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Prevalence and Distribution of Methicillin-Resistant *Staphylococcus Aureus* Among Clinical Isolates from Patients in Basra Province, Iraq

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ABSTRACT

Background: *To identify and characterize methicillin-resistant Staphylococcus aureus (MRSA) isolates from clinical specimens in Basra Province, Iran.* ***Methods:*** *MRSA isolates were identified and tested for antimicrobial susceptibility by using the VITEK® 2 system. Antibiotic resistance was further confirmed using the standard disk diffusion method and detection of the mecA gene via PCR. Inducible clindamycin resistance was detected using a modified disk diffusion assay following CLSI guidelines.* ***Results:*** *Of 108 Staphylococcus aureus isolates, 41 (38%) were identified as MRSA and 67 (62%) as methicillin-susceptible S. aureus (MSSA). Methicillin resistance was confirmed in all MRSA isolates by PCR detection of the mecA gene (310 bp). MRSA isolates showed higher resistance rates to erythromycin (51% vs. 24%), tetracycline (49% vs. 21%), and clindamycin (42% vs. 19%) than MSSA isolates. Methicillin resistance was the most prevalent in sputum isolates (85.7%), followed by wounds (35.6%), and blood samples (30%). Among the erythromycin-resistant MRSA isolates, 33.3% exhibited inducible clindamycin resistance (iMLSB phenotype), compared to 12.7% of the MSSA isolates.* ***Conclusion:*** *This study demonstrated a notable prevalence of MRSA in Basra, with high resistance to commonly used antibiotics. The detection of inducible clindamycin resistance underscores the importance of routine D testing. Continued surveillance, antimicrobial stewardship, and infection control measures are essential to effectively manage the MRSA burden.*

Keywords: Antibiotic Resistance, Methicillin-Resistant Staphylococcus Aureus.

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INTRODUCTION

Staphylococcus aureus is a versatile pathogen responsible for a wide range of infections, from mild skin and soft tissue infections (SSTIs) to life-threatening conditions, such as endovascular diseases, toxic shock syndrome (TSS), bacteremia, pneumonia, and sepsis (1). The emergence of methicillin-resistant S. aureus (MRSA) has severely complicated treatment options, particularly in healthcare settings across Iraq, where MRSA prevalence often exceeds 50% among clinical isolates (2, 3, 4). A recent study conducted at Al-Sader Teaching Hospital showed that S. aureus represented 31.57% of skin infections and 31.57% of bloodstream infections, with 91% of isolates resistant to methicillin and oxacillin (5).

Globally, MRSA colonization and infection continue to pose significant challenges. In the broader Middle East and North Africa (MENA)

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region, nasal carriage of MRSA among healthcare workers ranged from 10% to 22%, while community carriage varied across countries (6). In Iraq's Kurdistan region, healthcare workers were found to have over 60% nasal carriage of MRSA compared to approximately 22% among community members. These figures are mirrored in Duhok and Basra, where MRSA not only circulates in healthcare facilities but also contaminates food sources. One study found MRSA in 14.8% of locally sold food items (7). The widespread presence of MRSA in both hospital and community settings highlights the pressing need for robust surveillance, infection control, and antibiotic stewardship programmes in Iraq. Limited access to diagnostic infrastructure, inconsistent hygiene practices, and antibiotic misuse exacerbate the transmission and burden of MRSA (8, 3).

In conclusion, MRSA is a major threat to public health in southern Iraq. The high prevalence rates, both in clinical specimens and among carriers, underscore the importance of coordinated regional efforts. Future strategies should include routine screening (especially among healthcare professionals), improved infection control protocols, molecular typing to monitor clonal spread, and tailored antimicrobial stewardship policies to reduce the impact of MRSA infection across the country. Our study aimed to characterize MRSA isolates collected from outpatients or patients upon admission to hospitals in Basra Province.

