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Original Article

Prevalence and Antibiotic Resistance Pattern of Methicillin-Resistant *Staphylococcus aureus* Isolated from Iraqi Hospitals

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria are often multi-drug resistant, resulting in a high rate of treatment failure. This study aimed to identify the antibiotics resistance profile and molecular characteristics of MRSA strains isolated from patients' samples, including skin, wounds, and burns, which are the most common infections, and collected from hospitals. The samples included 34 MRSA isolates gathered from January 2020 to September 2020. All isolates were tested using the Kirby-Bauer method to determine MRSA susceptibility against antibiotics using the minimum inhibitory concentration protocol and the E-test. The polymerase chain reaction was used for the detection of antibiotic resistance genes, including tetracycline, erythromycin, linezolid, gentamicin, rifampicin, ciprofloxacin, quinupristin-dalfopristin, clindamycin, and *mec*A. Staphylococcal Cassette Chromosome *mec* (SCC*mec*) was determined by multilocus sequence typing of all isolates; accordingly, the findings indicated that the sensitivity of linezolid, quinupristin-dalfopristin, rifampin, daptomycin, and vancomycin differed. Moreover, multidrug resistance of MRSA was shown to be more than 90% for penicillin and 91.1% for erythromycin. It was revealed that SCC*mec* III was resistant to at least four to five different antibiotics. ST585 (2.9%), ST240 (8.8%), ST45 (14.7%), ST22 (17.6%), and ST239 (higher rate) were the five sequence types found in STs (55.8%). Finally, it was indicated that the emergence of MRSA in these Iraqi hospitals highlighted further research to better understand how the infection might be effectively controlled.

Keywords: MRSA, MLST, resistance gene, SCCmec

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria are often multi-drug resistant, resulting in a high rate of treatment failure (1). Methicillin-resistant *Staphylococcus aureus* isolates, originally found in earlier decades, are responsible for hospital-acquired infections that can lead to serious infection of various systems in the human body, resulting in shock and severe inflammations that threaten the life of patients (2). Additionally, other harmful factors contribute to MRSA infection, such as prolonged hospitalization, drug abuse, contamination, secondary illness, and a weakened immune system (3).

Methicillin-resistant *Staphylococcus aureus* can also be acquired as a community-acquired disease (4). Despite the fact that *S. aureus* is a prevalent commensal bacteria in humans (5), MRSA prolongs hospital stays, increases hospital expenditures, and may result in significant increases in morbidity and mortality in patients, compared to methicillin-resistant *S. aureus* (6). MRSA are recognized as health risks, particularly in large hospitals because they employ common medications (7). *Staphylococcus aureus*, MRSA, and other antibiotics are

generated by a genetic component transmitted to the chromosomal material of the bacterium. Staphylococcal Cassette Chromosome *mec* (SCC*mec*) is a characteristic indication of these bacteria (8).

To the best of our knowledge, there are little data relating to MRSA genotypes in Iraq. It has been discovered that utilizing genotypic and phenotypic detection, which may help prevent the spread of common genetic elements while also giving information about the kinds of infection within hospitals, leads to the reception of the appropriate therapy by patients (9). Multilocus sequence typing (MLST) and SCC*mec* are the terms used to refer to two different types of genetic testing. Despite the fact that all of these methods have pretty excellent discriminating capabilities, it has been established that combining genotyping methods to determine distinct MRSA clones may be advantageous (10). To sum up, this work described the resistance pattern and molecular characteristics of an MRSA isolate collected from the wound, burn, and pus samples in patients in Iraq.

2. Materials and Methods

2.1. Collection of the Specimen and Bacterial Culture

From January to September 2020, 150 samples were collected from 3 hospitals in the northern, central, and southern districts of middle Iraq for this local study. The isolates utilized in this investigation were gathered in the amounts of 55, 45, and 50 from hospitals in the mentioned regions, respectively. All agreements were obtained from all patients, including wounds, pus from the skin, and exudates from burns samples. All samples were collected in sterilized vials and immediately transferred to the Medical Laboratories Techniques, Department of Suwaira, Technical Institute, Laboratory of Microbiology, Middle Technical University, Iraq, for work-appropriate collection tests and were processed immediately; subsequently, they were cultured on nutrient agar and incubated for 1 day at 37°C. The bacterial isolates were identified based on the morphology and microscopic appearance of the colony (Oxoid, UK). After adding bacteria to nutrient broth (Himedia, India), 20% glycerol was employed to keep bacteria in the freezer for subsequent testing (11).

