

## Incidence of Methicillin Resistant *Staphylococcus aureus* and its antibiotic susceptibility pattern in a tertiary care hospital

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### Abstract

A total of 250 *Staphylococcus aureus* strains were isolated from various clinical samples over a period of 9 months at M. P. Shah Medical College, Jamnagar to identify the incidence rate and antibiogram of Methicillin Resistance *Staphylococcus aureus* (MRSA). All strains of *S. aureus* were tested for susceptibility to methicillin / oxacillin by disk diffusion method and minimum inhibitory concentration (MIC) of methicillin was determined by agar dilution method as per standard procedures. Antibiogram study of MRSA strains was performed. Out of total 250 *S. aureus* strains, 145 (58%) strains were methicillin and oxacillin resistant, having methicillin MICs of  $\geq 16 \mu\text{g} / \text{ml}$ , 100 (40%) strains were methicillin and oxacillin susceptible having methicillin MICs of  $\leq 4 \mu\text{g} / \text{ml}$ . 5 (2%) strains were borderline susceptible having methicillin MICs of  $8 \mu\text{g} / \text{ml}$ . Borderline susceptible strains were susceptible to co-amoxiclavate and were not considered as methicillin resistant strains. All 145 (100%) MRSA strains were resistant to penicillin G, 139 (96%) were resistant to erythromycin, 136 (94%) were resistant to gentamicin, 80 (55%) were resistant to amikacin, and 35 (24%) were resistant to rifampicin. All MRSA strains were susceptible to vancomycin.

**Keywords:** *Staphylococcus aureus*, MRSA, MIC, Antibiogram, Antibiotic Resistance.

### Introduction

*Staphylococcus aureus* is a common bacterium that often resides harmlessly on the skin, but it is also a formidable pathogen, causing infections ranging from boils to life threatening infections. It is the leading cause of nosocomial infections and deaths in the hospital worldwide. It is also one of the commonest human pathogens in community acquired infections.<sup>(1)</sup>

Strains of *S. aureus* resistant to both oxacillin and methicillin have most commonly been referred to as methicillin-resistant *S. aureus* (MRSA). The main bacterial targets of the  $\beta$ -lactam antibiotics in *S. aureus* are so-called penicillin-binding proteins (PBPs), which have a functional role in the biosynthesis of the bacterial cell wall. The antibacterial effect of  $\beta$ -lactam is mediated primarily by inactivation of the high-molecular weight PBPs (1, 2 and 3), which have the highest affinity for  $\beta$ -lactam antibiotics. The strains of *S. aureus* that is highly resistant to methicillin produce an additional low-affinity PBP2a (or PBP2'), encoded by *mecA* gene. Production of PBP2a confers intrinsic resistance to all  $\beta$ -lactams. Expression of *mecA* gene depends on gene regulatory systems. A family of *fem* genes (factors essential for expression of methicillin resistance) is also required for homogeneous expression of methicillin resistance.<sup>(2)</sup> Although acquisition of *mecA* by *S. aureus* is essential for the expression of high-level methicillin resistance, at least three other mechanisms explain low-level methicillin resistance: over expression of  $\beta$ -lactamase in some *S. aureus* also called Borderline *S. aureus* (BORSA); overproduction of PBP4; a normal PBP with lower affinity for  $\beta$ -lactams or alteration through acquired mutations of the PBP2 that could lower affinity for  $\beta$ -lactams, known as Modified *S. aureus*

(MODSA).<sup>(2)</sup> MRSA strains are also resistant to multiple antibiotics, antiseptics and disinfectants.<sup>(3)</sup> Vancomycin has been the last uniformly effective antibiotic available for serious MRSA infections but emergence of resistance to vancomycin heralds a potentially frightening era in infectious disease.<sup>(1)</sup>

MRSA is an important universal problem in hospitals. MRSA is usually introduced into an institution by a colonized or infected patients or health care workers. The principal mode of transmission is via the transiently colonized hands of hospital personnel. It is difficult to eliminate MRSA from the hospital once established.<sup>(4)</sup> The epidemic strains of MRSA (EMRSA) are also capable of causing endemic and epidemic problems.<sup>(5)</sup>

