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# Drug Resistance Pattern in Methicillin-Resistant Staphylococcus Aureus (MRSA) Isolates from Lahore, Pakistan

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## Abstract

**Background:** Methicillin resistant Staphylococcus aureus (MRSA) infections are of great risk to health because they are resistant to most common antibiotics and affixed to hospital acquired and community acquired infections. An evaluation of the antimicrobial resistance patterns of MRSA isolates from blood samples collected in Lahore, Pakistan is performed and prevalence as well as susceptibility trends for MRSA are reported. **Objective:** The purpose of this study was to determine the drug resistance patterns of MRSA isolates and assess their susceptibility to different agents, both beta lactam and non-beta lactam. **Methods:** A total of 103 fresh blood sample specimens were investigated using the standard protocols. Colonies were cultured on blood agar plates and Gram stained and the following biochemical tests (catalase and coagulase tests) for the identification of S. aureus were performed. Kirby Bauer method was used for antimicrobial susceptibility testing on Muller Hinton agar using antibiotic discs for ciprofloxacin, clindamycin, piperacillin/tazobactam, gentamicin, linezolid, penicillin, teicoplanin, vancomycin, and cefoxitin. **Results:** Of 103 blood samples of MRSA, 30 had growths of MRSA. The results indicated that all MRSA isolates were resistant to cefoxitin and penicillin with 100% beta lactam resistance. On the other hand, vancomycin, teicoplanin and linezolid showed 100% susceptibility, which makes them good therapeutic options. In this study, resistance rates for clindamycin, tazobactam and gentamicin were found to be 33.3%, 23.3% and 26.7%, respectively. The resistance rate of ciprofloxacin was 63.3%. **Conclusions:** Beta lactam antibiotics resistance of MRSA isolates of Lahore are very high therefore alternative agents like vancomycin, teicoplanin and linezolid are used. Too often, MRSA is treated as a highly primitive bacteria, which has it wrong; these findings highlight the need for more surveillance, routine testing and antibiotics judiciously."

## Lay abstract

Antibiotic resistance is becoming a global issue. One of the bacterial illnesses that leads to greater health and financial consequences is methicillin-resistant Staphylococcus aureus (MRSA) infection. There is currently no reliable information on the prevalence of MRSA infection in Nepalese youngsters. Thus, a pediatric hospital served as the setting for the present study.

Additionally, we found that MRSA is not frequently tested, thus we advise that, like other bacterial illnesses, it be constantly checked.

## Introduction

Staphylococci, gram positive cocci colonies which are usually white with regular edge (1). Non spore forming nonmotile facultative anaerobes usually oxidase negative and catalase positive (H) (2). Staphylococcus aureus is one of the commonest bacterial human pathogens that produces a wide spectrum of clinical symptoms (3). Because multidrug-resistant strains of MRSA (Methicillin-resistant Staphylococcus aureus) are becoming more prevalent, treating hospital-acquired and community-acquired MRSA is still challenging. Most healthy individuals normally have S. aureus in their surroundings as well as in their normal human flora (blood and skin) (4).

A greater incidence of severe infections in previously healthy non-hospitalized people is caused by MRSA strains. A kind of MRSA infection known as community-associated MRSA infections (CA-MRSA) affects healthy individuals who have not been admitted to the hospital in the previous 12 months (5). Direct touch is usually the source of transmission. Some illnesses, nevertheless, are spread by other means (6). The most prevalent bacterium, Staphylococcus aureus, is primarily responsible for skin, respiratory, and joint infections, among other illnesses. Numerous hard surfaces, like handrails and doorknobs, contribute to the spread of pathogenic bacteria in our surroundings (7).

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most prevalent strains of bacteria that are resistant to antibiotics (beta-lactams). Methicillin, penicillin, amoxicillin, and oxacillin are examples of beta-lactam antibiotics. A penicillin-binding protein 2A that is resistant to all beta-lactam drugs is produced by MRSA. MRSA spreads quickly via skin-to-skin contact and hands, and it may be more common in certain populations. Because MRSA is resistant to medications meant to precisely kill it, it is challenging to treat (8). According to recent research, CA-MRSA is primarily found among inmates, athletes, daycare providers, military personnel, and groups of individuals who often exchange infected objects or reside in crowded regions (9).