2.2. Diagnosis of Methicillin-Resistant *Staphylococcus aureus* by Chromogenic Agar

To confirm the diagnosis of MRSA, all colonies from cultures on Mannitol salt agar (MSA; Himedia, India), were purified by being cultured on MRSA Chromogenic Modified Agar Base (Candalab, Spain), which was combined with Cefoxitin MRSA Supplement, and placed in an incubator for 1 day at 35±2°C according to the manufacturer instructions.

2.3. Antibiotics Susceptibility Testing

All isolates of MRSA determined by the Kirby-Bauer method based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (12) included gentamicin (10 µg), cefoxitin (30 µg), rifampin (5 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), ciprofloxacin (5 µg), penicillin (10 u), linezolid (30 µg), and quinupristin-dalfopristin (15 µg) (Bioanalysis, Turkey), as shown in table 3. Cefoxitin 30 µg discs on agar (Himedia, India) were employed to identify MRSA isolates. E-test was also used to determine the minimum inhibitory concentration for vancomycin and daptomycin (bioMe'rieux) isolates (13). The control was *S. aureus* ATCC 29213.

2.4. Extraction of DNA

BYF DNA (i-genomic™) Extraction Mini Kit (Labotaq, Korea) was used for DNA extraction from gram-positive bacteria according to the manufacturer's instructions for MRSA isolates, and the concentration could be determined by measuring the absorbance at 260 nm in a spectrophotometer. For the greatest accuracy, readings needed to be between 0.1 and 1.0.

2.5. Genotypic Detection for Antibiotic Resistance Genes

Polymerase chain reaction (PCR)-based molecular diagnosis was employed to identify the existence of the following antibiotic resistance genes of various sorts, including erythromycin, tetracycline, gentamicin, and others as mentioned in references (14, 15).

2.6. Genotyping of MRSA based on SCC*mec*, and Multilocus Sequence Typing

Staphylococcal Cassette Chromosome *mec* typing was employed to genotype all MRSA isolates. Staphylococcal Cassette Chromosome *mec* types were established by comparing the banding patterns of MRSA to ATCC 10442 (SCC*mec* type I), N315 (SCC*mec* type II), 85/2082 (SCC*mec* type III), and WIS (SCC*mec* type V) (16) as reference strains (Table 1). All isolates were typed using MLST genotyping, which involved the amplification and sequencing of seven housekeeping genes (i.e., *arcC*, *aroE*, *glpF*, *gmK*, *pta*, *tpiA*, and *yqiL*) (17) according to the mentioned methodology.

2.7. Statistical Analysis

Statistical analysis was performed in Microsoft Excel 2017.

Table 1. Sequence of oligonucleotide primers of genes used in the study by Goudarzi, Kobayashi (10)

Target	F&R Primers	5′- 3′	Products
SCC*mec*	F -5R*mec*A R -5R431	TATACCAAACCCGACAACTAC CGGCTACAGTGATAACATCC	359 bp
	F R	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAAGTGTATTGGATAGCAAAAGC	433 bp
	F- 1272 R- 1272	GCCACTCATAACATATGGAA CATCCGAGTGAAACCCAAA	415 bp
	F-β R -α3	ATTGCCTTGATAATAGCCYTCT TAAAGGCATCAATGCACAAACACT	937 bp
	F -ccrC R -ccrC	CGTCTATTACAAGATGTTAAGGATAAT CCTTTATAGACTGGATTATTCAAAATAT	518 bp

3. Results

The results of this investigation revealed that a total of 150 clinical swabs included wounds and pus from the skin; exudates from burns were obtained from patients at 3 hospitals, with 64 *S. aureus*, and 34 isolates of MRSA, as shown in table 2.

Table 2. Spread of the MRSA and *Staphylococcus aureus* in samples from January 2020 to September 2021

Samples	Number	*Staphylococcus aureus* percentage	MRSA percentage
Swab of wounds	94	33 (35.1%)	17 (51.5%)
Pus from skin	20	12 (60%)	8 (66.6%)
Exudates from burn	36	19 (52.7%)	9 (47.3%)
Total	150	64 (42.6%)	34 (53.1%)

MRSA: Methicillin-resistant *Staphylococcus aureus*

Despite the fact that these bacteria cause infection in hospitalized patients and are commonly transmitted from one person to another, 150 samples were collected from people visiting three hospitals in central Iraq between January and September 2020. The isolates of MRSA were obtained from 17 (51.5%) wound swabs, 8 (66.6%) pus from the skin, and 9 (47.3%) exudates from burns. Figure 1 displays the prevalence of MRSA isolates of each specimen from patients' swabs.