MATERIAL AND METHODS

A total of 108 non-duplicated Staphylococcus isolates were collected between September (2024) and March (2025), from Al Sadir Teaching Hospital, Al Basrah Maternity & Children's Teaching Hospital and AL-Fayhaa Teaching Hospital in Basrah. These samples were collected from patients with wound infections (n = 73), sputum (n = 7), bloodstream infections (n = 23), diabetes swabs (n = 1), ear swabs (n = 3), and abscess swabs (n = 1). This study was approved by the Research and Development and Ethics Committee/Health Authority of Basra Province.

Bacterial Isolation

Collected samples were placed on blood agar at 37 ^{o}C for 24 h. The bacterial isolates were assessed for shape, size, color, pigment synthesis, and hemolytic capacity. The isolates were then transferred to mannitol salt agar, a selective and differential medium used to isolate and identify Staphylococcus species, and their ability to ferment mannitol was tested. All the plates were incubated at 37°C for 24 h. A single, pure colony from each isolate was transported into the VITEK 2 system to confirm bacterial identification and perform antibiotic susceptibility tests.

Bacterial Identification and Antibiotic Susceptibility Testing

Bacterial identification and antimicrobial susceptibility testing were conducted according to CLSI guidelines (9) using the VITEK® 2 System (BioMérieux, USA), following the manufacturer's recommended protocol.

Additional Confirmation of Antimicrobial Susceptibility Testing

In addition to the VITEK 2 system's automatic susceptibility testing, manual confirmation testing was carried out on Mueller-Hinton agar plates using the standard disk diffusion method in accordance with CLSI guidelines (9).MRSA isolates were identified using oxacillin (1 µg) and cefoxitin (30 µg) disks, and their susceptibility profiles were determined using a variety of antibiotics (Oxoid, Basingstoke, UK): gentamycin (10 µg), tetracycline (30 µg), clindamycin (2 µg), erythromycin (15 µg), ciprofloxacin (5 µg), moxifloxacin (5 µg), linezolid (30 µg), rifampicin (5 µg), and trimethoprim/sulfamethoxazole (5 µg), all of which were supplied by Mast Group Ltd., UK.

Detection *Mec*A gene in MRSA isolates

Resistance to methicillin was confirmed by detecting a 310 bp PCR product of the *mec*A gene using the forward primer GTA GAA ATG ACT GAA CGT CCG ATAA and reverse primer CCA ATT CCA CAT TGT TTC GGT CTAA (10). PCR reactions were prepared in a final volume of 25 µL, consisting of 12.5 µL of Taq Green PCR Master Mix (2X) (Promega, USA), 2 µL of bacterial DNA, 1 µL of each primer (10 µM), and 8.5 µL of nuclease-free water (Promega, USA). The thermal cycling conditions included initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 1 min. The final extension step was performed at 72°C for 5 min. A negative control (reaction without the DNA template) was included. The PCR products were analyzed by electrophoresis on a 2% (w/v) agarose gel stained with gel green and visualized under a UV transilluminator alongside a 100 bp DNA ladder.

Inducible Clindamycin Resistance (D-Test)

The D test was performed by a modified disk diffusion assay based on CLSI guidelines (9), placing a 15-µg erythromycin disk in close proximity (usually 15–20 mm) to a 2-µg clindamycin disk on a Mueller-Hinton agar plate that was inoculated with the test isolate. The plate was incubated aerobically at 35–37°C for 16–18 h. A positive result is indicated by a blunted or flattened zone of inhibition around the clindamycin disk, adjacent to the erythromycin disk, forming a characteristic "D" shape. A positive D-test suggests that the isolate exhibits inducible clindamycin resistance (iMLSB phenotype), and clindamycin should be reported as resistant despite being susceptible to routine testing.

RESULTS

A total of 108 isolates from the collected samples were identified as *S. aureus*, of which 41 (38%) isolates were MRSA and 67 (62%) were methicillin susceptible *S. aureus* (MSSA). Resistance to methicillin was confirmed by detecting a 310 bp PCR product of the *mec*A gene in all MRSA isolates (Figure 1). MRSA was highly resistant to erythromycin compared with MSSA (51VS 24%), tetracycline (49% VS 21%), and clindamycin (42% vs. 19%) **(Figure 2)**.

