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# **RESEARCH ARTICLE**



# Antibiotic Susceptibility Pattern of Methicillin Sensitive and Resistant *Staphylococcus aureus* from Clinical Isolates in a Tertiary Care Hospital at Mathura, Western Uttar Pradesh

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# Abstract

Staphylococcus aureus causes infection ranging from mild skin infection to fatal life threatening infections. Nowadays, Methicillin resistant Staphylococcus aureus (MRSA) which do not respond to commonly used antibiotics has emerged posing serious threat in health care settings which aimed to study the susceptibility pattern of MSSA and MRSA among inpatients and outpatients in our hospital. This study included 159 strains of S. aureus isolated from various clinical specimens collected from April 2018 – March 2019. Standard isolation techniques and identification protocols were followed. Among the total 159 Staphylococcal isolates, 134 isolates and 25 isolates were isolated from inpatients and outpatients respectively. Methicillin resistance was seen in 67.3% (107/159) of the total isolates, among which 69.4% (93/134) were from inpatients and 56% (14/25) were from outpatients. Among these total MRSA, 38% were reported from urine followed by pus and blood samples. Antibiotic susceptibility pattern revealed that 75% of MSSA strains were sensitive to doxycycline and Clindamycin. Among the MRSA isolates 95.3% were resistant to Ampicillin and 86.9%, 73.8%, 60.7%, 52.3% and 50.4% isolates were resistant to erythromycin, Co-trimoxazole, Ciprofloxacin, Moxifloxacin and Clindamycin respectively. All MRSA isolates were sensitive to vancomycin while 97.2% were sensitive to Linzolide. S. aureus isolated from urine showed high resistance of 89% and sensitivity of 92.7% to norfloxacin and nitrofurantoin respectively. This study reports high prevalence of MRSA. This study helps to select the appropriate antibiotic for proper patient care in this area and insist upon to follow strict hospital Infection control practices in this hospital.

Keywords: Staphylococcus aureus, MRSA, Cefoxitin, MSSA, Antimicrobial resistance

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#### INTRODUCTION

Staphylococcus aureus is a leading cause of pyogenic bacterial infection in humans worldwide and is well known for its ability to develop drug resistance. S. aureus has traditionally been the leading cause of skin and soft tissue infections and the severity may vary from benign impetigo to life threatening infections. It is commonly isolated from cutaneous abscesses, purulent cellulitis and surgical site infections. In the early 1960s, Methicillin Resistant S.aureus (MRSA) emerged as a potential pathogen causing nosocomial colonization, several outbreaks and difficult-to-treat infections like bacteremia, infective endocarditis, osteoarticular and pleuropulmonary infections. They also causes device related infections like central line associated blood stream infections, ventilator associated pneumonia and catheter associated urinary tract infections<sup>1-5</sup>. It always remains a challenge for physicians to treat and control MRSA infections. While infections by Methicillin Sensitive S.aureus (MSSA) remain stable, infections due to MRSA fluctuated. Patients in intensive care units (ICUs) and with chronic diseases like diabetes, cancer, vascular diseases and eczema are at greater risk of acquisition of MRSA infections due to hospital stay after invasive procedures and weekend immune system<sup>5</sup>. Further, people with open draining wounds and infections spread the MRSA in the community. As reviewed by Gupta et al.,6 nosocomial infections caused by MRSA increased from 30% in 1990's to 80% in 2010 in many countries. In India, it varies from 29% in 2009 to 47% in 2014<sup>3</sup>. This increasing rate of infections caused by MRSA pose a serious treat causing extensive difficult to control outbreaks. Moreover the drugs used to treat MRSA infections are highly expensive and potentially toxic. Hence this study aimed for the early detection of MRSA by screening and to determine the invitro susceptibility pattern to various antimicrobial agents which helps to choose the appropriate drug for treatment.