Even though MRSA is present everywhere, infection rates vary by country. Research indicates that MRSA is more prevalent in India and Pakistan than in northern Europe. In Pakistan, methicillin-resistant S. aureus has been shown to be 42–51% common. As MRSA-associated infections are on the increase, glycopeptides like teicoplanin and vancomycin are now being used more often. The irrational and negligent use of glycopeptides causes S. aureus to acquire even low-level vancomycin resistance. Despite being uncommon, this resistance poses a growing hazard because to its capacity for quick dissemination (10). The research focuses on the drug resistance isolation among the samples collected from different areas of Lahore.

## Methodology:

**Sample Collection**: A total of 103 blood samples were collected in the blood culture vials and transferred to the lab by following protocols and samples were incubated in machine (Bact/ALERT 3D) at 37 C for 24 hours.

**Figure 1: Sample collected in blood culture vials**

**Figure 2: Sample incubation in BacT/ALERT 3D**

**Inclusion Criteria:**

No Visible Contamination: Blood culture bottles or tubes should not have obvious contamination (e.g. cloudiness, incorrect appearance, abnormal culture growth), and there should be no appearance of culture growth outside of expected conditions.

**Exclusion Criteria:**

Contaminated Samples: Only non-contaminated blood cultures with visible signs of contamination (e.g. bacterial growth in non-sterile conditions) should be included in the study.

**Isolation and identification**: After incubation the samples were cultured on the blood agar media and plates were incubated for 24-48 hours at 37 C.

**Blood agar composition**

| Ingredients | Gram / % |
|---|---|
| Peptone | 10.0 |
| Beef Extract | 5 % |
| Agar | 5.0 |
| Sodium Chloride | 15.0 |
| pH | 7.4 |

The gram staining of bacterial isolates was carried out by applying crystal violet for 1 minute, gram iodine for 1 minute, decolorizer for 20-30 seconds and safranin for 1 minute and then observed the slide on microscope under 100X lens to see Staphylococcus which show gram positive cocci.

**Gram staining**

| | |
|---|---|
| Crystal violet | 1 minute |
| Gram iodine | 1 minute |
| Decolorizer | 20-30 seconds |

| | |
|---|---|
| Safranin | 1 minute |

**Biochemical identification:** Then we performed biochemical tests like catalase (shows the presence of catalase enzyme by producing the gas bubbles) and coagulase test (EDTA or citrate-treated rabbit plasma that has been rehydrated with sterile distilled water) for the confirmation of Staphylococcus aureus.

**Antibiotic susceptibility testing**: We did antibiotic susceptibility testing by following Kirby Baur's method. We prepared the muller Hinton agar for AST.

**Muller Hinton agar composition**

| Ingredients | Gram |
|---|---|
| Beef Extract | 2.00 |
| Acid Hydrolysate of Casein | 17.50 |
| Starch | 1.50 |
| Agar | 17.00 |

We inoculated the colony from blood agar media on MH agar and then we added different antibiotic discs which are Ciprofloxacin (CIP), Clindamycin (DA), Piperacillin/Tazobactam (TZP), Gentamicin (CN), Linezolid (LZD), Penicillin (P), Teicoplanin (TEC), Vancomycin (VA) and Cefoxitin (FOX).

## Results

### Isolation and identification:

Among total blood culture sample 30 samples showed the growth of Staphylococcus. Staphylococcus aureus colonies were observed on blood agar after 24-48 hours of incubation at 37˚C and showed shiny, convex, hemolytic white colonies. After gram staining, we observed the purple cocci shaped colonies under microscope at 100X.