A high rate of resistance to methicillin was detected in **85.7%**%, 35.6%, and 30% of the sputum, wound, and blood isolates, respectively **(Figure 3)**. Furthermore, a total of 21 (51.2%) MRSA isolates were resistant to erythromycin, among which 7 (33.3%) were D test-positive (iMLSB phenotypes) compared to 12.7% of MSSA exhibiting iMLSB phenotypes.

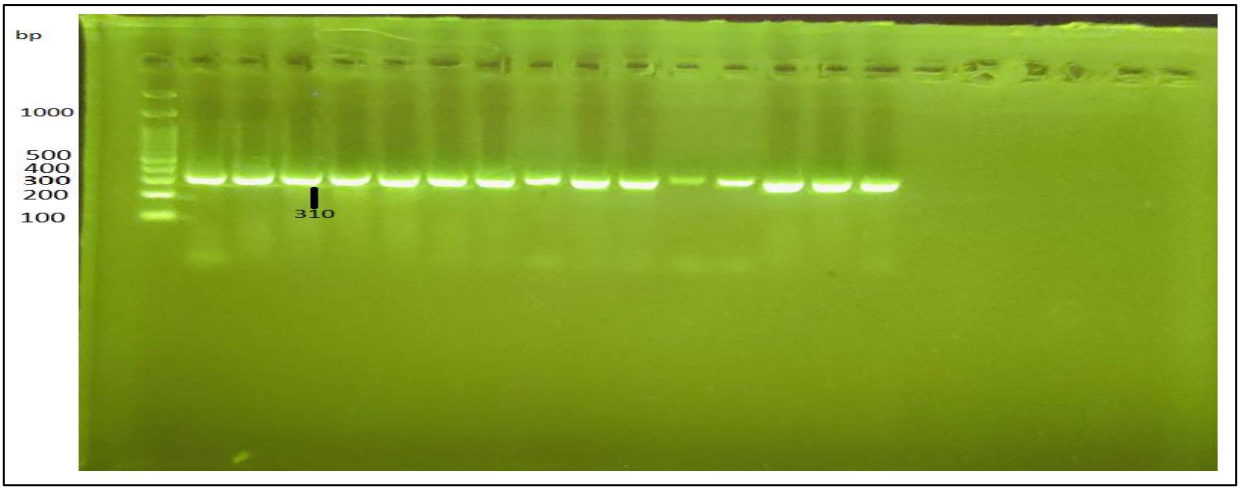

Figure 1: Gel electrophoresis showing *mecA* gene bands (310 bp) of representative MRSA isolates.