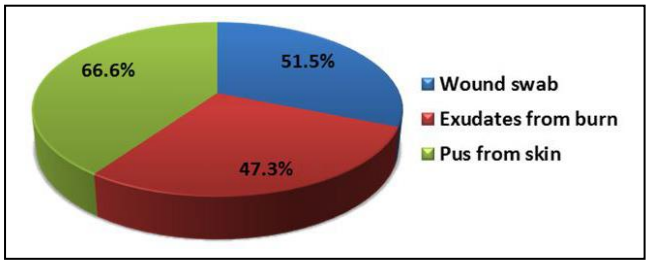

Figure 1. Prevalence of MRSA isolates of each specimen from patients

All isolates were subjected to Gram stain, catalase, and oxidase assays after being cultivated aerobically on MSA to demonstrate the capacity of the bacteria to ferment sugars in a medium. This was followed by mannitol fermentation

in some isolates, which turned 150 of them yellow; subsequently, a culture on MRSA Chromogenic Modified Agar Base medium with Cefoxitin MRSA Supplement was performed to confirm the presence of 34 MRSA isolates, which showed good growth under incubation conditions of 3°C for 18-24 h, as each manufacturer's instructions. The color of the colony ranged from magenta to rose to mauve, as depicted in figure 2.

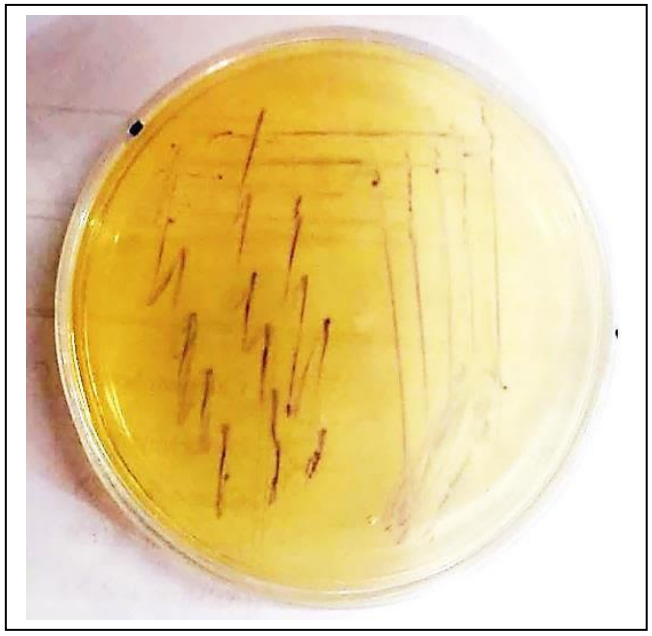

Figure 2. Magenta (rose to mauve) color for the colony of MRSA isolates

The resistance patterns for MRSA isolates showed penicillin (100%), cefoxitin (100%), gentamicin (85.2%), erythromycin (91.1%), tetracycline (64.7%), ciprofloxacin (26.4%), clindamycin (47.05%), rifampicin (5.8%), linezolid (5.8%), and quinupristin-dalfopristin (5.8%), as is presented in table 3. The MIC results for several antibiotics against MRSA isolates are summarized in table 4, and only one isolate showed resistance to vancomycin antibiotic at a percentage of 2.6%, while 6 isolates were sensitive to linezolid at a percentage of 16.05%, and the majority of isolates revealed an increased resistance rate at 64 µg/ml.

