Iranian Journal of Microbiology
Volume 17 Number 2 (April 2025) 229-238

ORIGINAL ARTICLE

Resistance profiles of *Staphylococcus aureus* isolates against frequently used antibiotics at private sector laboratories in Jordan

Rania Al-Groom[1,2*], Ghina Al-Saraireh[1], Sultan Ayesh Mohammed Saghir[3], Mohd Sajjad Ahmad Khan[4], Areej M. Almanaseer[1], Laila Alswalha[2], Wesal Alraei[5], Dalia Abu Al-Haijaa[5], Maha Hdaib[2], Anas Da'meh[1], Shereen Z Burjaq[6], Omar Al-Dmour[7], Fuad Alhawarat[8]

[1]Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, Zarqa University, Zarqa, Jordan
[2]Department of Allied Medical Sciences, Zarqa University College, Al-Balqa Applied University, Zarqa, Jordan
[3]Department of Medical Analysis, Princess Aisha Bint Al-Hussein College of Nursing and Medical Sciences, Al-Hussein Bin Talal University, Ma`an, Jordan
[4]Department of Basic Sciences, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
[5]Department of Diet Therapy Technology and Dietetics, Faculty of Allied Medical Sciences, Zarqa University, Al-Zarqa, Jordan
[6]Department of Medical Laboratory Sciences, Faculty of Science, Al-Balqa Applied University, Salt, Jordan
[7]Department of Allied Medical Sciences, Zarqa University college, Al-Balqa Applied University, Salt, Jordan
[8]Department of Applied Medical Sciences, Al Hussein Bin Abdullah II Academy for Civil Protection, Al-Balqa Applied University, Salt, Jordan

Received: October 2024**, Accepted:** December 2024

ABSTRACT

Background and Objectives: *Staphylococcus aureus (S. aureus)* is one of the most important pathogens, responsible for a range of infections. This study aimed to assess resistance patterns in *S. aureus* isolates obtained from certain private-sector laboratories against commonly used antimicrobial agents.

Materials and Methods: The process involved collecting various samples from several private laboratories and then identifying *S. aureus* isolates using biochemical characterization. The antibiotic susceptibility of these isolates was determined by disc diffusion method . Furthermore, Rt-PCR was employed to identify two genes namely the methicillin/oxacillin resistance genes (*mecA*), and (*SCCmec*).

Results: The findings of the current study exhibited that females constituted a larger proportion of the participants (59.1%) compared to males (40.9%), with a mean participant age of 40.82 years. Gram-positive bacteria were more prevalent (71.3%) than Gram-negative bacteria (18.3%), with *S. aureus* being the most frequent isolate (60.9%). Urine samples represented the highest collected sample type (47.8%). Out of the 115 bacterial isolates, 85.2% exhibited multidrug resistance to antibiotics such as cefazolin, gentamicin, vancomycin, and ceftazidime. Clindamycin was the most effective antibiotic, with a sensitivity rate of 62.9%, followed by teicoplanin and meropenem, each with a sensitivity rate of 52.9%. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were susceptabile to vancomycin and teicoplanin. The methicillin/oxacillin resistant isolates

*Corresponding author: Rania Al-Groom, Ph.D, Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, Zarqa University, Zarqa, Jordan; Department of Allied Medical Sciences, Zarqa University College, Al-Balqa Applied University, Zarqa, Jordan.
Tel: +96-2795743948 Fax: +96-253491110 Email: raniaalgroom@bau.edu.jo

showed significant association with *mecA* and *SCCA* genes.

Conclusion: This study highlighted the multi-drug resistance in *S. aureus* isolates, stressing the need for stringent antibiotic stewardship, continuous surveillance, and further research into alternative treatments, including novel antibiotics and combination therapy, to combat resistant strains.