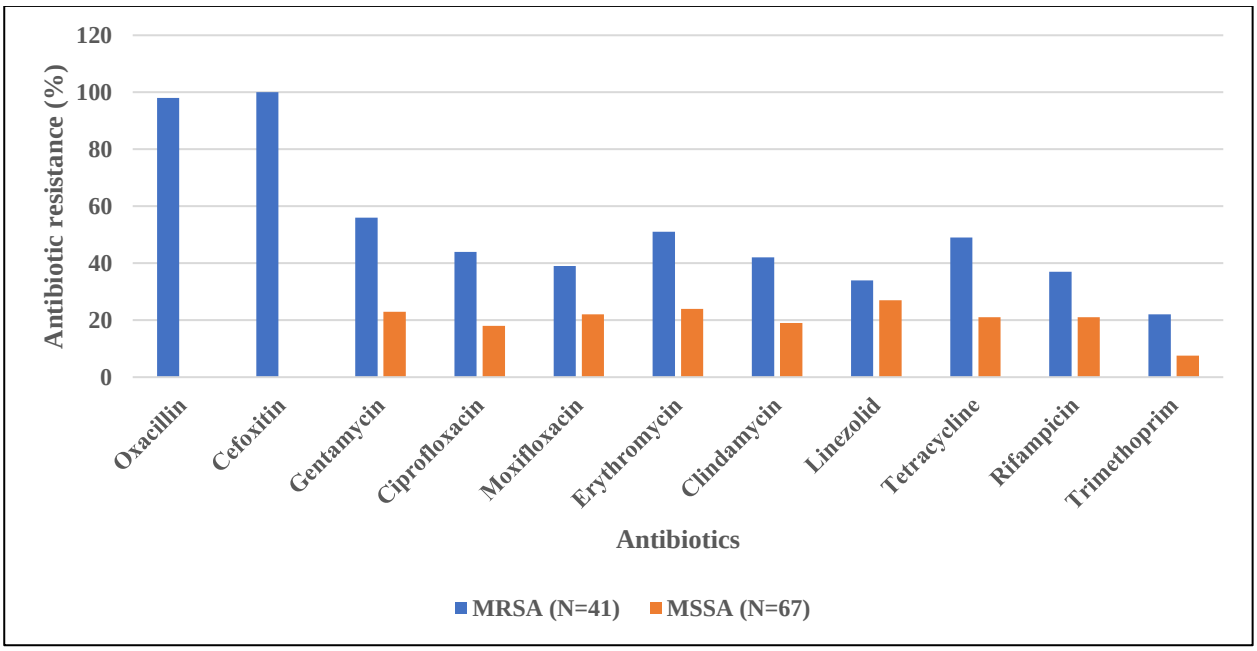

Figure 2: Antibiotic resistance pattern of MRSA and MSSA isolates.

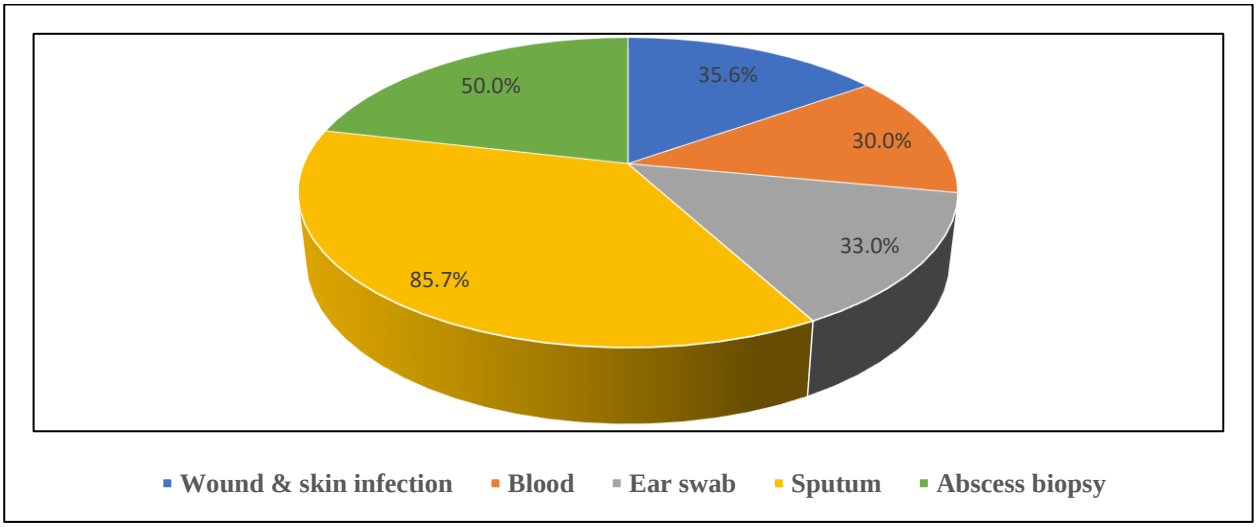

Figure 3 : Distribution of MRSA isolates by source of infection.