The detection of methicillin has been hampered due to variability in the standard techniques used to determine methicillin resistance. The resistant strains are often heteroresistant to  $\beta$ -lactam antibiotics in that two subpopulations (susceptible and resistant) coexist in culture. Each cell in the population may carry the genetic information for resistance, but only a small fraction ( $10^{-8}$  to  $10^{-4}$ ) can actually express the resistance phenotype. The resistant subpopulations usually grow much more slowly than the susceptible subpopulation and therefore may be missed when in vitro testing is performed. The successful detection of heteroresistance strains depends largely on promotion of the growth of the resistant subpopulations, which is favoured by neutral pH, cooler temperatures ( $30\text{-}35^{\circ}\text{C}$ ), the presence of NaCl (2-4%), and possible prolonged incubation (up to 48 h).<sup>(6)</sup>

Detection of MRSA is a complex issue and one single method may not reliably detect methicillin resistance in all strains of *S. aureus* so one or two

methods should be selected that give reliable results with local strains.<sup>(7)</sup>

Detection of methicillin resistance in *S. aureus*, as well as a complete antibiogram is important to monitor the increasing antibiotic resistance among *S. aureus* strains. Keeping the above mentioned points in view, the present study was undertaken.

### Material and Method

The study was conducted at M. P. Shah Medical College, Jamnagar, India. A total of 250 *Staphylococcus aureus* strains were isolated from various clinical samples received from different wards over a period of 9 months. Specimens were screened by Gram stain and were cultured on blood agar, MacConkey's agar, and nutrient agar. *S. aureus* strains were identified on basis of morphology, colony characters, and biochemical tests like catalase, coagulase and mannitol fermentation test. Susceptibility to methicillin (5 µg) and oxacillin (1 µg) were performed on Mueller Hinton agar by disk diffusion test.<sup>(7)</sup> Borderline susceptible strains were checked for co-amoxiclavate (3µg) susceptibility by disk diffusion test.<sup>(7)</sup> Agar dilution method was performed to determine the Minimum Inhibitory Concentrations of methicillin for all *S. aureus* isolates.<sup>(8,9)</sup> Mueller Hinton agar medium with 2% NaCl was mixed with serially diluted methicillin solutions. Methicillin agar containing different concentrations of methicillin from 2 to 64 µg/ml were prepared. Bacterial suspensions were prepared in nutrient broth with a concentration of 10<sup>8</sup> CFU/ml organisms using opacity standards. Spot inoculations of 1 µl of bacterial suspension (10<sup>5</sup> CFU/spot) were done on methicillin agar. 10-12 Staphylococcal isolates with standard sensitive and resistant strains were tested at a time and plates were incubated at 35°C aerobically for full 24 hours. Plates were observed for inhibition of growth. The lowest concentration of the methicillin, giving no visible growth was taken as the minimum inhibitory concentration (MIC) of particular strain. Strains exhibiting MICs ≥ 16 µg/ml were considered as resistant and MICs ≤ 8 µg/ml were considered as susceptible.<sup>(8,9)</sup> Antibiogram study was performed by Kirby - Bauer disk diffusion method for vancomycin (30 µg), rifampicin (5 µg), amikacin (30 µg), erythromycin (15 µg), gentamicin (10 µg), and penicillin G (10 units).<sup>(10,11)</sup>

### Observations & Results

A total of 250 *Staphylococcus aureus* strains comprised of 219 (87.6%) isolates from pus samples, 20 (8%) isolates from blood samples and 11 isolates from other samples. Out of 250 strains 145 (58%) *S. aureus* strains were resistant to oxacillin and methicillin, 100 (40%) were methicillin susceptible strains. 5 (2%) strains were borderline susceptible, which were found to be co-amoxiclavate susceptible and were not considered as true methicillin resistant strains. Out of 145 MRSA strains, 135 (93.1%) were from pus samples, 5 (3.45%)

were from blood samples and 5 were from other samples (Table 1).

**Table 1: Distribution of *S. Aureus* & MRSA strains**

Sample	<i>S. aureus</i> (N = 250)	MRSA strains (N = 145)
Pus	219 (87.60%)	135 (93.10%)
Blood culture	20 (08.00%)	5 (03.45%)
Conjunctival swab	5 (02.00%)	2 (01.38%)
Urine	3 (01.20%)	2 (01.38%)
Vaginal swab	2 (00.80%)	1 (00.69%)
Cerebrospinal fluid	1 (00.40%)	0 (0%)
Total	250 (100%)	145 (100%)

MRSA strains showed MICs of > 16µg/ml. Methicillin susceptible strains showed MICs of < 4 µg/ml and the borderline susceptible strains showed MICs of 8 µg/ml. Distribution of strains as per their MIC is shown in Table 2.

