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Isolation, Molecular Characterization and Antibiotic Resistance Pattern of MDR-Staphylococcus aureus Isolated from Clinical Samples

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I. Abstract

Antibiotic resistance has emerged as a major global health crisis, advancing faster than the development of new antimicrobial drugs. Staphylococcus aureus is a significant human pathogen capable of causing a wide spectrum of infections, ranging from mild skin wounds to severe diseases such as osteomyelitis, bacteremia, and endocarditis. This study aimed to isolate and identify S. aureus from various clinical samples and to determine its antibiotic resistance profile. A total of 100 clinical isolates were collected from hospitals in Thi-Qar Province, Iraq. Bacterial identification was performed using selective media, biochemical tests, and the VITEK-I system. Antibiotic susceptibility testing was conducted using the Kirby–Bauer disc diffusion method and confirmed with the VITEK 2 system according to CLSI guidelines. Molecular investigation was conducted for the presence β-lactam resistance genes (mecA and blaZ). Results revealed that most isolates were multidrug-resistant (MDR). The highest resistance rates were observed against ampicillin and erythromycin (90% each), while resistance to oxacillin and cefoxitin reached 100%, confirming methicillin resistance (MRSA). Molecular analysis demonstrated that all MRSA isolates carried the mecA and blaZ genes responsible for β-lactam resistance. Vancomycin remained the most effective antibiotic among those tested. This study highlights the alarming incidence of MDR-S. aureus in clinical infections within Thi-Qar province, emphasizing the urgent need for rational antibiotic use, improved infection control, and continuous monitoring of resistance genes in hospital settings.

Keyword: Staphylococcus, Antibiotics resistance, MDR, PCR, and XDR

II. INTRODUCTION

Antimicrobial resistance (AMR) has become one of the most critical global health threats of the 21st century, as stated by the World Health Organization (WHO) (Coque *et al.,* 2023). The increasing rates of morbidity and mortality associated with bacterial infections are largely due to the emergence of MDR and extensively drug-resistant (XDR) pathogens (Fatima *et al.,* 2023). The irrational and excessive use of antibiotics in both clinical and community settings has accelerated the evolution of resistant strains by promoting genetic mutations and horizontal gene transfer (Wang *et*

al., 2017; Catalano *et al.,* 2022). Among the various resistant pathogens*, S. aureus* represents one of the most adaptable and dangerous bacterial species. It has developed multiple resistance mechanisms, including enzymatic inactivation of antibiotics, modification of drug targets, efflux pump activity, and antibiotic trapping (Hanif and Hassan, 2019). The rapid emergence of methicillin-resistant *S. aureus* (MRSA) strains has significantly reduced the effectiveness of β-lactam antibiotics and poses serious challenges to clinical treatment options (Nandhini *et al.,* 2022).

Staphylococcus aureus is a Gram-positive coccus that typically forms grape-like clusters and can grow under both aerobic and anaerobic conditions. It produces various virulence factors such as hemolysins, proteases, and exotoxins responsible for severe infections including septicemia, osteomyelitis, pneumonia, and endocarditis (Tam and Torres, 2019; Divyakolu *et al*., 2019). Furthermore*, S. aureus* is a leading cause of hospital-acquired and community-acquired infections and is responsible for significant food-borne illnesses worldwide (Tigabu and Getaneh, 2021). Despite global efforts to control antibiotic resistance, developing countries such as Iraq continue to face increasing rates of hospital-acquired and community-acquired infections caused by *S. aureus*. Limited surveillance programs, over-the-counter antibiotic availability, and lack of molecular monitoring contribute to the widespread misuse of antibiotics and the emergence of multidrug-resistant strains (Salam *et al.,* 2023).

II. MATERIALS AND METHODS

Sampling

A total of one hundred clinical samples were collected from various hospitals in Thi-Qar Province, Iraq. Samples were obtained from wounds, cerebrospinal fluid (CSF), blood, burns, sputum, and pus. All laboratory work was performed in the Microbiology Laboratory, College of Veterinary Medicine/ University of Shatrah.

Isolation and Identification

All Samples were cultured on Blood Agar and Mannitol Salt Agar (MSA) media to isolate and differentiate *Staphylococcus* species. Plates were incubated at 35°C for 24 hours. Morphological characteristics, including colony appearance and β-hemolysis on Blood Agar, were recorded. Biochemical tests (catalase, and oxidase), and Gram staining were performed for preliminary identification. Also, *S. aureus* isolates were confirmed using the VITEK 2 compact system (bioMérieux, France) for automated biochemical identification (Abdulhusein and Kadim, 2024).