#### MATERIALS AND METHODS

This prospective work was carried out in the microbiology laboratory of a tertiary care teaching hospital at western Uttar Pradesh for a period of one year from April 2018 to March 2019 following the ethical guidelines. Various clinical specimens like blood, pus, sputum, pleural fluid, urine, vaginal and ear swabs were included. **Bacterial isolates** 

A total of 159 *S.aureus* strains were isolated from these specimens. Standard techniques<sup>7</sup> like Gram staining and coagulase test were used for the identification of *S. aureus*. **Antimicrobial susceptibility testing** 

Inoculum was prepared from the colonies grown on blood agar plates and antibiotic susceptibility testing was carried out on Mueller Hinton Agar after adjusting the turbidity to 0.5McFarland standard. Modified Kirby Bauer disc diffusion method was followed for antibiotic susceptibility testing using antibiotic discs like Ampicillin (10µg), Ciprofloxacin (5µg), Clindamycin (2µg) Co-trimoxazole (1.25/23.75 µg), Doxycycline (30µg) Erythromycin (15µg), Linezolid (30µg) Moxifloxacin (5µg), Nitrofurantoin (300µg) Norfloxacin (10µg), Teicoplanin (30µg) and Vancomycin (30µg) procured from Hi-media. Zone of inhibition was measured and results were interpreted as Susceptible, Intermediate and Resistant following the recommendations of CLSI guidelines<sup>8</sup>. Screening for MRSA was done using cefoxitin discs (30µg) (Hi Media) on Mueller Hinton agar and zone of inhibition  $\leq$  22mm indicates methicillin resistance and was reported as MRSA. S. aureus ATCC 25923 was used as standard control strain. Chi-square test was used for the statistical analysis of the data and p values which were < 0.05 were considered to be statistically significant.

#### RESULTS

A total of 159 S. gureus was isolated of which 32.7% were MSSA and 67.3% were MRSA. Among the MSSA isolates 38.5% (20/52) was isolated from pus, 32.7% (17/52) from urine and 21% (11/52) from blood whereas, among the MRSA isolates 35.5% (38/107) was obtained from urine, 29% (31/107) from pus followed by 26.2% (28/107) from blood. Distribution of MRSA and MSSA isolated from different clinical specimen is listed in Table: 1. Regarding the susceptibility pattern of MSSA, 42.3% of isolates were sensitive to Ampicillin, 38.5% were sensitive to Erythromycin, 46.2% to Ciprofloxacin, 44.2% to Co-trimoxazole and 48.1% to Moxifloxacin. Among the MRSA isolates only 4.7% isolates were sensitive to Ampicillin, 13.1% to erythromycin, 39.3% to

Ciprofloxacin, 26.2% to Co-trimoxazole, 47.7% to Moxifloxacin, 86% to Teicoplanin, and 97.2% were sensitive to linezolid. Doxycycline and Clindamycin sensitivity was seen in 75% of the MSSA strains, but among MRSA 64.5% were sensitive to Doxycyline and 49.6% were sensitive to Clindamycin. As far as the sensitivity pattern of MRSA is concerned, it showed sensitivity of 100% and 86% for Vancomycin and Teicoplanin respectively. Among urinary pathogens 94.1% of MSSA and 86.8% of MRSA were resistant to Norfloxacin, while only 11.8% and 5.2% were resistant to Nitrofurantoin respectively. The resistance patterns of MSSA and

 Table 1. Distribution of MRSA in different clinical samples

Clinical samples	S. aureus (%)	MRSA (%)	P value
Blood	39 (24.5)	28 (26.2%)	0.76
Ear Swab	01 (0.6)	1 (0.9%)	0.7
Pleural fluid	02 (1.3)	1 (0.9%)	0.80
Pus	51 (32)	31 (29%)	0.59
Sputum	8 (5)	5 (4.7%)	0.89
Urine	55 (34.6)	38 (35.5%)	0.87
Vaginal swab	3 (1.9)	3 (2.8%)	0.62
Total	159 (100)	107 (100)	
	Clinical samples Blood Ear Swab Pleural fluid Pus Sputum Urine Vaginal swab Total	Clinical samples         S. aureus (%)           Blood         39 (24.5)           Ear Swab         01 (0.6)           Pleural fluid         02 (1.3)           Pus         51 (32)           Sputum         8 (5)           Urine         55 (34.6)           Vaginal swab         3 (1.9)           Total         159 (100)	Clinical samplesS. aureus (%)MRSA (%)Blood39 (24.5)28 (26.2%)Ear Swab01 (0.6)1 (0.9%)Pleural fluid02 (1.3)1 (0.9%)Pus51 (32)31 (29%)Sputum8 (5)5 (4.7%)Urine55 (34.6)38 (35.5%)Vaginal swab3 (1.9)3 (2.8%)Total159 (100)107 (100)

MRSA – Methicillin Resistant Staphylococcus aureus.