**Table 2: MICs of methicillin for *S. Aureus* strains (N= 250)**

MIC (mcg/ml)	<i>S. aureus</i> Strains
≤ 2	70 (28.0%)
4	30 (12.0%)
8	5 (02.0%)
16	0 (0%)
32	22 (08.8%)
64	33 (13.2%)
> 64	90 (36.0%)
Total	250 (100%)

All 145 (100%) MRSA strains were uniformly susceptible to vancomycin, 35 (24%) MRSA strains were resistant to rifampicin, 80 (55%) strains were resistant to amikacin, 136 (94%) strains were resistant to gentamicin, 139 (96%) strains were resistant to erythromycin, while all 145 (100%) MRSA strains were resistant to penicillin G (Table 3).

**Table 3: Antibiogram of MRSA strains (N = 145)**

Antibiotic (Concentration)	Susceptible	Resistant
Vancomycin (30 µg)	145 (100%)	0 (0%)
Rifampicin (5 µg)	110 (76%)	35 (24%)
Amikacin (30 µg)	65 (45%)	80 (55%)
Gentamicin (10 µg)	9 (6%)	136 (94%)
Erythromycin (15 µg)	6 (4%)	139 (96%)
Penicillin G (10 units)	0 (0%)	145 (100%)

### Discussion

The local prevalence of MRSA strains varies significantly with the presence of certain isolates associated with epidemic, disease condition, size of the institution, the patient population at risk for infection or

colonization, movement of colonized or infected patients between institution, infection control practices and the laboratory methods employed to identify MRSA.<sup>(12)</sup>

The problem of methicillin resistance in *S. aureus* emerged in 1960. In the initial phase, i.e. during 1960s the incidence rates of MRSA were low, reflecting a general low frequency of methicillin resistance.<sup>(13)</sup> Jevons (1960, London)<sup>(14)</sup> and Pal (1964, New Delhi)<sup>(15)</sup> have reported Incidence of MRSA 0.06% and 11.84% respectively.

1970s was the quiescent period for MRSA.<sup>(13)</sup> MRSA re-emerged in 1980s with increased scope assuming a problem of global concern. Higher incidence rates of MRSA are seen worldwide after 1980, including the present study. Judy (1985, Illinois),<sup>(16)</sup> Hashimoto (1993, Japan),<sup>(17)</sup> Rohini (1995, Mumbai),<sup>(18)</sup> and Jesudason (1996, Vellore)<sup>(19)</sup> have reported incidence of MRSA 50%, 60%, 87%, and 45.7% respectively.

The probable reasons are, for higher incidence of MRSA may be; Improvement in the laboratory methods for detection of MRSA.<sup>(20)</sup> Extensive and often indiscriminate use of B-lactam agents including cephalosporins and aminopenicillins.<sup>(21)</sup> Emergence of particular strains of MRSA called epidemic strains. EMRSA have capability to spread widely, to cause more colonization and infection and to persist for longer period of time.<sup>(22)</sup> Difficulty in eradication of MRSA / EMRSA strains.<sup>(20)</sup> Health care system and MRSA transmission: Overcrowding of indoor patients, lack of barrier nursing, inappropriate disposal of infected materials and improper disinfection of equipments and instruments facilitates MRSA transmission in hospital.<sup>(23)</sup> Multiple invasive procedures.<sup>(24)</sup> Advances in medical field which extends the survival life of patients. The extraordinary advances in medical field especially respiratory medicine have kept patients alive who would have died much earlier. These patients are repeatedly treated with multiple antibiotics, ultimately leading to multi-resistant bacteria.<sup>(20)</sup>

Antibiotic susceptibility pattern of MRSA indicates that they are in a sense multiresistant Staphylococcus aureus. All MRSA strains are susceptible to vancomycin, so vancomycin still remains choice of drug for serious infections. Resistance to vancomycin has been reported in America and Japan.<sup>(25,26)</sup>

### Conclusion

- The incidence of methicillin resistant Staphylococcus aureus was 58% in the institution.
- MRSA strains were resistant to a number of antibiotics commonly used in institution, but uniformly susceptible to vancomycin.

### Recommendation

- Routine screening for MRSA along with its susceptibility pattern is very important especially to identify the emergence of vancomycin resistance in MRSA.

- Since prevention is always better than cure, the infection control measures aimed at minimizing nosocomial transmission of MRSA as much as possible should be developed and implemented.

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