Antibiotics sensitivity

Antibiotics susceptibility screening was performed using ten antibiotics, which included: Ampicillin (25µg), Rifampin (5µg), Cefixime (5µg), Cephalexin (30µg), Erythromycin (10µg), Clindamycin (10µg), Fusidic Acid (20µg), Oxacillin (10µg), Tigecycline (5µg), and Tetracycline (10µg) against all isolated *Staphylococcus* spp. following the Clinical and Laboratory Standards Institute (CLSI) guideline. The Kirby-Bauer disc diffusion technique and interpretation rules were used to conduct antibiotic sensitivity testing (AST) (CLSI, 2020). In order to compare and modify their turbidities to the 0.5 McFarland standards, five to six bacterial colonies were suspended in five milliliters of sterilized distillation water and then mixed by a vortex. This was done by adding more colonies or water. A bacterial suspension's 0.5 McFarland standard corresponds to 1 x 10^8 CFU/ml. The bacterial suspension was then swabbed onto Muller-Hinton agar. After the plates were allowed to dry at room temperature, sterile forceps were used to place antibiotic disks on the agar surfaces, and the plates were incubated for 24 hours at 37°C. The results were recorded using standard interpretative measures of the inhibition zone (Kadim, 2024).

Molecular Profiling

Genomic DNA was extracted from confirmed *S. aureus* isolates using a Geneaid Bacterial DNA Extraction Kit (Taiwan) according to the manufacturer's instructions. The *mecA* and *blaZ* resistance genes were amplified using conventional polymerase chain reaction (PCR) as shown in table (1).

Table 1. The specific primer used, annealing temperature and the products.

No.	Genus	GENE	Primer Sequence	Annealing temp	Product size
1	*S. aureus*	*mecA-F*	5CCTAGTAAAGCTCCGGAA3	54 ℃	314 bp
		mecA-R	CTAGTCCATTCGGTCCA		
2		*blaZ-F*	ACTTCAACACCTGCTGCTTTC	57 ℃	240 bp
		blaZ-R	TGACCACTTTTATCAGCAACC		

PCR products were analyzed by 1.5% agarose gel electrophoresis using Safe-Green dye and visualized under UV transillumination. The presence of amplification bands corresponding to *mecA* (314 bp) and *blaZ* (240 bp) confirmed the presence of these resistance genes (Parastan *et al*., 2020).

III. RESULTS AND DISCUSSION

A total of 100 clinical samples were collected from patients of both genders and various age groups, including specimens from burns, wounds, cerebrospinal fluid (CSF), sputum, and pus. Out of these, 71 samples (71%) showed positive bacterial growth, while 29 samples (29%) exhibited no

growth. The absence of growth may be attributed to prior antibiotic use, improper sampling techniques, or insufficient bacterial load at the time of collection.

S. aureus represented 39 isolates (54.9%) of the total Gram-positive isolates, consistent with previous findings reported by Chaudhary *et al,.*(2019). On Blood Agar, *S. aureus* colonies exhibited clear β-hemolysis with medium to large, smooth, and convex colonies having shiny margins. On Mannitol Salt Agar, the isolates fermented mannitol, changing the medium color from pink to yellow, indicating the presence of *S. aureus* (Tam & Torres, 2019). All 39 *S. aureus* isolates were tested against ten antibiotics using the Kirby–Bauer disk diffusion method. The results revealed high resistance rates to ampicillin (90%) and erythromycin (90%), while resistance to oxacillin and cefoxitin reached 100%, confirming methicillin resistance (MRSA). Lower resistance rates were recorded for gentamicin (56%) and fusidic acid (51%).A summary of antibiotic susceptibility results is shown in Table (1).