Keywords: *Staphylococcus aureus*; Multidrug-resistant organisms; Methicillin-resistant *Staphylococcus aureus* strains; *MecA*; *SCCmec*

INTRODUCTION

Staphylococcus aureus (S. aureus) belongs to the group of Gram-positive cocci that are non-spore forming and nonmotile (1). It is considered as an opportunistic human pathogen that can cause a wide range of related illnesses (2). It is responsible for minor skin diseases such as cellulitis, folliculitis, as well as abscesses and it also causes more threatening conditions such as pneumonia, endocarditis, osteomyelitis, toxic shock syndrome, and sepsis (1, 3). *S. aureus* can employ various adaptive strategies through its mechanisms that enhance invasion, colonization, and enable it to evade host defense systems facilitating its spread (4). Studies have shown that *S. aureus* can infect three skin sites including the axillae, perineum, and anterior nares, which serve as the main reservoirs for the microorganism, facilitating its reproduction and dissemination throughout the body (3, 5). Exposure to methicillin-resistant *S. aureus* (MRSA) or other antibiotic-resistant *S. aureus* strains within households notably elevates the risk of infection (6).

S. aureus is a primary cause of infections in both community and clinical settings. Methicillin-Sensitive *Staphylococcus aureus* (MSSA) is the main agent, showing sensitivity to all antibiotics used against Staphylococcal infections (7). Also, MRSA bacteria are found to be resistant to nearly all known antibiotics except vancomycin and teicoplanin (8). Several studies have shown that MRSA infections result in higher fatality rates than MSSA infections. Numerous basic health conditions that predispose patients to *S. aureus* infections are associated with failed liver transplants. The active surveillance culture (ASC) seems to be of the greatest utility in countries where MRSA is greatly prevalent, and for high-risk patients, namely immunosuppressive individuals, residents of intensive care units (ICUs), patients in long-term care or hemodialysis facilities (8-10).

The excessive and improper use of antibiotics are commonly regarded as major contributors to the emergence and dissemination of antibiotic resistance. Moreover, prior antibiotic treatments and extended exposure have been associated with a heightened risk of MRSA colonization (11). Nevertheless, microbial species frequently exposed to specific treatments can evolve more resistant strains through natural mutations or acquired resistance mechanisms. Consequently, some bacteria may develop resistance to multiple antibiotics (12, 13). Acquired antibiotic resistance involves either temporary or permanent alterations in bacterial genetic material (14). This resistance can arise from spontaneous DNA mutations or through the transfer of genetic material between organisms. DNA mutations are chromosomal modifications that may occur through insertion/, deletion, or substitution of one or more nucleotides within the genome (15). The resulting mutation could be permanent, replaced by the organism, or fatal to the cell. Some spontaneous mutations have limited influence on the organism's sensitivity to antimicrobial drugs (12-14). Resistance characteristics are often expressed in extrachromosomal R factors (resistance plasmids). Most resistance genes are plasmid mediated, even though these features can be added to host bacterial DNA. Plasmids can enter cells through mechanisms such as transduction (phage-mediated), transformation, or bacterial conjugation (15).

Resistance to methicillin is determined by the *mecA* gene, which encodes the low-affinity penicillin-binding protein PBP 2A and is contained in a mobile genetic element called staphylococcal cassette chromosome *mec* (*SCCmec*) (16). *SCCmec* was first described in an MRSA strain, whose sequencing set the bases for the study of *SCCmec* basic characteristics, as well as its diversity (17). The *mecA* gene, which lies in the *SCCmec* resistance island, is carried by 95% of the isolates that display a phenotype of methicillin resistance and is detected in all multiresistant *S. aureus* isolates (18). Rapid methicillin-resistant *Staphylococcus aureus* (MRSA) tests are based upon either the multiple-locus approach, which tar-

gets both the resistance determinant *mecA* and an *S. aureus* species- specific target, or the single-locus approach that targets the junction between the staphylococcal cassette chromosome *mec* element (*SCCmec*) and *orfX* (19).

The global and local spread of antibiotic-resistant bacteria including in Jordan has sparked significant interest in researching these bacteria and the various types of antibiotics involved. Such data created an interest to coduct the present study to investigate the prevalence of MRSA and to determine the resistance patterns of *S. aureus* isolates in private laboratories. Additionally, it aimed to detect the presence of *mecA* and *SCCmec* genes in MRSA using the Xpert SA Complete Assay Real-time PCR.

