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Determining the Antibiotic Resistance of *Staphylococcus aureus* Strains Isolated from Patients Hospitalized in Zahedan, Southeastern Iran

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ABSTRACT

Background & Objective: *Staphylococcus aureus* is one of the most important public health risks as it causes many forms of infection. Treatment options for infections caused by this pathogen are limited due to the development of resistance to commonly used antibiotics. This study aimed to investigate the antibiotic resistance patterns in *Staphylococcus aureus* strains.

Materials & Methods: In this cross-sectional study conducted between 2020 and 2023, 348 *Staphylococcus aureus* specimens were isolated from clinical samples at Aliebne Abitaleb Hospital in Zahedan, Iran. Following the identification of the strains, their antibiotic resistance to eight antibiotics was assessed by the disk diffusion method based on Clinical Laboratory Standard Institute 2022, and the MRSA isolates were screened. Additionally, Vancomycin's MIC was evaluated using the E-test. Statistical analysis was conducted using the chi-square test using SPSS, version 16 (IBM SPSS, Armonk, NY, USA).

Results: Of the 348 isolated *S. aureus* strains (171 from males and 177 from females), 163 (50%) were from blood cultures, and 53 (15.2%) were from tracheal samples. The strains showed the highest resistance to erythromycin (77.9%) and penicillin (100%). The samples had the lowest resistance to cotrimoxazole (36.2%) and nitrofurantoin (29.6%). Additionally, all strains were susceptible to vancomycin. In this study, 76% and 30% of the examined strains could be classified as MDR and XDR, respectively. There was no significant association between age, gender, and MRSA infection ($P > 0.05$).

Conclusion: In this study, cotrimoxazole showed considerable activity against MRSA strains. Moreover, since all isolates were susceptible to vancomycin, this antibiotic could be the primary treatment choice. The high prevalence of MDR strains is a significant cause for concern.

Keywords: **Antibiotic resistance, *Staphylococcus aureus*, MRSA**

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1. Introduction

Nosocomial infections are a significant global public health issue. The increasing antibiotic resistance of pathogens associated with hospital spaces has become a significant treatment challenge for doctors (1). Infectious diseases rank as the second most important cause of human mortality globally (2). Nosocomial diseases are defined as the factors that develop locally or systemically after 48 hours of hospitalization. The development of these infections is increasing significantly, and there seems to be problems in solving this problem. Infectious diseases are most commonly encountered in the form of sepsis (bloodstream culture), urinary tract infection (UTI), surgical site injury (SSI), and respiratory tract infection (RTI) (3). Regarding the treatment of diabetic patients, the increasing population, using more drugs for treatment, and using medical devices leads to risks in the treatment of patients (4). Also, with advances in knowledge, specific monitoring studies now aim to identify the type of pathogens responsible for infections and the pattern of antibiotic resistance to help doctors choose the correct antibiotic (5). The rise of antibiotic-resistant bacteria in health care is a serious crisis (6). However, the excessive and irrational use of

antibiotics increases the pathogens' resistance to them, which is also associated with an increase in treatment costs (6). The most common strains isolated from hospital clinical samples are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae, Enterococcus, and Acinetobacter baummannii* strains (7,8). Hospitals are the main place for bacteria resistant to antimicrobial agents, and they play an essential role in the emergence and spread of these infections. Public health is seriously threatened by antibiotic resistance (9).

