5(26.3%) were non-multi-resistant.mMRSA are defined as MRSA resistant to two or more classes of antibiotics in addition to ß-lactams;mMRSAaccounts for 75.0% of isolations in 2001.Literatures suggest HA-MRSA strains tend to be more multidrug resistant in contrast to CA-MRSA strain with hallmark resistance to fluoroquinolones.The distribution of MRSA by the site of infection was as follows; 3(50.0%) blood stream, 10(14.3%) skin and soft tissue, 1(50.0%) genital tract and 1 (11.1%) ENT. Only 1(100.0%) strain of respiratory tract infection yielded MRSA which was collected from laboratory stock culture. No MRSA was detected from body fluids other than blood. Maximum number of MRSA was isolated from Skin soft tissue followed by blood stream and urinary tract infections. From 57 ICU specimens 7(12.3%) were *S.aureus* isolates of which 4(57.1%) came out to be MRSA. If larger number of ICU sample could have been collected the real situation of MRSA related infection could have been possible.MRSA accounts for >50% of *S. aureus* isolates from patients in ICUs in the NNIS system; in 2003, 59.5% of *S.aureus* isolates in NNIS ICUs were MRSA (NNIS, 2003). By 2004, this figure increased to 63% (CDC, 2006).

MSSA and MRSA isolates' pattern of antibiotic susceptibility varied considerably. MSSA isolates were prone to most of the medicines tested, although some resistance was noted to penicillin, tetracycline, tobramycin and, to some extent, clindamycin, amikacin and kanamycin. Multiple drug resistance was prevalent in the event of MRSA, however, and only a few antibiotics were effective against these isolates. MRSA strains have been discovered to be more resistant than MSSA strains to other antibiotics. In the case of erythromycin, ciprofloxacin, tobramycin, tetracycline, gentamicin, and amikacin, significant difference ($p<0.05$) was noted. There was a important distinction between MRSA sensitivity patterns and MSSA isolates patterns.

**Conclusion**

According to antibiotic profiles, the bulk of MRSA are multi-resistant. All strains, however, are susceptible to vancomycin and linezolid. Skin and soft tissue infection is the most prevalent location of MRSA infection. A large-scale multicenter survey should be conducted to assess Bangladesh's actual condition.

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