

## Antibiotic Resistance Pattern of Methicillin Resistant *Staphylococcus aureus* Isolated From Clinical Specimens

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**Abstract:** Methicillin Resistant *S. aureus* poses serious threat to human health since the mortality and morbidity rate due to *S. aureus* infection is significant. In the present study, a total of 37 *S. aureus* strains isolated from 1078 clinical were tested against various antibiotics. Antibiotic susceptibility of the isolated *S. aureus* was carried out using disk diffusion method. The MIC and MBC of different antibiotics were determined by tube dilution assay and disk diffusion technique. The maximum prescribed antibiotic groups were cephalosporins and penicillins. The most resistant group was penicillin followed by aminoglycoside, cephalosporins and quinolones. The order of resistance of *S. aureus* against various antibiotics was ampicillin (92.10%), cloxacillin (61.29%), cefradine (60%), ciprofloxacin (59.35%), gentamicin (58.06%). There was no resistant isolate against vancomycin; however, four isolates (10.52%) had shown intermediate sensitivity. MIC and MBC of five major groups of antibiotics namely, penicillins, cephalosporins, aminoglycosides, quinolones and vancomycin were also determined. In-vitro study of MBC of the five groups of antibiotics showed 2 to 3 fold two-fold dilution higher value than in MIC of each group of antibiotic. Only MBC of vancomycin was in the range of 1 to 2 two-fold dilutions higher than their MICs.

**Keywords** - Antibiotic resistance, MRSA, MIC, MBC, *Staphylococcus aureus*

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### I. Introduction

Multidrug resistance is now a very common norm in *S. aureus*. The mortality of *S. aureus* bacteremia remains approximately 20–40% despite the availability of effective antimicrobials, which indicates the propensity of *S. aureus* to develop resistance. Some strains that infect hospitalized patients are resistant to most of the antibiotics used to treat infections, vancomycin being the only remaining drug to which resistance has not developed [1]. There are also reports of MRSA strains with less susceptibility toward Vancomycin [2]. Some identified genes to be the cause of such resistance. *Staphylococcus* cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus* [3]. *S. aureus* resistance to penicillin is mediated by *blaZ*, the gene that encodes  $\beta$ -lactamase. Methicillin resistance requires the presence of the chromosomally localized *mecA* gene. The cause behind the resistance of quinolones to be the stepwise acquisition of chromosomal mutations. Vancomycin resistance has resulted from the probable conjugal transfer of the *vanA* operon from a vancomycin-resistant *E. faecalis* [4]. The study was thus conducted to observe the pattern of antibiotic resistance of *S. aureus* isolated from clinical samples.

### II. Material and Methods

A total of 1078 samples received in the microbiology laboratory of Chattagram Maa Shishu O General Hospital, which include blood, pus and miscellaneous samples (swabs/sputum/body fluids).

#### 2.1 Sample Processing

##### 2.1.1 Blood

Blood samples were collected from patients visiting OPD (Out Patients Department), different wards (IDP) and were brought to the pathology laboratory. Freshly drawn, 3-5 mL blood was immediately transferred to 50 mL of Tryptone Soya broth (CM0129-OXOID), and incubated at 37° C for 24 hours. Then sub-cultured on Blood Agar (CM 0055- OXOID), Chocolate agar (by heating Blood Agar at 63°C) and MacConkey Agar (CM 0007-OXOID) plates, and incubated for 24 hours at 37° C.

##### 2.1.2 Pus

Pus samples were plated directly onto Blood Agar (CM 0055- OXOID), Chocolate agar (by heating Blood Agar at 63°C) and MacConkey Agar (CM 0007-OXOID), and incubated for 24 hours at 37° C.

### **2.1.3 Miscellaneous Samples**

Body fluids, sputum and swab samples were cultured on Blood agar, MacConkey agar and Chocolate agar, respectively and then incubated for 24 to 48 hours at 37°C.

### **2.2 Sub-Culturing and Maintenance of Bacterial Isolates**

Randomly selected isolates were sub-cultured and preserved for further studies. For this purpose mid exponential phase bacterial isolates were inoculated into 10% non-fat Skim Milk (DIFCO) and maintained at 4°C. In this condition, bacterial culture can be preserved for as long as one month.

### **2.3 Identification**

All of the isolates were identified by morphological and biochemical properties as outlined in the Bergey's Manual of Determinative Bacteriology (9th edition). The isolates were additionally investigated by PCR amplification of species specific parts of the gene encoding the 23S rRNA with the oligonucleotide primers [5]

### **2.4 Antibiotic Susceptibility Test**

The resistance pattern among these isolates against different groups of antibiotics, which are commonly in use, was determined by disk diffusion technique [6]. Commercially available disks were applied on Mueller Hinton Agar (CM0337-OXOID) as recommended by the manufacturer.

### **2.5 Determination of MIC and MBC**

Agar dilution method was used to determine the minimum inhibitory concentrations (MIC), or the lowest concentration of antimicrobial agent required to inhibit the microorganism. Serial two fold dilution concentrations of ampicillin, cefradine, gentamicin, ciprofloxacin and vancomycin active pharmaceutical ingredient (API) and their respective brands were made (TABLE 1) and tested against *S. aureus* from the clinical specimens. Inoculum of MIC isolates and control *S. aureus* were subcultured on blood agar plates and incubated overnight at 35° C.