This is a natural process by which bacteria have evolved over billions of years to adapt to environmental threats, either by horizontal gene transfer or genetic mutation (10). Meningitis and bacteremia are two bacterial infections that can now be treated thanks to antibiotics. Antibiotic-resistant bacteria have become more widespread in recent decades due to the overuse of antibiotics, combined with social and economic factors; at least 700,000 people worldwide die each year as a result of antibiotic resistance. Without new and effective antibiotics, the World Health Organization estimates that this number will increase to 10 million untreated cases by 2050, creating a public health concern (11). A list of pathogens with the acronym ESKAPE (*Staphylococcus aureus*, *Enterococcus faecium*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa, Acinetobacter baumannii*, and *Klebsiella pneumoniae*) was released by the World Health Organization (WHO) in February 2017 in response to the growing prevalence of antibiotic resistance (12). Although antibiotic treatment for all patients can reduce mortality to some extent, blood culture infection and sepsis are two of the severe and life-threatening factors that can still lead to death in hospitalized patients. These days, examining and determining patients infected with pathogens such as methicillin-resistant *Staphylococcus* (MRSA), *Pseudomonas aeruginosa*, and other pathogens resistant to a wide range of antibiotics are receiving attention (13). The human pathogen *Staphylococcus aureus* is highly prevalent and can cause fatal pneumonia, endocarditis, osteomyelitis, bacteremia, and infections of the skin and soft tissues. The rate of staphylococcal MRSA infections has increased. The bacterium's drug resistance has increased due to bacterial evolution and overuse of antibiotics in the past few decades (2).

Serious infectious diseases such as food poisoning, pyogenic endocarditis, otitis media, osteomyelitis, pneumonia, and pyogenic skin and soft tissue infections are frequently caused by MRSA in humans (14).

The primary pathogen that causes illnesses that start in the community and hospitals is MRSA. The World Health Organization (WHO) recently identified MRSA as one of the twelve priority pathogens threatening public health (15). Furthermore, in several regions across the globe, including the Middle East, North Africa, the United States, Europe, and East Asia, MRSA has emerged as the most common antibiotic-resistant pathogen. MRSA infections also have a higher death rate than AIDS, Parkinson's disease, and homicides, according to the US Centers for Disease Control (CDC, 20). MRSA infections acquired in hospitals now affect 50.4% of the population in China. Moreover, identifying inducible clindamycin resistance merely through a D-test can result in treatment failure (16). Infections with MRSA can be harder to treat than other bacterial infections. For a long time, vancomycin has been regarded as the best medication for treating invasive infections like pneumonia, sepsis, and severe MRSA infections. We conducted this study using clinical samples from the Ali Ebne Abitaleb Hospital in Zahedan to ascertain the prevalence of MRSA strains, *Staphylococcus aureus* isolates, and inducible clindamycin resistance.

2. Materials and Methods

This descriptive and analytical study collected 30000 clinical samples from blood cultures, urine, tracheal aspirates, synovial fluid, knee fluid, abdominal fluid, catheters, pleural fluid, wounds, abscesses, and the skin.

The samples were collected from patients admitted to Ali Ebne Abitaleb Hospital in Zahedan, Southeastern Iran, from March 2020 to February 2023. Among the collected samples, 348 *Staphylococcus aureus* infections were identified. The isolates were cultivated on blood and chocolate agar. Gram staining, catalase activity, and biochemical tests (MSA test, DNase test, and slide and tube coagulase tests) were used to confirm their identification (17).

2.1 Antibiogram susceptibility test

All *Staphylococcus aureus* isolates underwent an antibiotic susceptibility test using the disc diffusion test (DDT) following CLSI 2022 (Clinical Laboratory Standard Institute, 2022) guidelines. The isolates were categorized as sensitive, resistant, or intermediate by measuring the growth inhibition zone for each antibiotic. The results were then interpreted following CLSI 2022 guidelines. The disc diffusion test uses discs impregnated with drugs, including ciprofloxacin, cotrimoxazole, clindamycin, erythromycin, and penicillin doxycycline (MAST, UK).

2.2 E-test method

E-test strips (Liofilichem, Italy) were used to determine vancomycin susceptibility and were finally interpreted according to CLSI 2022 guidelines (18).

2.3 Microbiological processing of MRSA clinical samples

We used cefoxitin (30 ug) and oxacillin (1 ug) disks (Mast, UK) to identify MRSA isolates. Cefoxitin discs have also been used to identify methicillin resistance. Other studies have also demonstrated the superiority of cefoxitin discs (19). MRSA is indicated by a growth inhibition zone surrounding the oxacillin disk of 10 mm or less. Regarding the cefoxitin disk, MRSA was identified as the growth inhibition zone less than or equal to 21 mm (20).