Table (1): Antibiotics susceptibility test for 39 *S. aureus*

Antibiotics	R%	I%	S%
Ampicillin	35(90%)	0	4
Cefixitin	36(92%)	0	0
Ciprofloxacin	30(76%)	1	8
Erythromycin	35(90%)	0	9
Clindamycin	28(72%)	0	11
Fusidic Acid	20(51%)	2	15
Oxacillin	39(100%)	0	0
Gentamicin	22(56%)	0	17
Tetracycline	31(79%)	0	16
Rifampin	20(51%)	1	18

The results found that *S. aureus* was 100% resistant to the antibiotics cefoxitin and oxacillin, so that it is considered to be methicillin-resistant bacteria (MRSA). A study by Thamer and Alsammak (2021) in Erbil showed highly resistance (93.42%) to Methicillin. These studies support the results of the current study. As a result to appear of cefixime and ampicillin resistance in percentage 92%, 90% this consistent with (Mama *et al*., 2019) who found that 82.28% of wound infection causing by *S. aureus* was MRSA. Also, isolates appeared low resistance against antimicrobial agents such as Rifampin and gentamicin in percentage 51% and 56% respectively, this is consistent with Mama *et al*., (2019) who mentioned that *S. aureus* showed high sensitivity to

ciprofloxacin (96.9%), and gentamycin (94%) while other study by Dilnessa *et al*., (2016) recorded that the percentage of ciprofloxacin and gentamycin resistance were 32.4% and 30.2 % respectively. Remarkable susceptibility of isolates may be due to lesser use of these antibiotics in the treatment. These differences in resistance and susceptibility rates may be due to the influence of many factors like the source and the site of samples from which the isolates were collected, the age of the patient, the season of specimens collection, the wrong or incomplete treatment also participate significantly in the development of bacterial resistance against antimicrobials, and how much this antibiotic was used in the community and this differs from person to another.

Molecular screening of *Staphylococcus aureus* isolates revealed that all 39 isolates (100%) harbored both *mecA* and *blaZ* resistance genes (Figure 2 and 3). The amplification of *mecA* (314 bp) and *blaZ* (240 bp) by PCR confirmed their genetic presence in all methicillin-resistant isolates, as shown in figure 1 and 2.

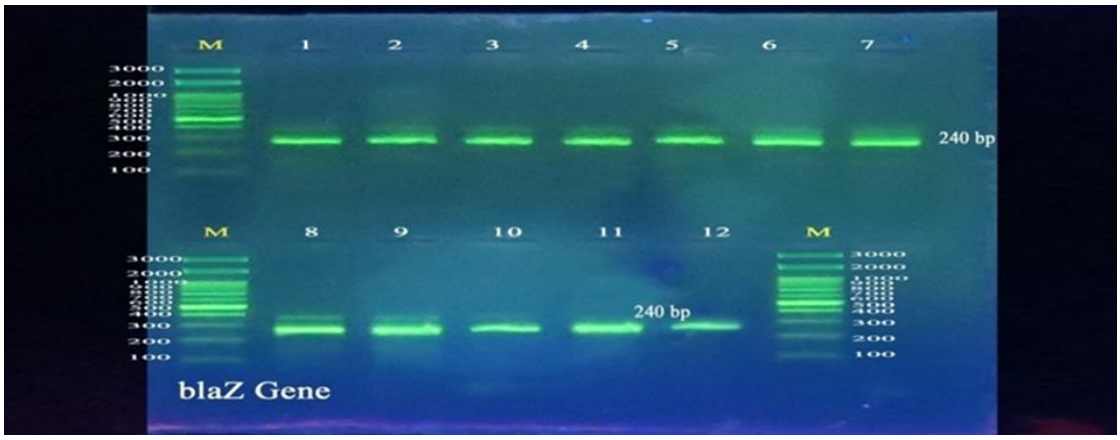

Figure (1). Gel Electrophoresis of amplified PCR product of *blaZ* gene in *S. aureus*

