



## PREVALENCE AND ANTIBIOGRAM OF MRSA ISOLATED FROM VARIOUS CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL IN PESHAWAR

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### ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major hospital-acquired infection, yet its current epidemiology in Pakistan remains unknown. This study aimed to determine MRSA prevalence across wards in Peshawar's Rehman Medical Institute. Microbiologic culture and susceptibility testing were performed on 160 total samples. *S. aureus* was isolated from 18 samples (11.3%), of which 17 (94.4%) were MRSA. Other pathogens like *Enterococcus*, *Pseudomonas*, *Klebsiella*, and *Escherichia coli* were also detected at lower frequencies. No bacterial growth occurred in 60% of tested samples. The extremely high MRSA positivity rate among isolated *S. aureus* demonstrates this multidrug-resistant strain is flourishing in the hospital and underscores the need for improved infection control. Continued surveillance of antibiotic resistance rates is vital to guide interventions against nosocomial MRSA transmission as a public health threat in Pakistan. Further studies should explore genetic and phenotypic bases of local MRSA strains to elucidate antibiotic evasion mechanisms and virulence traits. The study revealed a substantial 94.4% prevalence of MRSA in *S. aureus* positive samples from hospital patients, indicating antibiotic resistant strains now overwhelmingly dominate in this setting. Such extensive resistance has dire implications for treatment options and underscores how deeply entrenched MRSA has become within the hospital ecosystem, necessitating more aggressive infection control to protect public health.

**Key Words:** Clinical and Laboratory Standards Institute (CLSI), European centre for disease control (ECDC), Methicillin-resistant *Staphylococcus aureus* (MRSA).

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## INTRODUCTION

*Staphylococcus aureus* is a coagulase positive, non-motile gram-positive *S. aureus* is certainly the most clinically significant bacteria. *Staphylococcus aureus* is most commonly present in the nasal mucosa. When the body's cutaneous and mucosal barriers are compromised, such as due to chronic skin diseases, chronic wounds, or surgical procedures then *Staphylococcus aureus* can penetrate the body and invade the underlying tissues or the circulation and spread infection. *S. aureus* infection is more likely to affect people with weakened immune system or hospitalized patients with chronic diseases. MRSA was initially identified in the United Kingdom in 1961, not long after methicillin was first used in clinical settings. Methicillin was once widely used as an antibiotic, but due to its toxicity, it is no longer available. Instead, related, more stable penicillin-like antibiotics for example oxacillin, and flucloxacillin are used as antibiotics. However, the phrase "*Methicillin-resistant Staphylococcus Aureus*" is still used. MRSA has been shown to cause diseases in livestock. Numerous cases were found since the beginning of the 21<sup>st</sup> century. A number of *S. aureus* clones have evolved into MRSA through the horizontal gene transfer of the staphylococcal cassette chromosome mec (SCCmec), a mobile genetic element that encodes the genes *mecA* and *mecC*, which give resistance to methicillin and, consequently, to the majority of  $\beta$ -lactam antibiotics. MRSA frequently exhibits resistance to multiple other antibiotic groups. The amazing ability of *S. aureus* to show resistance to any antibiotic has important effects on both the current and future treatments available for this microorganism (1).

MRSA infections are not restricted to humans but they can also cause infection in domestic and farm animals. MRSA is distributed globally but its frequency is different in various regions. The prevalence of MRSA is quite high in Pakistan as compared to European countries. It has been reported that the prevalence of MRSA in Pakistan is 42 to 51 % (2).

The nasal carriage of *S. aureus* is known to increase the likelihood of developing future endogenous infections and to act as a pathogen transmission vector in medical facilities. In this regard, healthcare professionals, who serve as the link between the hospital and the community, have the potential to make it more difficult to control *S. aureus* in healthcare settings. They might act as reservoirs, victims, or perhaps as vectors of MRSA cross-transmission in the healthcare setting. *S. aureus* was susceptible to practically every type of antibiotic created at the beginning of the antibiotic

era. But as time went on, many *S. aureus* strains developed a greater level of antibiotic resistance. The appearance of MRSA clones in the 1960s marked a turning point in the development of *S. aureus* resistance. MRSA is now widespread in many medical setups and hospitals all over the world. (3)