2.4 Inducible clindamycin resistance test

D-shaped susceptibility patterns to clindamycin were regarded as D-test positive (D+). The isolates underwent susceptibility testing to erythromycin (15 µg) and clindamycin (2 µg) (Mast, UK).

Muller-Hinton agar (MHA) plates were incubated with a 0.5 McFarland pathogen suspension following CLSI 2009 guidelines.

Disks containing erythromycin and clindamycin were arranged 15–26 mm apart on the MHA plates. Inducible clindamycin resistance was identified as flattening the D-shaped growth inhibition zone surrounding clindamycin following an 18-hour incubation period at 37 °C (21).

3. Results

3.1 Characteristics of S. aureus strains identified in the clinical samples

From the 3000 clinical samples, 348 *S. aureus* cases were isolated, 171 isolates (49.1%) from male patients and 177 (50.9%) from female patients. Also, strains of *S. aureus* were isolated from different clinical samples, including 163 strains (50%) from blood, 42 (12.1%) from urine, 41 (11.8%) from wounds, 54 (15.2%) from the trachea, 8 (2.3%) from the throat, 4 (1.1%) from pleural fluid, 4 (1.1%) from abscesses, 2 (0.6%) from abdominal fluid, and 12 (5.8%) from other fluids. The results for MRSA isolates are shown in Table 1. Among the 348 *S. aureus* isolates, 221 were MRSA, and 127 were MSSA (Table 1).

Table1. Frequency of MRSA and MSSA strains in various *S. aureus* clinical samples

Clinical strain	MRSA[a] (*n* = 221)		MSSA[b] (*n* = 127)	
	Female	Male	Female	Male
Wound	5	10	10	6
Blood	18	17	75	64
Urine	6	3	25	8
Tracheal	10	12	15	16
Throat	1	2	5	0
Pleural fluid	0	3	0	1
Abscess	0	1	2	1
Abdominal fluid	0	1	0	1
Other	4	3	6	4

MRSA: methicillin-resistant *S. aureus*
[b]MSSA: methicillin-susceptible *S. aureus*

3.2 Prevalence of clinical strains in different wards

The isolates from patients in intensive care units 30 (23.9%) and emergency rooms 30(21.6%) had higher rates of MRSA detection (Figure 1) (Table 2).

3.3 Prevalence of antibiotic resistance

Figure 1 shows the details of antimicrobial resistance of strains with very high antimicrobial resistance levels. Out of 348 *S. aureus* isolates, 77.9% were resistant to erythromycin, 43.6% to ciprofloxacin, 52.7% to doxycycline, 36.2% to trimethoprim/sulfamethoxazole, and 29.6% of the urine samples were resistance to nitrofurantoin. All strains (100%) were sensitive to vancomycin (E-test < 2). However, 76% and 30% of strains were identified as MDR and XDR strains, respectively (Figure 2).

Table 2. Prevalence of MRSA isolates in different wards

	Em.	ICU	CCU	Int.	Op.	Sur.	Burn	Co.	Hem.	Mat.	Neo.	Ped.	Tra.	Out.
MRSA	30	30	1	13	4	1	2	2	7	3	1	14	1	1
MSSA	29	24	1	21	2	4	1	8	9	5	5	10	1	4

Em.: emergency, Int.: internal, Op.: operation room, Sur.: surgery, Co.: covid, Hem.: hematology, Tra.: transplant