Table 2. Antibiotic susceptibility pattern of MRSA to various antibiotics

MRSA isolated from the outpatient and inpatient samples is presented in Table: 2 and 3.

#### DISCUSSION

MRSA poses a serious and constant threat to health care institutions. Data on the prevalence of MRSA is not uniform. Literature has documented a significant variation in the prevalence rate from different parts of India and between countries<sup>1-6,9-28</sup>. Studies from India report MRSA prevalence ranging from 6.9% to 87%<sup>9</sup>. A higher percentage have also been reported from other countries like Sudan (45%), Kenya (53.4%), Nigeria (73.8%), Peru (80%), Rwanda (82%), and in Colombia (90%)<sup>4,10-14</sup>. In the current study we report a prevalence of 67.3% in this hospital. Other studies have documented 20.3% of MRSA from Gujarat, 26.14% from Nepal, 29% from Mumbai and Mangalore, 53% from Pune and around 80% from Bubaneswar showing the varying prevalence of MRSA<sup>15-20</sup>. This wide variation in prevalence rate might be due to study design, inclusion and exclusion criteria, types of specimen, laboratory protocols, study duration, population included, phenotypic or genotypic characteristics studied etc. All these factors may sometimes overestimate the prevalence of MRSA generated from a single centre study, and this overestimated prevalence rate is generalized throughout the country.

Antibiotics	Isolate fro (93	om in pa <sup>.</sup> 3)*	tient	ls	olate from (14	Total resistance (IP + OP cases) n=107				
	S	%	R	%	S	%	R	%	R	%
Ampicillin	5	5.3	88	94.6	0	0	14	100	102	95.3
Erythromycin	14	15	79	85	0	0	14	100	93	86.9
Doxycycline	65	69.9	28	30.1	4	28.6	10	71.4	38	35.5
Clindamycin	47	50.5	46	49.5	6	42.8	8	57.1	54	50.4
Ciprofloxacin	38	40.9	55	59.1	4	28.6	10	71.4	65	60.7
Co-trimoxazole	26	28	67	72	2	14.3	12	85.7	79	73.8
Vancomycin	93	100	0	0	14	100	0	0	0	0
Linezolid	90	96.8	3	3.2	14	100	0	0	3	2.8
Moxifloxacin	46	49.5	47	50.5	5	35.7	9	64.3	56	52.3
Teicoplanin	82	88.2	11	11.8	11	78.6	3	21.4	14	13
Norfloxacin	5	19.2	21	80.8	0	0	12	100	33	86.8
Nitrofurantoin	24	92.3	2	7.7	12	100	0	0	2	5.2

MRSA - Methicillin Resistant *Staphylococcus aureus*, IP – inpatient OP - Outpatient, \*Norfloxacin and Nitrofurantoin was tested for 55 isolates obtained from urine among which 38 isolates were MRSA.

Some authors<sup>18,20-22</sup> have predominantly isolated MRSA from skin and soft tissue infections. In our study majority, 35.5% (38/107) of MRSA from urine and 38.5% (20/52) of MSSA from pus samples were reported, however the difference was not statistically significant. Studies<sup>6,21,23-26</sup> conducted report approximately 90% of resistance to β lactams and erythromycin drugs among MRSA which is consistent with our report. In this study, MRSA isolated from outpatients were 100% resistant to ampicillin and erythromycin whereas among MSSA it is 45.5% and 72.7% respectively. Similarly, higher level of resistance to Co-trimoxazole and Ciprofloxacin is also noted. In this study MRSA from outpatients had high resistance to Ampicillin, Erythromycin, Co-trimoxazole and Ciprofloxacin compared to inpatients (Table: 2 and 3). This increased drug resistance among outpatients might be because the hospital caters mainly to rural population and patients who were referred from peripheral clinics without proper infection control practices. Also, self medication and facilitated self medication through pharmacists may be the reason for increased drug resistance.