*S. aureus* can quickly develop resistance to broad-spectrum antibiotics, including quinolones, aminoglycosides, and lactam antibiotics, used in clinical practice to treat severe infections. The majority of *S. aureus* clones in circulation are unsusceptible to methicillin and several other antibiotics. In hospitals and other healthcare settings, as well as in healthy populations who have never been hospitalized, invasive *methicillin-resistant Staphylococcus aureus* (MRSA) infections are a huge challenge. Mec genes (*mecA*, *mecC*) on the mobile genetic element known as staphylococcal cassette chromosome mec are linked to methicillin resistance in staphylococci (SCCmec). MRSA makes up a sizable fraction of nosocomial infections in Pakistan, and numerous medical kinds of research have shown the growing clinical importance of MRSA (4).

*S. aureus* infections can range from minor to serious skin and soft tissue infections to invasive, even fatal systemic infections. The risk of spreading the disease to other patients and medical personnel increases when individuals who are carrying MRSA are admitted. Particularly if there are several strains of antibiotic-resistant bacteria present, such diseases may have grave complications and lethal outcomes. Additional identified risk factors for MRSA acquisition include extended hospital stays, outpatient clinic visits, skin or soft tissue infections in patients, employment in healthcare settings, and a history of antibiotic usage. (5).

MRSA is a global problem causing nosocomial infections. According to WHO MRSA infections affect 150,000 patients annually in European countries. MRSA infection puts an additional economic burden of 380 million euros per year on European countries (6).

It has been investigated that the MRSA mortality rate is 20,000 in the USA. MRSA mortality rates are higher than AIDS and tuberculosis combined. In the past 10 years, very high increase in the prevalence of MRSA has been found. (7).

MRSA infection risk is higher in children, aged individuals, sportsmen, military service members, drug abusers, people living in undeveloped backward urban areas, people with HIV AIDS or cystic fibrosis, and people who are healthcare providers or frequently interact with healthcare

providers, and those living in prison. MRSA infection rates spiked from the 1990s to the 2000s. Before the emergence of *Community-acquired MRSA*, it was assumed that only hospitalized populations are infected by MRSA, but in the early 1980s cases were reported in those people who have never been hospitalized. (8)

**Phenotypic methods:** The disk-diffusion method can be used to test for methicillin resistance in pure *Staphylococcus aureus* cultures that were produced by plating clinical samples on suitable culture media. This procedure comprises either adding a cefoxitin disc to Mueller-Hinton agar or, as advised by the Clinical and Laboratory Standards Institute (CLSI), supplementing MHA with 4 % NaCl and 6 micrograms per millilitre of oxacillin. Oxacillin was first used as the marker antibiotic to find MRSA, however, CLSI now advises cefoxitin. To avoid misleading negative results, the disk-diffusion method must strictly adhere to incubating samples at 37° degrees centigrade for 24 hours.

**Non-phenotypic methods:** RT PCR tests are used to identify *S. aureus* and the presence of the *mecA* gene is being used for direct MRSA identification from clinical samples. These assays have received widespread validation. In around 1.5 hours, results are obtained (1).

Broad-spectrum antibiotics should not be used indiscriminately to treat MRSA infections; instead, the best course of action should be determined by the conclusive findings of an antibiotic sensitivity test. Telavancin and tedizolid, are two new antibiotics that are considered to be more effective against MRSA infections. Moreover, a different strategy would be to find compounds that can obstruct the efflux process (9).

## 2. MATERIAL AND METHODS

The study design was an observational cross-sectional study, conducted at Rehman medical institute (RMI) Peshawar. The duration of our research studies was 3 months from August 2022 to October 2022. Samples were collected only from different wards of RMI hospital, Peshawar. Other than wards for example pharmacy, OPD, Radiology, and Laboratory were excluded. The sample size was collected through the formula  $n = \frac{z^2 \times p(1-p)}{e^2}$  and our sample size was 160. The age distribution of patients comprised from 1 to 76 years. All samples were collected by non-probability convenient sampling in sterile containers by clinicians using an aseptic technique and transported to the laboratory without delay. All samples were processed according to CLSI guidelines.

### 2.9 Culture and bacterial identification

Collected samples were inoculated into Chocolate agar (CHA) blood agar (BA), and MacConkey media (MA), Selective media for *S. aureus* was not used. *S. aureus* was identified by standard microbiological techniques.