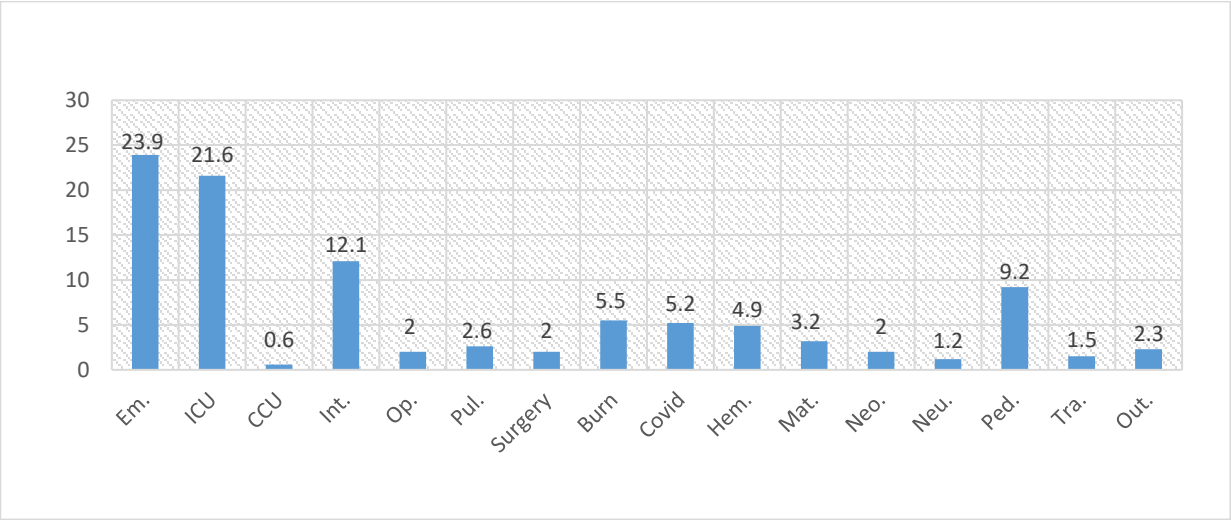


Figure1. Prevalence of clinical strains in different wards
Em.: emergency, Int.: internal, Op.: operation room, Hem.: hematology, Mat.: maternity, Neo.: neonatology, Neu.: neurology, Ped.: pediatric, Tra.: transplant, Out.: outpatient

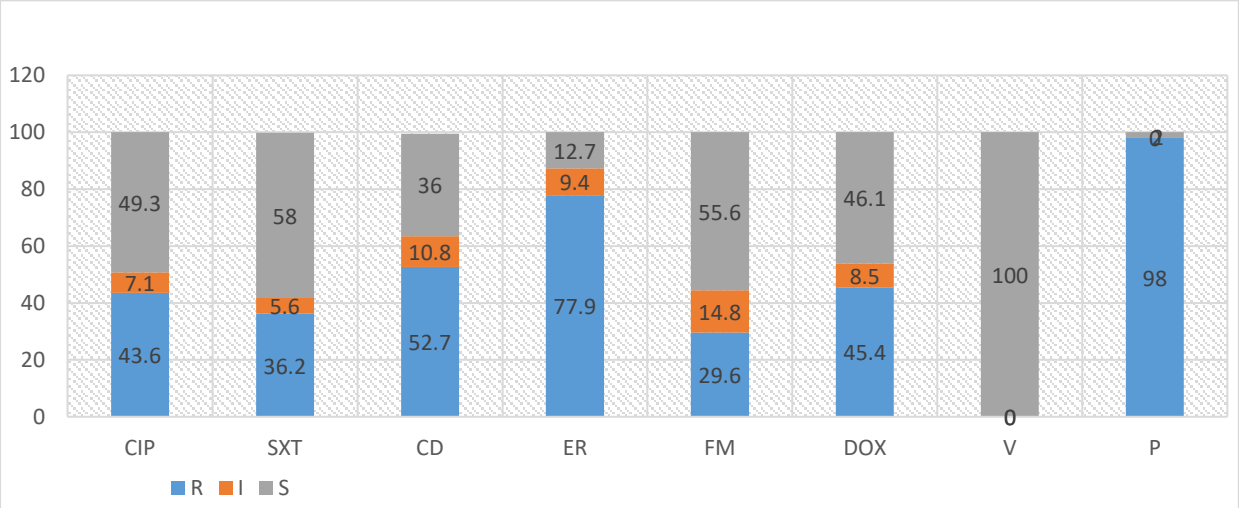


Figure2. Pattern of antibiotic resistance in S. aureus clinical isolates
CIP: ciprofloxacin, SXT: trimethoprim/sulfamethoxazole, CD: clindamycin, ER: erythromycin, FM: nitrofurantoin, Dox: doxycycline, V: vancomycin, P: penicillin