**Figure 3: Colonies of S. aureus**

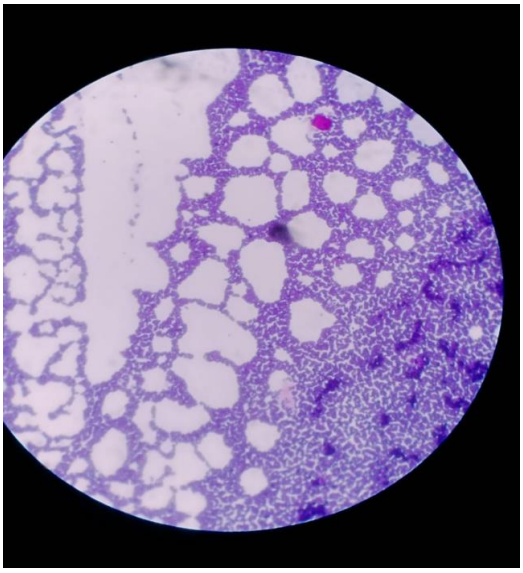

**Figure 4: Gram staining results**

**Biochemical Testing:**

**Catalase Test:**

The catalase test was carried out to determine the presence of Staphylococcus aureus. Gas production shows the presence of Staphylococcus aureus in the samples.

**Figure 5: Catalase test's result**

**Coaggulase Test:**

The coagulase test was carried out to determine the presence of Staphylococcus aureus. Clumping shows the presence of Staphylococcus aureus in the samples/ Isolates.

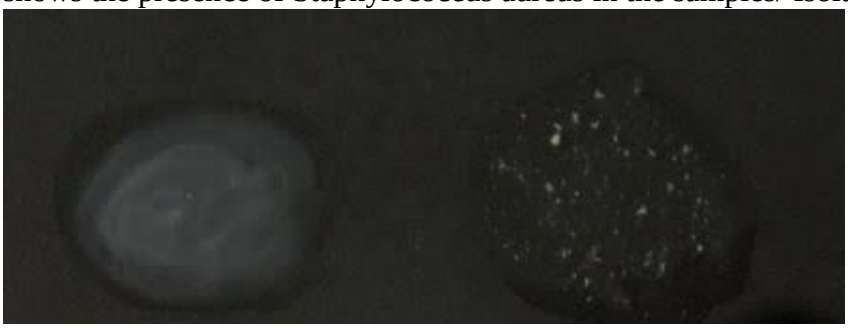

**Figure 6: Coagulase test results**

**Drug Susceptibility Testing:**

In order to determine Antibiotic Susceptibility, bacterial colony was inoculated on muller Hinton agar plate. After 24 hours of incubation, we observed growth of bacteria and antibiotic zone against the Methicillin resistant Staphylococcus aureus.

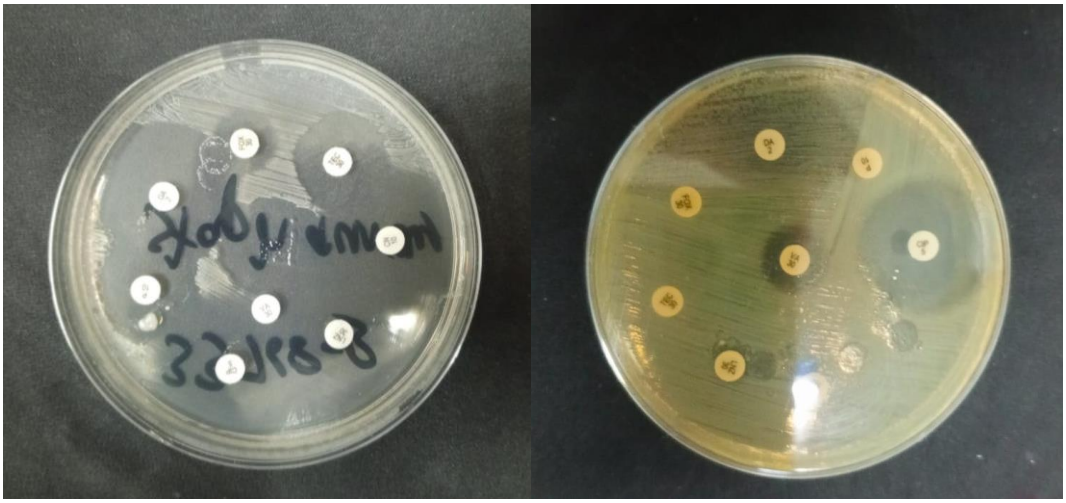

**Figure 7: Antibiotic susceptibility test results**

An overview of MRSA isolates' antimicrobial resistance (AMR) trends against antimicrobial agents is shown in Table 4. There were just a few MRSA isolates that tested positive for clindamycin (33.3%), tazobactam (23.3%), and gentamycin (26.7%). It was also shown that β-lactam antibiotics, such as cefoxitin (100%) and penicillin (100%) were ineffective against MRSA.