MATERIALS AND METHODS

Study design and ethical consideration. This cross-sectional study was performed to isolate and characterize *S. aureus* isolates. 115 clinical isolates were obtained from various clincal samples such as nasal carriage, wounds, skin, ear, pus, swabs, urine, and catheters from the patients who had attended the 7 branches of PMLAB Group Laboratory and 2 branches of Nour-Amman Laboratory in Jordan. . This study was conducted between May 2024 and July 2024 and was approved by the research ethical committee at Zarqa University and the Ministry of Health before collecting the samples (MOH/ REC/2024/240).

Isolation and characterizataion of *S. aureus*. Staining characteristics were examined for all clinical isolates (16). Mannitol salt agar (MSA) which is a selective and differential media was employed to distinguish pathogenic Staphylococci capable of fermenting mannitol, indicated by a yellow halo around the colonies. The bacterial samples were streaked onto the agar and incubated at 37°C for 24 hours. Samples that failed to grow on the mannitol salt agar were subsequently excluded. The other bacterial isolates in these samples were identified at species level by the private laboratories (17). The catalase enzyme found in the cells of bacteria removes oxygen from hydrogen peroxides. To assess the catalase production in test isolates, a 3% (v/v) hydrogen peroxide solution was prepared and poured into tiny test tubes in quantities of 2 ml. Using a clean glass rod, a tiny portion of the culture (1 to 2 colonies) was transferred into test tubes containing 3% (v/v) hydrogen peroxide solution and tested for bubble formation (18).

To assess the ability of isolate for coagulase production, a 1:6 dilution of sterile human plasma in saline (0.85% NaCl) was prepared, and 1 mL of the diluted plasma was poured in tiny test tubes. Few colonies of each sample was aseptically transferred to these tubes with diluted plasma. The tubes were then incubated at 37°C for 1, 2, and 4 hours before being turned 90 degrees to check for clotting (19).

Antimicrobial susceptibility testing. As shown in Table 1, twenty five antimicrobial agents purchased from Bioanalyse (Ankara, Turkey) were used for susceptibility testing against all *S. aureus* isolates. Disc diffusion assay as recommended by the Clinical and Laboratory Standards Institute (CLSI) documents M02-A12 (20) was used with some modifications to assess the sensitivity of the bacterial strains. Briefly, 100 µL of 0.5 McFarland cell suspension was spread onto nutrient agar plates. Next, antibiotic drug discs

Table 1. Details of antibiotcs used in this study.

Antibiotic	Symbol	Concentration (µg)
Ampicillin	AM	10 µg
Azetreonam	ATM	30 µg
Amikacin	AK	30 µg
Cefotaxime	CTX	30 µg
Ceftriaxone	CRO	30 µg
Cefuroxime	CXM	30 µg
Cefoxitin	FOX	30 µg
Ciprofloxacin	CIP	5 µg
Co-Trimoxazole	SXT	25 µg
Gentamicin	CN	10 µg
Imipenem	IPM	10 µg
Levofloxacin	LEF	5 µg
Nitrofurantion	F	300 µg
Norfloxacin	NOR	10 µg
Tazocin	TPZ	
Cephalothin	KF	30 µg
Cefixime	CFM	5 µg
Ertapenem	ETP	10 µg
Pipercallin	PRL	100 µg
Amoxicillin	AMC	30 µg
Clavulanic Acid		
Ceftazidime	CAZ	30 µg
Meropenem	MEM	10 µg
Cefazolin	CZ	30µg
Oxacillin	OX	1 µg

were placed over the agar surface and incubated at 37°C for 24 h. Each experiment was conducted in triplicate and the average diameter of the inhibition zone around the discs was calculated in mm. The isolates were classified into resistant, intermediate, and sensitive accordonig to the guidelines of CLSI and previous literature (21).