### 2.10 Antibiotic susceptibility testing

Antibiotic susceptibility tests of the *S. aureus* isolates were performed by the Kirby-Bauer disk diffusion method in compliance with Clinical and Laboratory Standards Institute (CLSI) guidelines using Mueller-Hinton agar (MHA) standard media. The inhibition zone standards for antimicrobial susceptibility were considered from tables for interpretative zone diameters of CLSI.

### 2.11 Identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains

MRSA was identified by using cefoxitin (30 µg) discs Muller Hinton agar. Plates were inoculated with test organisms and cefoxitin (30µg) discs were inserted in the plate and incubated at 37°C. Plates containing cefoxitin discs were read following a 24-hour incubation period. The diameter of the zone of inhibition (ZOI) of growth was recorded and interpreted as susceptible or resistant according to the criteria of CLSI. *S. aureus* isolates were deemed methicillin-resistant when the Zone of inhibition was  $\leq 21$  mm with the cefoxitin disc.

### 2.12 Ethical Consideration

Institutional consent was taken from the department of microbiology at Rehman Medical Institute (RMI) Peshawar, Pakistan. Data is kept confidential and only used for academic purposes.

### 2.13 Data Analysis

The Statistical Package for Social Sciences software (SPSS) was used to analyse all of the data. Quantitative data were presented in the form of a Pie Chart. A histogram is used for the presentation of Data.

## 3. RESULTS

The present study showed that prevalence of MRSA in different wards of RMI hospital Peshawar. Out of 160 patients, 74 (46.25 %) were males' patient, and 86(53.75) were female patients. The age distribution of patients comprised from 1 to 76 years. We divided our study population into different age groups. The age group 1 to 15 years comprises 22.5 % of the whole study population while the age groups 16 to 30 years, 31 to 45 years, 46 to 60 years, and 61 to 75 years encompasses 15

%, 15.6 %, 21.3 %, and 18.1 % of our study population respectively.

Out of 160 samples collected, 18 (11.25 %) samples were found *Staphylococcus aureus* positive. Out of 18 *S.aureus* specimens, 17 (94.44 %) were found MRSA positive. By using clindamycin disk, moxifloxacin disk, cloxacillin, and erythromycin disks the isolates were detected as MRSA

Other bacteria found were *Enterococcus* (1.9 %), *Pseudomonas aeruginosa* (5.6 %), *Klebsiella pneumonia* (5.6 %), *Enterobacter aerogenes* (3.8 %), *Escherichia Coli* (7.5 %), *Acinetobacter baumannii* (1.9 %), *Citrobacter braakii* (0.6 %), *Proteus mirabilis* (1.3 %) while no growth was reported in 60 % of samples.

**Table 1: Gender distribution of the Study population**

Serial No.	Patient	Frequency	Percent	Valid Percent	Cumulative Percent
1	Male	73	45.6	45.6	45.6
2	Female	86	53.8	53.8	99.4
3	Gender	1	.6	.6	100.0
4	Total	160	100.0	100.0	100.0

**Table 2: Age distribution of the study population**

Serial No.	Patients age (Years)	Frequency	Percent	Valid Percent	Cumulative Percent
1	1 to 15	36	22.5	22.5	22.5
2	16 to 30	24	15.0	15.0	37.5
3	31 to 45	25	15.6	15.6	53.1
4	46 to 60	34	21.3	21.3	74.4
5	61 to 75	29	18.1	18.1	92.5
6	above 76	11	6.9	6.9	99.4
7	Age	1	.6	.6	100.0
8	Total	160	100.0	100.0	100.0