**Table 4: Antibiotic susceptibility test results**

| Antibiotic | µg/Disk | S (mm) | R(mm) | S% | R% |
|---|---|---|---|---|---|
| **CIP** | 05 | >21 | <15 | 36.7 | 63.3 |
| **DA** | 02 | >21 | <14 | 66.7 | 33.3 |
| **CN** | 10 | >15 | <12 | 73.3 | 26.7 |
| **LZD** | 30 | >26 | <22 | 100 | 0 |
| **TZP** | 30 | >22 | <21 | 76.7 | 23.3 |
| **TEC** | MIC | <08 | >32 | 100 | 0 |
| **P** | 10 | >29 | <28 | 0 | 100 |
| **VA** | MIC | <02 | >16 | 100 | 0 |
| **FOX** | 30 | >22 | <21 | 0 | 100 |

**Susceptibility pattern towards Cefoxitin and Penicillin**

Cefoxitin is a second generation cephalosporins, beta-lactam antibiotics which binds to the penicillin or transpeptidase. Among 30 Methicillin resistant Staphylococcus aureus isolates, all (100%) showed resistance against cefoxitin. Penicillin is also a beta-lactam antibiotic which is more effective against gram positive bacteria. All of the samples (100%) were found to be resistant against Penicillin.

**Susceptibility pattern towards Vancomycin, Teicoplanin and Linezolid**

A glycopeptide antibiotic called Vancomycin is used to treat bacterial infections like MRSA that are severe yet susceptible. In the present study, all 30 isolates (100%) showed susceptibility to Vancomycin. Teicoplanin is a long-acting semi synthetic glycopeptide antibiotic that works well against infections caused by gram-positive bacteria, similar work as Vancomycin. This antibiotic also showed 100 susceptibilities. Linezolid, an antibiotic that belongs to the oxazolidinone family, is very good in treating severe Gram-positive infections (MRSA) similar as Vancomycin. Like VA, LZD also showed 100% susceptibility.