DISCUSSION

In the present study, out of 108 *Staphylococcus aureus* isolates, 41 (38%) were identified as methicillin-resistant *S. aureus* (MRSA), while 67 (62%) were methicillin-susceptible *S. aureus* (MSSA). The MRSA proportion aligns with some regional trends, but varies considerably both globally and across the Middle East. The global prevalence of MRSA among *Staphylococcus aureus* infections varies significantly, not only between continents, but also within individual countries and regions. According to the World Health Organization's Global Antimicrobial Resistance and Use Surveillance System (GLASS), MRSA prevalence rates range from less than 5% in Northern Europe to approximately 50% in Southern Europe and the United States, with even higher rates of 56–86% reported in parts of East Asia (11, 12, 13, 14). In Africa, most available data are from the Mediterranean region, where MRSA prevalence ranges from 19% to 52%. In Nigeria, reported rates vary between 11% and 31%, while in South Africa, the prevalence is

approximately 24%. However, data from many Sub-Saharan African countries remain limited and are often based on single-center studies (15, 11). In the Middle East, MRSA rates tend to be higher than in many Western countries. A recent meta-analysis estimated MRSA prevalence in Gulf Cooperation Council (GCC) countries to be around 15–55% (16). In Iraq, the reported MRSA rates have varied. A study by Baghdad found MRSA prevalence among *S. aureus* clinical isolates to be 33% (17), which is comparable to the 38% prevalence found in our study. Other studies conducted in Mosul reported a 52.8% MRSA rate (18), and in Duhok, Kurdistan, and Iraq 50.4% (7), suggesting a variable prevalence range in different Iraqi regions. Resistance to erythromycin, tetracycline, and clindamycin was significantly higher in MRSA than in MSSA isolates in the current study:

- Erythromycin: 51% (MRSA) vs. 24% (MSSA).
- Tetracycline: 49% (MRSA) vs. 21% (MSSA).
- Clindamycin: 42% (MRSA) vs. 19% (MSSA).

These findings are in line with global patterns where MRSA strains generally show higher resistance to multiple non-β-lactam antibiotics. Recent data indicate that the pooled global prevalence of resistance to erythromycin, clarithromycin, and azithromycin across 76 countries is 57.3%, 52.6%, and 57.9%, respectively. The highest rate of erythromycin resistance was reported in Oceania (72%), whereas Europe had the lowest rate (40.7%). Subgroup analyses showed significant variation in resistance patterns by bacterial species, with MRSA exhibiting higher resistance rates than MSSA, and coagulase-negative staphylococci (CoNS) showing greater resistance than other species. Notably, a slight decline in erythromycin resistance was observed over time, decreasing from 59.6% between 2015 and 2019 to 55% between 2020 and 2023 (19, 12).

Similar resistance trends were observed for Iraq. Sami and Qassim (2022) reported clindamycin resistance in 47.05% of MRSA isolates and erythromycin resistance as high as 91.1%, indicating a notably higher level of erythromycin resistance than the findings of the current study (8).

The highest methicillin resistance was observed in sputum isolates (85.7%), followed by wounds (35.6%) and blood (30%). This pattern may reflect hospital-acquired respiratory infections, particularly in patients admitted to the ICU. A study from Egypt reported MRSA prevalence in respiratory samples, ranging from 60% among ICU patients (20). Similar high MRSA rates in lower respiratory tract infections have been reported by Defres et al. (2016) (21).

In the present study, the D-test revealed inducible clindamycin resistance in:

- 33.3% of erythromycin-resistant MRSA isolates.
- 12.7% of erythromycin-resistant MSSA isolates.

These findings underscore the importance of D testing in clinical laboratories. Comparable frequencies of inducible clindamycin resistance have been reported previously. For instance, Mokta et al. (2015) found iMLSB phenotypes in 28.39% MRSA and 9.29% MSSA) (22). In Africa, a systematic review showed that a high number of iMLSB phenotypes were observed in MRSA isolates (3.6–77.8%) than MSSA (0–58.8%) (23).

CONCLUSION

This study contributes to the growing body of evidence highlighting the ongoing challenge of MRSA infection in both global and regional contexts. The observed resistance patterns, particularly to erythromycin and clindamycin, were consistent with findings across the Middle East, including Iraq. The high rate of inducible clindamycin resistance emphasizes the necessity for routine D-testing to guide effective antimicrobial therapy. Ongoing surveillance, antimicrobial stewardship, and

infection control are critical in managing the MRSA burden.

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