**Table 3: Sample nature of samples collected**

Serial No.	Patients reports	Frequency	Percent	Valid Percent	Cumulative Percent
1	Bile Culture report	1	0.6	0.6	0.6
2	Bronchial Wash Culture Report	1	0.6	0.6	1.3
3	Catheter Tip Culture Report	7	4.4	4.4	5.6
4	CSF Culture Report	9	5.6	5.6	11.3
5	End bronchial Secretion Culture Report	1	0.6	0.6	11.9
6	Fluid Culture Report	5	3.1	3.1	15
7	Fluid Culture sensitivity report	1	0.6	0.6	15.6
8	High Vaginal Swab Culture Report	3	1.9	1.9	17.5
9	Pleural Fluid Culture report	1	0.6	0.6	18.1
10	Pus Culture	2	1.3	1.3	19.4
11	Pus Culture Report	9	5.6	5.6	25
12	Pus Swab Culture	1	0.6	0.6	25.6
13	Pus Swab Culture Report	15	9.4	9.4	35
14	pus swab Culture sensitivity report	2	1.3	1.3	36.3
15	Sample nature	1	0.6	0.6	36.9
16	Sputum Culture Report	8	5	5	41.9
17	Stool Culture Report	11	6.9	6.9	48.8
18	Stool Culture sensitivity report	2	1.3	1.3	50
19	Tissue Culture	1	0.6	0.6	50.6
20	Tissue Culture Report	4	2.5	2.5	53.1
21	Tissue Culture sensitivity report	1	0.6	0.6	53.8
22	Tracheal Secretion Culture Report	4	2.5	2.5	56.3
23	Urine Culture Report	61	38.1	38.1	94.4
24	Urine Culture sensitivity Report	9	5.6	5.6	100
24	Total	160	100	100	100

**Table 4: Different bacterial species identified**

Serial No.	Valid	Frequency	Percent	Valid Percent	Cumulative Percent
1	<i>Enterococcus species</i>	3	1.9	1.9	1.9
2	<i>Staphylococcus aureus</i>	18	11.3	11.3	13.1
3	<i>Pseudomonas aeruginosa</i>	9	5.6	5.6	18.8
4	<i>Klebsiella pneumonia</i>	9	5.6	5.6	24.4
5	<i>Enterobacter aerogenes</i>	6	3.8	3.8	28.1
6	<i>Escherichia coli</i>	12	7.5	7.5	35.6
7	No growth	96	60.0	60.0	95.6
8	<i>Acinetobacter baumannii</i>	3	1.9	1.9	97.5
9	<i>Citrobacter braakii</i>	1	.6	.6	98.1
10	<i>Proteus mirabilis</i>	2	1.3	1.3	99.4
11	<i>species identification</i>	1	.6	.6	100.0
12	Total	160	100.0	100.0	100.0

#### 4. DISCUSSION AND RECOMMENDATION

Methicillin-resistant *S. aureus* also called superbug or bad bug has created a serious concern for healthcare planners as a limited options available to treat MRSA. Problems with MRSA are not only confined to industrialized countries an alarmingly high rate of MRSA infections was also observed in Pakistan in the past decade. Early MRSA incidence was reported in Pakistan in 1989 and then its prevalence has been observed to be increasing. Different studies have depicted variations in the prevalence rates of MRSA in different countries. Differences in the length of the study period, number of study sites, sample size, sample type, and the laboratory procedures employed may be factors that could contribute to variations in the prevalence rate of MRSA. The rate of MRSA obtained in this study, however, was nearly the same as the MRSA prevalence rate recorded in different research studies in different countries.

In the present study, we found a 94.44 % frequency of MRSA among *S. aureus* isolates. In comparison with another study performed by Tekalign Kejela et al in 2022 about the prevalence of MRSA among patients of a hospital in Ethiopia. The point prevalence of MRSA was investigated by a cross-sectional investigation. 384 patients in total, of which 201 were men and 183 were women, were admitted to the different wards of the Mettu Karl Hospital in Ethiopia. The study's sample size was 384 samples, including 166 swabs from wounds and 218 swabs from the nasal cavities. Based on visual and biochemical characteristics, *Staphylococcus aureus* was separated, characterized, and identified. Its identity was then confirmed using Polymerase Chain Reaction amplification of the nuc gene. The isolates were tested against 12 different antibiotics, and MRSA was recognized by cefoxitin (30 g) disc, which was then verified by the amplification of the mecA gene. *Staphylococcus aureus* was found in 126 out of 384 Samples (32 %). MRSA was present in

clinical specimens at a point prevalence of 18.8 % (72/384). MRSA was present in 72 out of the 126 *Staphylococcus aureus* samples (57.1). MRSA was isolated from 35 out of the collected 166 samples taken from patients in the surgical unit. Patients admitted to surgical wards had a higher rate of MRSA isolation than those to medical and paediatric wards. (10).