Xpert® SA complete PCR. The GeneXpert instrument system automates and combines sample purification, nucleic acid amplification, and target sequence identification in simple or complex samples using real-time PCR (RT-PCR). The systems require single-use, disposable cartridges that hold the PCR ingredients. The cartridge's self-contained design avoids cross-contamination of the samples. The systems include an instrument, a computer, and pre-loaded software for conducting tests and analyzing the data (22). The Xpert SA Complete Assay was used according to the manufacturer's instructions, which comprised of MRSA and SA detection assays as well as a sample processing control (SPC) to ensure that the target bacteria were properly processed. The Probe Check Control (PCC) confirmed probe integrity, PCR tube insertion into the cartridge, and dye stability (23). The primers and probes employed in the Xpert SA Complete Assay found proprietary sequences for staphylococcal protein A (spa), the methicillin/oxacillin resistance gene (*mecA*), and the staphylococcal cassette chromosome (*SCCmec*) inserted into the SA chromosomal attB site (Table 2).

Table 2. Primers used in this study

Loci	Primer sequences (5' → 3')	References
MecA	F: GGCATCGTTCCAAAGAATGT	(24)
	R: CCATCTTCATGTTGGAGCTTT	
SCCmec	F: CATTTGTGAAACACAGTACG	(22)
	R: GTTATTGAGACTCCTAAAGC	

Statistical analysis. Statistical and data analysis was performed for objective analysis of the specific outcomes. All analyses were performed using Statistical Package for Social Sciences (SPSS), version 22.0 (IBM Corporation, Armonk, NY).

RESULTS

Demographic data. The clinical isolates were obtained from different clinical samples during two months (May 2024 and July 2024). Females 68 (59.1%) were more frequent than males 47 (40.9%). The mean age of the participants was 40.82 years ranging from 7 to 95 years, with 45 (9.6%) being the most frequent age among the participants.

Regarding the sample types, the study revealed that a total of 115 clinical samples were collected from various sources such as nasal carriage, wounds, skin, ears, pus swabs, urine, and catheters, with frequencies of occurrence of 5 (4.3%), 2 (1.7%), 30 (26.1%), 9 (7.8%), 5 (4.3%), 55 (47.8%), and 4 (3.5%), respectively. Urine samples were the most frequently collected as illustrated in Fig. 1.

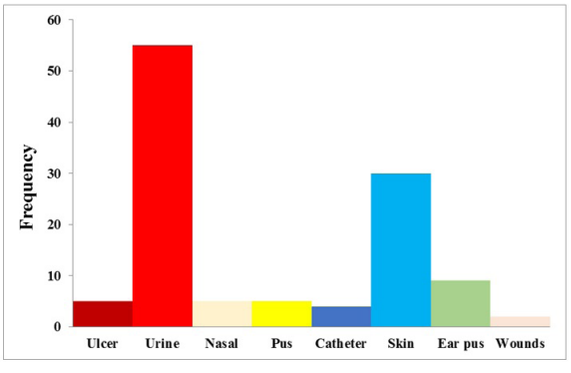

Fig. 1. Frequency of each sample type among different samples

Bacterial growth. In the present study, out of the 115 samples analyzed, Gram-positive bacteria were the most prevalent, accounting for 82 samples (71.3%). In contrast, Gram-negative bacteria were found in 21 samples (18.3%), whereas 12 samples (10.4%) showed either insignificant or no pathogenic growth.

Multi-drug resistant organisms (MDRO). Of the total 115 isolates, 98 (85.2%) demonstrated resistance to multiple antibiotics, including cefazolin, gentamycin, vancomycin, and ceftazidime, identifying them as multi-drug resistant strains. In contrast, 17 isolates (14.8%) did not exhibit multi-drug resistance (data presented here for 115 isolates, include observation from our work on *S. aureus* isolates and records from private laboratories for other bacterial isolates).

Frequency of isolated organisms. Among the isolates, *S. aureus* was the most prevalent species with a frequency of 70 (60.9%), followed by *E. coli* as 12 (10.4%), *S. epidermidis* as 12 (10.4%), *Serratia* as 6

(5.2%), and *Klebsiella* as 5 (4.3%) as shown in Fig. 2 (as mentioned in previous sections, *S. aureus* isolates were isolated from our work and data for other bacterial isolates were obtained from the private laboratories).