## **III. Results and Discussion**

### **Susceptibility Pattern of *S. aureus***

The susceptibility of 38 strains of *Staphylococcus aureus* were assessed against various antimicrobial agents by disk diffusion method. Most of the *S. aureus* were multi drug resistant and Vancomycin was found to be most effective drug against these clinical isolates. There was no resistant isolate against vancomycin and only 4 (10.52%) isolates had intermediate sensitivity. Gentamicin was the second most effective drug against these isolates, with 41.94% sensitive strains. Ciprofloxacin and cefradine were found to be effective antimicrobial drug against these *S. aureus* strains exhibiting 38.06% and 32.27% sensitivity, respectively. Ampicillin demonstrated only 5.26% sensitivity. The order of resistance of *S. aureus* against 5 groups of antibiotics was ampicillin (92.10%), cefradine (60%), ciprofloxacin (59.35%), gentamicin (58.06%) and an intermediate resistance to vancomycin (38.06%). Cloxacillin resistance for these isolates was 61.29%. Most of the cloxacillin resistant strains were intermediate resistance towards vancomycin.

### **MIC & MBC of clinical isolates of *S. aureus* against five groups of antibiotics**

MIC and MBC were determined for all the 38 *S. aureus* isolates from clinical samples against five groups of antibiotics, namely penicillins, cephalosporins, aminoglycosides, quinolones and vancomycin (TABLE 2). The MIC obtained for each isolate against various antibiotics and their brands were compared with one another. Kinetics of bactericidal activity showed that the all brands of each antibiotic possessed almost similar activity against clinical isolates of *S. aureus* tested. The MBC value for ampicillin was 2 to 3 two-fold dilution higher than the MIC values. Cephalosporins had MIC range of 8 to 2048 µg/mL. MBC values of cefradine and the other brands of the cephalosporins were 2 to 3 two-fold dilution higher than MIC against all isolates. In the current study, bulk of the isolates were resistant to Gentamicin at the concentration 1024 µg/mL to 2048 µg/mL and most of them were inhibited at the range of 4 µg/mL to 512 µg/mL and certainly MIC lies in between for both standard powder and Gentacine. The MBC of gentamicin and gentacine was in range of 32 µg/mL to >1024µg/mL.

*Staphylococcus aureus* is recognized as one of the most important bacterial pathogens contributing towards hospital infection world wide. *S. aureus* causes localized infection spreading into the blood stream [7]. Antibiograms of the isolates, which were collected from CMOSH, had resistance against various antibiotics such as ampicillin, cloxacillin, ciprofloxacin, cholramphenicol, gentamicin, amikacin, and different generations of cephalosporins. Majority of the *S. aureus* strains from the present study were MDR, especially the isolates recovered from pus of the indoor patients of CMOSH. Such MDR pattern among these clinical isolates of *S.*

*aureus* has also been documented earlier [8, 9, 10] who have reported multidrug resistant *S. aureus* as a cause of nosocomial infection during 1992-1997. Methicillin resistant *S. aureus* were more prevalent in pus (30%) than blood (24%) and miscellaneous samples (27%). Based on results of culture sensitivity, ampicillin was found to be the most resistant (92.1%), than compared to the other antibiotics. Cloxacillin showed 61.3% insensitivity towards *S. aureus* and Vancomycin established 38% intermediate resistance. Among the 4 studied isolates that were intermediately resistant to Vancomycin were completely resistant to cloxacillin. The comparison of MIC values obtained for each isolate against various antibiotics showed that the all brands of each antibiotic possessed almost similar activity against clinical isolates of *S. aureus* tested. In the current study, bulk of the isolates was resistant to Gentamicin at the concentration and MIC lies in between for both standard powder and Gentacine. This clearly illustrates emergence and development of resistance against gentamicin by MDR strains of *S. aureus*. Most of the isolates lie in resistance range of 2 µg/mL to 64 µg/mL with Quinolones, ciprofloxacin in particular. The MBC was 2 to 3 two-fold dilution higher than MIC, which is in concurrence with the works of Brumfitt and Hamilton-Miller [11] and later others [10, 12] documented the similar result.

#### IV. Tables

**Table 1: List of Antibiotics used with brand name and manufacturers' name**

Sl. No	Group	Antibiotic	Manufacturer
1.	Penicillin	Ampicillin (SP)	Bayer Pharma
		Tycil (L)	Beximco Pharma (Bangladesh)
		Benzapenem (L)	Square Pharmaceuticals Ltd.
2.	Cephalosporin	Cefradine (SP)	Pharmagen Ltd.
		Ceftron 500 (L)	Square Pharmaceuticals
		Cefotil 500 (L)	Square Pharmaceuticals
		Turbocef 500 (L)	Beximco Pharma
3.	Aminoglycoside	Gentamicin (SP)	Eli Lilly
		Gentacine (M)	Hoechst Pvt Ltd.
4.	Quinolone	Ciprofloxacin (SP)	Crosschen International (Switzerland)
		Neofloxacin 500 (L)	Beximco Pharma
		Ciprocin 500 (L)	Square Pharmaceuticals
5.	Vancomycin	Vancomycin (SP)	Eli Lilly

**Table 2: MIC and MBC of five tested groups of antibiotics against the Clinical Isolates of *S. aureus***

Antibiotics used	MIC	MBC
Penicillin	.008 to 2.048 mg/L	.032 mg/L to >1.024 mg/L
Cephalosporin	.008 to 2.048 mg/L	.032 mg/L to >1.024 mg/L
Aminoglycosides	.004 to 2.048 mg/L	.016 mg/L to >1.024 mg/L
Quinolones	.001 mg/L to 128 mg/L	.004 mg/L to 1.024 mg/L
Vancomycin	.002 mg/L to 8 mg/L	.004 mg/L to .016 mg/L

#### V. Conclusion

This study clearly indicates the trend of *S. aureus* towards development of antibiotic resistance against various antibiotics and even the most effective ones are now becoming insensitive against *S. aureus*. So a more sustainable therapy must be in place to combat *S. aureus* infection. So there are provisions of undertaking a further research to analyze the factors affecting pathogenicity and the interaction with host to find a sustainable solution.

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