Researchers have also reported varying level of resistance to clindamycin and doxycycline among MRSA. Gupta *et al.*<sup>6</sup> in his

studies reported 75.5% of MRSA isolates were resistant to clindamycin and in contrast, Mamtora et al.17 reported 73% of MSSA strains were resistant. In our study 50% of MRSA and 25% of the MSSA strains were resistant to clindamycin. For doxycycline, some authors<sup>6,15</sup> have reported resistance ranging from 56% to 60% among MRSA and 1.4% to 12.54% among MSSA. In this study 71.4% of MRSA isolates from outpatients were resistant to doxycycline. Among urinary pathogens 94.1% and 86.8% of MSSA and MRSA were resistant to Norfloxacin, while only 11.8% and 5.2% were resistant to Nitrofurantoin respectively. All MRSA and MSSA isolated from outpatients were 100% resistant to Norfloxacin and 100% sensitive to nitrofurantoin, hence nitrofurantoin remain being the drug of choice for urinary tract infections. The varying susceptibility pattern of MRSA to different antibiotics may also get influenced by the antibiotic prescription practice of a physician.

Due to the development of resistance and emergence of MRSA, nowadays vancomycin and linezolid remains the most reliable therapeutic agent for MRSA infections. Most of the studies<sup>5,6,18</sup> from India including our study report 100% susceptibility to vancomycin, where only few studies<sup>17</sup> report resistance. Among MRSA, authors

Antibiotics	Isolate from inpatient (41)					Iso	Isolate from outpatient (11)			Total resistance (IP + OP cases) n=52		
	S	%	R	%	Ι	%	S	%	R	%	R	%
Ampicillin	16	39	25	61	0	0	6	54.5	5	45.5	30	57.7
Erythromycin	15	36.6	24	58.5	2	4.9	3	27.3	8	72.7	32	61.5
Doxycycline	32	78	9	22	0	0	7	63.6	4	36.4	13	25
Clindamycin	28	68.3	12	29.3	1	2.4	10	90.9	1	9.1	13	25
Ciprofloxacin	18	43.9	22	53.7	1	2.4	5	45.5	6	54.5	28	53.8
Co-trimoxazole	19	46.3	22	53.7	0	0	4	36.4	7	63.6	29	55.8
Vancomycin	41	100	0	0	0	0	11	100	0	0	0	0
Linezolid	41	100	0	0	0	0	11	100	0	0	0	0
Moxifloxacin	19	46.3	22	53.7	0	0	6	54.5	5	45.5	27	51.9
Teicoplanin	38	92.7	3	7.3	0	0	9	81.8	2	18.2	5	9.6
Norfloxacin	1	9.1	10	90.9	0	0	0	0	6	100	16	94.1
Nitrofurantoin	9	81.8	2	18.2	0	0	6	100	0	0	2	11.8

 Table 3. Antibiotic susceptibility pattern of MSSA to various antibiotics

MSSA - Methicillin Sensitive *Staphylococcus aureus*, IP – inpatient, OP - Outpatient, \*Norfloxacin and Nitrofurantoin was tested for 55 isolates obtained from urine among which 17 isolates were MSSA.

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<sup>6,27,28</sup>, have reported approximately 99% sensitivity to linezolid and our study reports 97.2% sensitivity. The antibacterial activity of linezolid is around 100% which is comparable with that of vancomycin and can be used as an alternative in treating MRSA infections. Routine surveillance of hospitalassociated infections including antimicrobial susceptibility pattern of MRSA and formulation of a definite antibiotic policy may be helpful to reduce infection burden caused by MRSA in the hospital.

### CONCLUSION

The present study revealed that MRSA accounts for more than half of the staphylococcal infections, which emphasize to contain the spread by implementing proper infection control practices. This study highlights the high prevalence of MRSA resistant to ampicillin, erythromycin, cotrimoxazole and ciprofloxacin among outpatients and hence these drugs were not suitable for empirical therapy of suspected staphylococcal infections. Nitrofurantoin being sensitive to most of the staphylococcal isolate it remains the drug of choice for treating urinary tract infections. MRSA strains were highly susceptible to vancomycin and linezolid however appropriate prescription of drug based on the antibiotic susceptibility pattern is the need of the hour to avoid the emergence of resistant variants.

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## **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

#### FUNDING

None.

## AUTHORS' CONTRIBUTION

All authors have made a substantial and intellectual contribution to the work, and approved it for publication.

### DATA AVAILABILITY

All datasets generated and analyzed in this study are included in the manuscript and Table.

### ETHICS STATEMENT

This study does not contain any procedures performed within human participants or animals.

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