4. Discussion

The prevalence of infection with MRSA strains has steadily increased worldwide since their introduction in 1961 (22). MRSA strains account for over half of *S. aureus* infections in the US. Iranian reports also show that MRSA has become more common over time in clinical specimens. However, the results of different studies on MRSA infection rates are by no means similar (23). The application of various infection control strategies, the use of antibiotics, the population, the study design, and laboratory testing to identify methicillin resistance could all contribute to the heterogeneity. As seen in Fig. 2, this study indicated that the most prevalent strains were those susceptible to vancomycin and trimethoprim/sulfamethoxazole. Of the isolates of *S. aureus* found in this study, 55.6% were resistant to nitrofurantoin. Furthermore, none of the isolates had vancomycin-resistant strains, and every strain had 100% penicillin resistance. Although there is evidence of a decrease in the prevalence of MRSA isolates in some European countries, according to Dibah et al., this bacterium is still discussed as a major problem in public health (20). According to studies, patients admitted to ICUs in Ardebil had the highest percentage of MRSA strains (68.4%) isolated from them, all of which were vancomycin-susceptible (24). As stated by Rahimi et al., in studies examining the antibiotic resistance pattern of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*

isolates in Tehran, the highest antibiotic resistance belonged to penicillin, clindamycin, tobramycin, and tetracycline. No strain was resistant to vancomycin, and 98 percent of the MSSA isolates were resistant to penicillin (25). Also, Jafari et al. showed that out of 256 examined samples in Tabriz, 197 (76.95%) were MRSA with high resistance to penicillin (100%), co-amoxiclav (94.22%), and gentamicin (81.22%), and chloramphenicol induced the lowest resistance (16.75%)(26). Rezazadeh et al. revealed that 80 out of 100 *S. aureus* isolates were resistant to methicillin in Arak. Tetracycline (88.5%), penicillin (100%), ciprofloxacin (85.7%), and levofloxacin (85.7%) showed the highest rates of resistance, while chloramphenicol (5.70 %) showed the lowest (27). Additionally, a high prevalence of MRSA has been reported in other countries. Wang et al. reported that out of 409 *S.aureus* isolates, 260 (63.6%) were MRSA and 100% were resistant to penicillin. In addition, similar to our study, 81% and 79% were resistant to erythromycin and clindamycin, respectively (28). In Saudi Arabia and Kuwait, MRSA infection rates were reported as 33% and 32%, respectively (29). A review of 15 studies shows that *S. aureus* infection rates with MRSA are between 13% and 74% worldwide (30). In the United States, the incidence of invasive MRSA infections was 31.8% per 100,000 people in 2005 (31). More than 60% of *S. aureus* infections were MRSA-positive in Italy, Portugal, and Romania (32). Treatment recommendations for MRSA bacteremia typically include vancomycin or daptomycin (33). In our study, all of the isolates were sensitive to vancomycin. Thus, this antibiotic could be recommended for the treatment of MRSA infections. Also, in the present study, infection with MRSA was seen in patients with septicemia and short- and long-term catheters more than others (Table 1). Additionally, there was no relation between infections and gender. In Arora et al.'s study, 63 (54.8%) of the strains were MRSA in orthopedic wards and surgical units; in contrast, emergency and intensive care unit (ICU) wards had the highest percentage of MRSA isolates (Table 2). More than half of the *S. aureus* recovered from patients in intensive care units (ICUs), and roughly 40% of the *S. aureus* isolated from non-ICU patients are healthcare-associated MRSA (HA-MRSA) strains, which continue to be a significant issue in many institutions (34).

5. Conclusion

Effective antibiotic therapy and suitable infection control measures are imperative in light of the high prevalence of MRSA infections in hospitals.

6. Declaration

6.1 Acknowledgments

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6.2 Authors' Contribution

ZB: Conceived and designed the experiments; Critical revision of the article MJ: Analyzed and interpreted the data, Performed the experiments; Drafting of the Article

6.3 Conflict of Interest

The authors declare that they have no conflict of interest.

6.4 Funding

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6.5 Ethics Approval and consent to participate

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