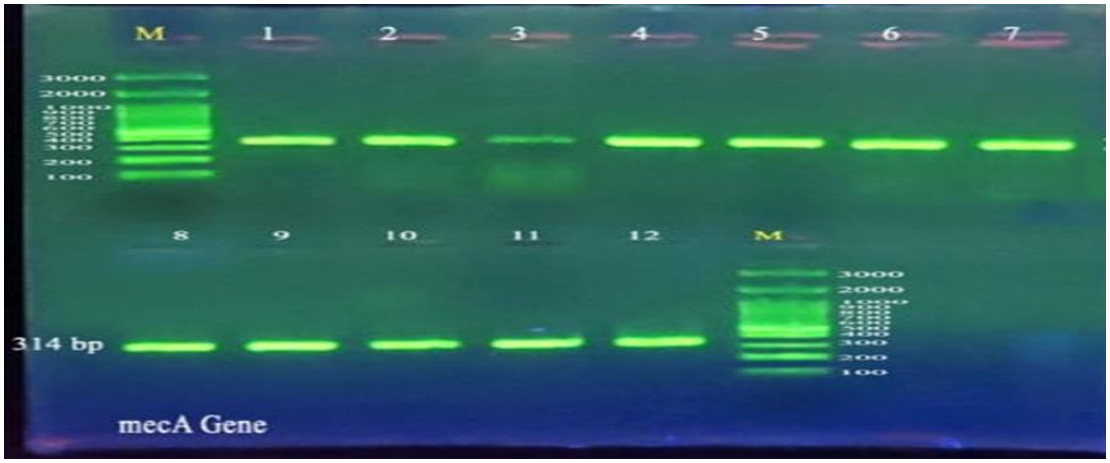

Figure (2). Gel electrophoresis of amplified PCR product of *mecA* gene in *S. aureus*

The *mecA* gene encodes the altered penicillin-binding protein (PBP2a), which has a low affinity for β-lactam antibiotics, thereby conferring resistance to methicillin and other β-lactam agents. Meanwhile, the *blaZ* gene encodes the β-lactamase enzyme responsible for hydrolyzing the β-lactam ring in penicillins and cephalosporins, further contributing to resistance (Guo *et al*., 2020).
The presence of both mecA and blaZ in all isolates correlates with the phenotypic resistance profile, particularly the complete resistance to oxacillin and cefoxitin and the high resistance to ampicillin, observed earlier, particularly the 100% resistance to oxacillin and cefoxitin and the 90% resistance to ampicillin. These findings suggest that the mecA and *blaZ* genes are the major genetic determinants of β-lactam resistance in *S. aureus* from clinical infections in Thi-Qar Province. Similar findings were reported by Mama *et al*., (2019) and Dilnessa and Bitew (2016), who also observed that *S. aureus* isolates carrying *mecA* showed complete resistance to methicillin and cefoxitin. Furthermore, the high prevalence of MDR isolates may be attributed to the widespread and uncontrolled use of antibiotics, particularly β-lactams and macrolides, within local healthcare facilities. The high rate of multidrug resistance (MDR) observed in this study emphasizes the urgent need for continuous molecular surveillance and rational antibiotic prescription policies to mitigate the emergence and dissemination of resistant *S. aureus* strains.

The detection of the *mecA* and *blaZ* genes in all tested *S. aureus* isolates provides a clear explanation for the high-level resistance to beta-lactam antibiotics, including penicillins and cephalosporins. The *mecA* gene encodes the altered penicillin-binding protein PBP2a, which confers methicillin resistance (MRSA), while *blaZ* encodes penicillinase. *Staphylococcus aureus* develops resistance primarily through mechanisms such as modification of ribosomal targets (e.g., erm-mediated methylation causing MLSB resistance), β-lactamase production (blaZ), and expression of the altered penicillin-binding protein PBP2a encoded by *mecA.* Since *S. aureus* is a Gram-positive bacterium, it lacks an outer membrane; therefore, resistance mechanisms involving outer membrane proteins do not apply. For example, the principle of resistance to Clindamycin and Erythromycin is caused by a modification in ribosomal RNA methylase (Guo *et al.*, 2020).This study focused on the current scenario of antimicrobial-resistant *Staphylococcus* spp. In Thi-Qar province the incidence of MDR *Staphylococcus* spp. was high. This high frequency of MDR in our study could be related to a high level of antibiotic use, which could be due to accessibility or low purchase prices of drugs.

IV. CONCLUSION

The present study demonstrated a high prevalence of multidrug-resistant *S. aureus* among clinical isolates collected from Thi-Qar Province, Iraq. Phenotypic and molecular analyses confirmed

that all MRSA isolates harbored both *mecA* and *blaZ* genes, which are directly responsible for resistance to β-lactam antibiotics, including methicillin, cefoxitin, and ampicillin. These findings indicate that *S. aureus* in this region poses a significant public health concern due to its ability to resist multiple antibiotic classes. The correlation between phenotypic resistance and genetic determinants emphasizes the importance of integrating molecular diagnostics into routine clinical microbiology to improve early detection and treatment strategies.

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