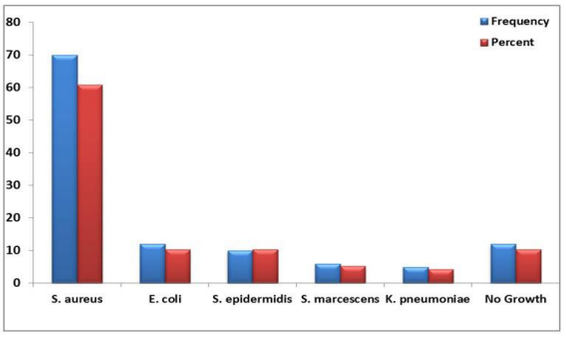

Fig. 2. Isolated organisim's frequency and percentages of distribution.

Pearson correlation analysis: Correlation between age and the isolated organism. Pearson correlation at α (p-value) = 0.05 was used in this study. Age and organism have a moderate positive correlation (r = 0.266). P-value = 0.004 < α: The differences between the means are statistically very significant.

Sample type and gram stain of the isolated organism. There is a weak positive correlation between sample type and Gram-stain (r = 0.063). P-value = 0.502 > α: The differences between the means were not statistically significant.

Sample type and the isolated organism. There is a weak positive correlation between sample type and the isolated organism (r = 0.073).

P-value = 0.438 > α: The differences between the means are not statistically significant.

Multi-drug resistant organisms and the isolated species. Multi-drug resistant organisms and the isolated species have a strong positive correlation (r = 0.867). The differences between the means are statistically significant (P-valu e≤0.001).

Antibiotic resistance and sensitivity. Antimicrobial resistant assay was performed for 70 isolates of *S. aureus* to determine susceptibility profile against 33 antimicrobial agents. *S. aureus* isolates were mostly sensitive to clindamycin (62.9%) followed by teicoplanin (52.9%), norfloxacin (51.4%) erythromycin and ceftriaxone (50%), meropenem and chloramphenicol (44.3%). Both cefoxitin and linezolid displayed sensitivity and resistance of 50.0% and 44.3% respectively. Whereas co-trimoxazole and gentamicin, each showed a sensitivity rate of 50.0% and a resistance rate of 47.1%. Amikacin and cefuroxime each exhibited sensitivities of 44.3% and resistances of 52.9%. Ciprofloxacin has a sensitivity of 47.1% and resistance of 44.3%. Amoxicillin and clavulanic acid, cefixime, and piperacillin each exhibited sensitivities of 42.9% and resistances of 48.6%. The sensitivity and resistance of isolates to other antibiotic including tigecycline imipenem, rifampin, nitrofurantoin aztreonam, cefotaxime, oxacillin and vancomycin are demonstrated in Table 3.

MRSA molecular detection. The primers and probes in the Xpert SA Complete Assay Real-time PCR and RT-PCR detected proprietary sequences for the staphylococcal protein A (spa), the gene for methicillin/oxacillin resistance (*mecA*), and the staphylococcal cassette chromosome (*SCCmec*) inserted into the SA chromosomal attB site. All of the methicillin / oxacillin resistant isolates showed significant association to *mecA* and *SCC A* genes (Figs. 3, 4 and Table 4).

DISCUSSION

The findings of this study offer significant information on the effects of usually employed antibiotics on the resistance trends of *S. aureus* isolates in private-sector laboratories. The issue of MRSA identified in this study fits well with the global tendencies of the bacterium's prevalence. Despite the facts that have shown the updates on the epidemiology and the virulence factors of MRSA, this pathogen still presents a public health issue due to its resistance to beta-lactam antibiotics (25).

This study has confirmed the high resistance of the *S. aureus* isolates to penicillin and oxacillin. These findings are in agreement with another study reporting high levels of MRSA infection (26). This is due to the over-prescription of antibiotics in communities, healthcare facilities, and elsewhere (27). Moreover, resistance to non-beta-lactam antibiotics was also high just as in other areas globally (28). However, the rate of drug resistance is proving that vancomycin is still effective in combating MRSA. Nevertheless, increasing reports of the vancomycin intermediate *S.*

Table 3. Antibiotic resistance and sensitivity pattern.