Ahmad Nikpey 2021 investigated the rate of MRSA in different wards of a hospital in Iran. This was a cross-sectional study. Samples were collected from 12 different wards of hospitals. Each hospital has 255 and 230 patient beds, and they are both in the north of the Iranian city of Qazvin. Gram-negative bacteria and *S. aureus* were found in 59.6 and 80 % of the samples, respectively. The most contaminated ward was the intensive care unit, which had a 7.5 % prevalence of MRSA. MRSA was recovered from 16.7 % of the surface samples, whereas *S. aureus* was found in 20 % of them. (11). Zainol Abidin et al 2020 conducted a research study about MRSA Infections in a tertiary care hospital in Malaysia. In this research experiment, he determined the prevalence of MRSA infection in the only a surgical ward. Out of the 598 patients who were *staphylococcus aureus* positive, 51 patients were found to have MRSA infection, making the frequency of MRSA in surgical wards 8.53 %. (12).

Raut S et al 2017 conducted a research study about the prevalence of MRSA in a hospital in Nepal. A total of 1981 samples were taken from patients attending the hospital for treatment. From the total of 1981 samples, 133 were confirmed as *S. aureus*. MRSA was detected by the disk diffusion method. Cefoxitin was used as an antibiotic disk for MRSA detection. Among 133 *Staphylococcus aureus* samples, 58 (43.6 %) samples were MRSA. (13). Pus (118, 55.4%) had the greatest rate of *S. aureus* isolation among clinical specimens.

CSF and urethral discharge did not contain any *S. aureus*. 194 *S. aureus* isolates were discovered; 34

(17.5 %) were MRSA and the remainder 160 (82.5 %) were MSSA. A total of 98 (50.5 %) *S. aureus* isolates tested positive for several drugs, with penicillin resistance at its greatest (187, 96.4 %) and clindamycin and vancomycin resistance at its lowest (23, 11.9 %, and 10.1 %, respectively). MRSA strains were completely resistant to penicillin G, erythromycin, trimethoprim-sulfamethoxazole, and vancomycin (10, 29.4 %), with the latter being the least resistant. 153, or 79.0 %, of the 194 *S. aureus* isolates were beta-lactamase producers. (14).

Shilpa Arora et al 2020 studied about the prevalence of MRSA in a tertiary care hospital in India. This research study included 250 coagulase-positive staphylococci isolated from a total of 6743 specimens (like blood, pus, urine, sputum, etc.). Using the oxacillin and cefoxitin disc diffusion method, the oxacillin screen agar method, and the minimum inhibitory concentration method with broth macro dilution, methicillin resistance was found. MRSA was discovered in 115 strains overall (46 %) 73 % of MRSA strains had multidrug resistance. (15).

In 2018 Tarequl Islam et al. investigated about the prevalence of MRSA in Hospitals in Chittagong, Bangladesh. The sample size of this research study was 100. Out of the 100 samples in total, 66 *Staphylococcus aureus* isolates were identified. Out of 66, 53 of them (80.3 %) tested positive for oxacillin but to prevent any false results the 53 oxacillin-positive isolates were then tested again for cefoxitin resistance. 43 (65.15 %) were confirmed to be MRSA, while the remaining 23 isolates (34.85 %) were determined to be methicillin-sensitive. (16).

The research was conducted in 2017 by Tuba Siddiqui et al. on the prevalence of MRSA and its susceptibility profile in healthcare facilities in Karachi, Pakistan. The frequency of MRSA was 52 % out of a total of 346 *S. aureus* strains. MRSA infection was shown to be more prevalent in people aged 21 to 30 (i.e. 30 %), followed by people aged 31 to 40 (i.e. 20 %). Male and female MRSA prevalence rates were 70 % and 30 %, respectively. MRSA was found in blood 20 % more frequently. MRSA exhibited strong resistance to Cefoxitin and Oxacillin and reported isolates with 25 % Teicoplanin resistance and 100 % Vancomycin-resistant *S. aureus* (VRSA) resistance. Minocycline resistance in MRSA was 16 %. (17)

## 5. CONCLUSIONS

According to our research studies the occurrence of MRSA in *Staphylococcus aureus* specimens was high which is a significant threat to public health in

hospitals. This high prevalence of MRSA highlights the need for periodic assessments to understand and characterize MRSA infections. In addition, the maintenance of proper hygiene by hospitalized patients and staff and avoidance of irrational use of broad-spectrum antibiotics could effectively reduce the rate and dissemination of multi-drug resistant strains .

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