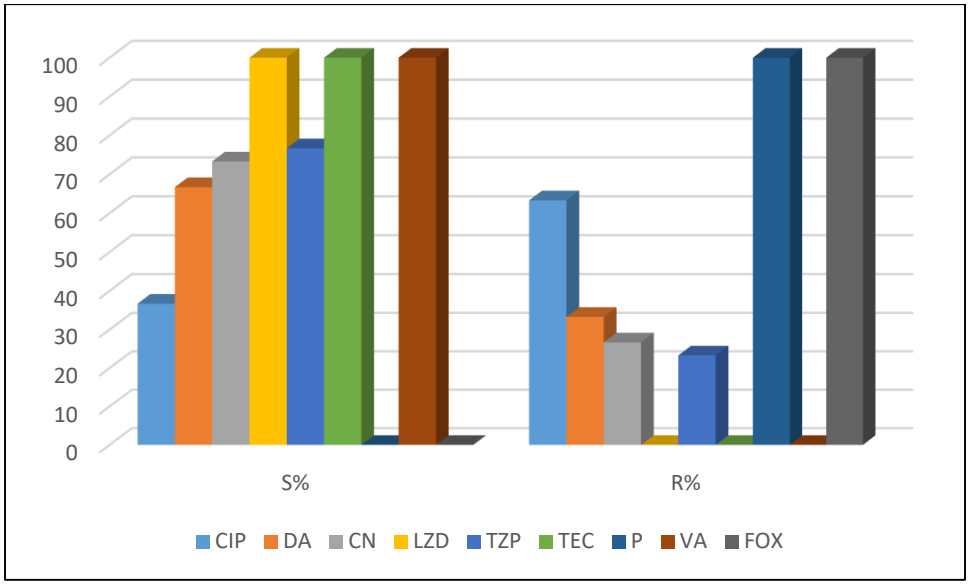


**Figure 8: Graphical representation of antibiotic susceptibility pattern**

## Discussion

S. aureus infections may be harmful to one's health, particularly in middle- and low-income countries. MRSA is emerging as a significant worldwide public health issue. MRSA has significantly increased rates of patient morbidity and mortality, as well as lengthier hospital admissions. S. aureus infections provide a significant clinical problem in medical practice since the bacterium is resistant to the commonly given first-line treatments. However, the incidence of MRSA differs significantly by nation. This research used a variety of sample types to examine the prevalence of S. aureus in patients. Every sample that contains clinically identified S. aureus might act as a reservoir for MRSA, which could spread the illness across a population. As a result, the likelihood of a community-acquired MRSA infection developing quickly is increased. Despite being a direct vital source, S. aureus infections may also be isolated from fomites. In hospitals, fomites might be regarded as an indirect means of spreading S. aureus and other infectious infections (11). Therefore, it is impossible to overlook environmental sources and fomites.

Methicillin-resistant S. aureus has been identified as a significant bacterial pathogen that causes hospitalization and community infections globally because of its elevated virulence and steadily increasing resistance to antibiotics. Although the worldwide incidence of MRSA is steadily rising, European nations typically have lower MRSA frequencies than other regions of the globe. The adoption of stringent infection control procedures and antibiotic prescription regulations in European nations may be the cause (10). In the present study, 36.1% of MRSA among the S. aureus isolates in our investigation. According to a research by Naeem et al., the prevalence of MRSA in Peshawar was 31.5% (12).

Vancomycin may be the preferred medication for treating multidrug-resistant MRSA infections since all of the MRSA isolates examined in this research were found to be completely responsive to it. Several earlier investigations revealed similar results (13). Furthermore, all isolates showed linezolid sensitivity, which is consistent with previous studies (14, 15). Since the FDA has authorized linezolid for the treatment of MRSA infections, it may be a viable substitute for vancomycin (16). Patients treated with linezolid had a much greater survival rate than those treated with vancomycin, according to a previous research (17). Cefoxitin resistance was seen in all MRSA isolates, which is consistent with earlier research (18).

## Conclusion

The contribution of this study is to demonstrate the alarming prevalence of multidrug resistant S. aureus in life the Lahore, some things that MRSA is posing to the clinical and community settings.

Beta lactam antibiotic resistance is complete in MRSA isolates, with the availability of alternative treatments manifested in vancomycin, teicoplanin and linezolid as reliable therapeutic options. MRSA requires regular monitoring of patterns of antimicrobial resistance in order to guide appropriate treatment and prevent further transmission. Additionally, in resource limited settings, improvements to outcomes really require stronger antibiotic stewardship and adoption of molecular diagnostic techniques.

**Perspective on the future**

Similar to previous medication resistance situations, this research emphasizes the significance of frequent MRSA testing, reporting, and monitoring. Health care professionals (HCWs) need to be more aware of nosocomial infections and AMR. Even if molecular approaches are not cost-effective on a small scale, they should be used in developing nations like Pakistan to validate the diagnosis of microbial diseases and MDR. To better understand the nature of specific bacterial infections, there should be a substantial increase in ongoing AMR surveillance and hospital associated infection (HAI) monitoring. It should be possible to prescribe combination medications as part of empirical treatment, especially in poor nations. Overall population is often more exposed to the outside world due to their active participation in outdoor activities.

**Author Contributions:**

Conceptualization, Hafiz Tanzeel Ahmed Qureshi; Writing—original draft preparation, Tanzeel Ahmad Qureshi, Aamir Amin, Muhammad Tayyab Sarwar, Afshan Nosheen; Writing—review and editing, Tanzeel Ahmad Qureshi, Muhammad Tayyab Sarwar, Afshan Nosheen, Aamir Amin; Supervision, Aamir Amin; All authors have read and agreed to the published version of the manuscript

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