Antibiotic	Sensitivity	Frequency	Percent
Vancomycin	R	49	70.0
	S	19	27.1
	I	2	2.9
Oxacillin	R	45	64.3
	S	25	35.7
Tigecycline	R	34	48.6
	S	31	44.3
	I	5	7.1
Teicoplanin	R	31	44.3
	S	37	52.9
	I	2	2.9
Erythromycin	R	35	50.0
	S	35	50.0
Amikacin	R	37	52.9
	S	31	44.3
	I	2	2.9
Linezolid	R	31	44.3
	S	35	50.0
	I	4	5.7
Gentamycin	R	33	47.1
	S	35	50.0
	I	2	2.9
Rifampin	R	38	54.3
	S	32	45.7
Levofloxacin	R	45	64.3
	S	25	35.7
Chloiamphenicol	R	34	48.6
	S	36	51.4
Clindamycin	R	26	37.1
	S	44	62.9
Ciprofloxacin	R	31	44.3
	S	33	47.1
	I	6	8.6
Ampicillin	R	34	48.6
	S	30	42.9
	I	6	8.6
Cefazolin	R	60	85.7
	S	10	14.3
Aztreonam	R	49	70.0
	S	19	27.1
	I	2	2.9
Cefotaxime	R	45	64.3
	S	25	35.7
Cefazolin	R	34	48.6
	S	31	44.3
	I	5	7.1
Meropenem	R	31	44.3
	S	37	52.9
	I	2	2.9
Ceftriaxone	R	35	50.0
	S	35	50.0
Cefuroxime	R	37	52.9
	S	31	44.3
Cefoxitin	R	31	44.3
	S	35	50.0
	I	4	5.7
Co-Trimoxazole	R	33	47.1
	S	35	50.0
	I	2	2.9
Imipenem	R	38	54.3
	S	32	45.7
Nitrofurantion	R	45	64.3
	S	25	35.7
Norfloxacin	R	34	48.6
	S	36	51.4
Tazocin	R	26	37.1
	S	44	62.9
Cephalothin	R	31	44.3
	S	33	47.1
	I	6	8.6
Cefixime	R	34	48.6
	S	30	42.9
	I	6	8.6
Ertapenem	R	60	85.7
	S	10	14.3
Pipercallin	R	31	44.3
	S	33	47.1
	I	6	8.6
Amoxicillin and Clavulanic acid	R	34	48.6
	S	30	42.9
	I	6	8.6
Ceftazidime	R	60	85.7
	S	10	14.3

aureus and vancomycin resistant *S. aureus* strains pose a significant threat (29). The resistance patterns obtained in this study coincide with earlier studies on antibiotic resistance *S. aureus* isolates from health-care settings across other regions (27) pointing out that the problem of antibiotic resistance is worldwide.

In our study 98 isolates (85.2%) showed resistance to various kinds of antibiotics (multi-drug resistance organisms, MDROs), whereas 17 (14.8%) were not multi-drug resistant. This high incidence of MDROs

GeneXpert PC

Test Report

Patient ID: sample 15
Sample ID: sample 15
Test Type: Specimen
Sample Type:

Assay Information

Assay	Assay Version	Assay Type
Xpert SA Nasal Complete G3	5	In Vitro Diagnostic

Test Result: MRSA NEGATIVE; SA POSITIVE

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
SPC	0.0	-16	NA	PASS
SPA	11.9	378	POS	PASS
mec	12.2	532	POS	PASS
SCC	0.0	3	NEG	PASS

Detail

Analyte Name	Prb Chk 1	Prb Chk 2	Prb Chk 3	Probe Check Result	2nd Deriv Peak Height	Curve Fit
SPC	25	46	24	PASS	0.0	NA
SPA	47	90	47	PASS	0.0	NA
mec	55	102	56	PASS	0.0	NA
SCC	163	165	163	PASS	0.0	NA

Fig. 3. MRSA negative and *S. aureus* positive isolate using Xpert PCR

GeneXpert PC

Test Report

Patient ID: sample 20
Sample ID: sample 20
Test Type: Specimen
Sample Type:

Assay Information

Assay	Assay Version	Assay Type
Xpert SA Nasal Complete G3	5	In Vitro Diagnostic

Test Result: MRSA POSITIVE; SA POSITIVE

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
SPC	0.0	-15	NA	PASS
SPA	10.5	316	POS	PASS
mec	10.6	195	POS	PASS
SCC	11.4	408	POS	PASS

Detail

Analyte Name	Prb Chk 1	Prb Chk 2	Prb Chk 3	Probe Check Result	2nd Deriv Peak Height	Curve Fit
SPC	22	40	22	PASS	0.0	NA
SPA	39	75	39	PASS	0.0	NA
mec	55	103	55	PASS	0.0	NA
SCC	128	128	127	PASS	0.0	NA

Fig. 4. MRSA positive and *S. aureus* positive isolate using Xpert PCR

Table 4. MRSA negative and MRSA positive percentage

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Neg	62	88.6	88.6	90.4
	Pos	8	11.4	11.4	100.0
	Total	70	100.0	100.0	

aligns with the global trends indicating an increase in antibiotic resistance, particularly among Gram-negative bacteria (30). The data suggests a pressing need for stringent infection control measures and the judicious use of antibiotics to curb the spread of resistance (31). Additionally, the substantial proportion of non-MDROs highlights the importance of accurate microbial identification and susceptibility testing to ensure effective treatment strategies (6). The persistence of MDROs emphasizes the necessity for ongoing surveillance and research to develop novel antimicrobial agents and alternative therapeutic approaches (32).

In *S. aureus* infections, antibiotics work primarily through blocking cell wall formation. Peptidoglycan chains are the strongest structure in the cell wall, and they are carried extracellularly by lipid carriers found in the cytoplasmic membrane (20, 21). Penicillin-binding protein (PBP) is the enzyme that links freshly generated peptidoglycan chains within the cell. Beta-lactams covalently bond to PBP, blocking peptidoglycan chains from forming cross bridges. Without an effective extracellular membrane, the cell breaks down, and *S. aureus* no longer survives (33). In this regard, the identification of *mecA* and *SCCmec* genes in the current study of the MRSA isolates supports the genetic factor that underlines this resistance. These genes are documented to be characteristic of MRSA and are very essential in enabling the pathogen to deter beta-lactam antibiotics (34). In a study using DNA microarray technology, *mecA* was detected in at least five divergent lineages, implying that horizontal *mecA* transfer has played a fundamental role in the evolution of MRSA (35). The transfer of *mecA* in the *S. aureus* isolates has been reported, suggesting that *mecA* may transfer more frequently to MSSA (18). A study conducted by Tao et al. (36) explained the dynamics of resistance in *S. aureus* as a subject of genetic mutations and horizontal gene transfer.

Antibiotic-resistant *S. aureus* is particularly prevalent, posing a big problem in public health. Consequently, MRSA infections are associated with a rise in disease severity, mortality levels, hospital length of stay and elevated cost of medical care (11). The discussion of the results in the light of previous research would offer the conclusions and recommendations for public health, clinical practice and for future research. These findings imply that MDR is a significant problem which needs to be tackled by proper implementation and enforcement of infection prevention and control measures in healthcare facilities. Most critically, renal patients, who are more prone to infections with MDR pathogens, require special attention in terms of management. Both strict adherence to the measures of hygiene and rational use of antibiotics is critical for reducing the prevalence of the MRSA strains (37).

CONCLUSION

The incidence of antibiotic resistance in *S. aureus* isolates from private laboratories underlines the need for strategies to control MRSA and its resistance to numerous antibiotics. A significant correlation was perceived between age and isolated organisms, as well as sample type and isolated organisms. Further researches including other healthcare facilities and different regions are highly recommended in order to understand the existing resistance patterns and identify the new threats. Furthermore, the use of molecular typing approaches like whole genome sequencing can add a layer of additional understanding of the relations between resistance typing, and transmission patterns of the strains.

ACKNOWLEDGEMENTS

The authors would like to thank the staff members of the Faculty of Allied Medical Sciences at Zarqa University.

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