Most isolates showed resistance against 11 agents of antibiotics at the rate of 5.8% (2/34), to 10 antibiotics at 41.1% (14/34), to 9 antibiotics at 17.6% in (6/34), to 8 antibiotics at 11.7% (4/34), to 7 antibiotics at 8.8% (3/34), to 6 antibiotics at 5.8% (2/34), and to 5 antibiotics at 8.8% (3/34). Molecular detection for SCC*mec* typing of MRSA isolates from clinical samples showed that type III was the most predominant SCC*mec* type revealed in 23 (67.6%) isolates, followed by types IV, I, and II showed in 7 (20.5%), 3 (8.8%), and 1 (2.9%) isolate, respectively. However, the MLST findings for the 34 strains revealed 5 different sequence types (ST239 in 19 strains, ST22 in 6 strains, ST45 in 5 strains, ST240 in 3 strains, and ST585 in 1). The molecular characterizations of 34 MRSA strains derived from clinical samples are presented in table 5.

The findings of the MRSA molecular detection revealed that the antibiotic resistance genes utilized in this investigation were discovered in some MRSA strains chosen randomly with the most predominant resistance genes shown with gel electrophoresis 1.5% agarose stained with Ethidium Bromide to detect genes, as depicted in figure 3.

Table 3. Profile antibiotic susceptibility for isolates in this study

Antibiotics	Micrograms content per disk	MRSA=34	
		R%	S%
Penicillin	10 U	100%	0%
Cefoxitin	30 µg	100%	0%
Gentamicin	10 µg	85.2%	17%
Erythromycin	15 µg	91.1%	8.9%
Tetracycline	30 µg	64.7%	35.3%
Ciprofloxacin	5 µg	26.4%	73.5%
Clindamycin	2 µg	47.05%	52.9%
Rifampin	5 µg	5.8%	94.2%
Quinupristin-Dalfopristin	15 µg	5.8%	94.2%
Linezolid	30 µg	5.8%	94.2%

MRSA: Methicillin-resistant *Staphylococcus aureus*

Table 4. Results of minimum inhibitory concentration for isolates used in this study

Antibiotics agents	MIC value	Breakpoints	MRSA (n=34)			
			R%	S%	MIC50	MIC90
Penicillin	0.125->256	≤0.12 / - / ≥0.25	100%	0%	64	128
Cefoxitin	0.25->128	≤4 / - / ≥8	100%	0%	64	128
Vancomycin	<0.063-16	≤2 / 4–8 / ≥16	2.7%	97.3%	1	8
Daptomycin	0.063-1	≤1 / - / -	0%	100%	0.25	0.5
Gentamicin	0.5->512	≤4 / 8 / ≥16	85.2%	17%	64	512
Tetracycline	0.5-256	≤4 / 8 / ≥16	64.7%	35.3%	32	128
Erythromycin	0.25->128	≤0.5 / 1– 4/ ≥8	91.1%	8.9%	128	128
Ciprofloxacin	0.25->128	≤1 / 2 / ≥4	26.4%	73.5%	4	32
Clindamycin	0.25->128	≤0.5 / 1–2/ ≥4	47.05%	52.9%	4	32
Rifampin	<0.063->128	≤1 / 2 / ≥4	5.8%	94.2%	4	64
Quinupristin-Dalfopristin	0.25->128	≤1 / 2 / ≥4	5.8%	94.2%	0.5	3
Linezolid	< 0.063-64	≤4 / - / ≥8	5.8%	94.2%	1	8

MRSA: Methicillin-resistant *Staphylococcus aureus*; MIC: Minimum inhibitory concentration

Table 5. Molecular characterizations and antibiotics resistant profile of MRSA isolates from different clinical samples at the three hospitals in Iraq

Variables	MRSA (n=34)
STs (n)	ST239 (19), ST22 (6), ST45 (5), ST240 (3), ST585 (1)
SCCmec (n)	Types III (23), IV (7), I (3), II (1)
Types of clinical samples (n)	Wound (17), exudates from burns (9), pus from the skin (8)
Antibiotic resistance profile (n)	P (34), CF (34), GEN (29), TE (22), E (31), CIP (9), CC (16), RIF (2), QD (2), LIN (2)
Antibiotic resistant genes profile (n)	*erm*A (20), *erm*B (2), *erm*C (1), *tet*K (12), *tet*M (16), *aac*A-*aph*D (30), *van*A (1), *van*B (1)

MRSA: Methicillin-resistant *Staphylococcus aureus*; ST: Sequence; P: Penicillin; CF: Cefoxitin; GEN: Gentamicin; TE: Tetracycline; E: Erythromycin; CIP: Ciprofloxacin; CC: Clindamycin; RIF: Rifampin; QD: Quinupristin-Dalfopristin; LIN: Linezolid

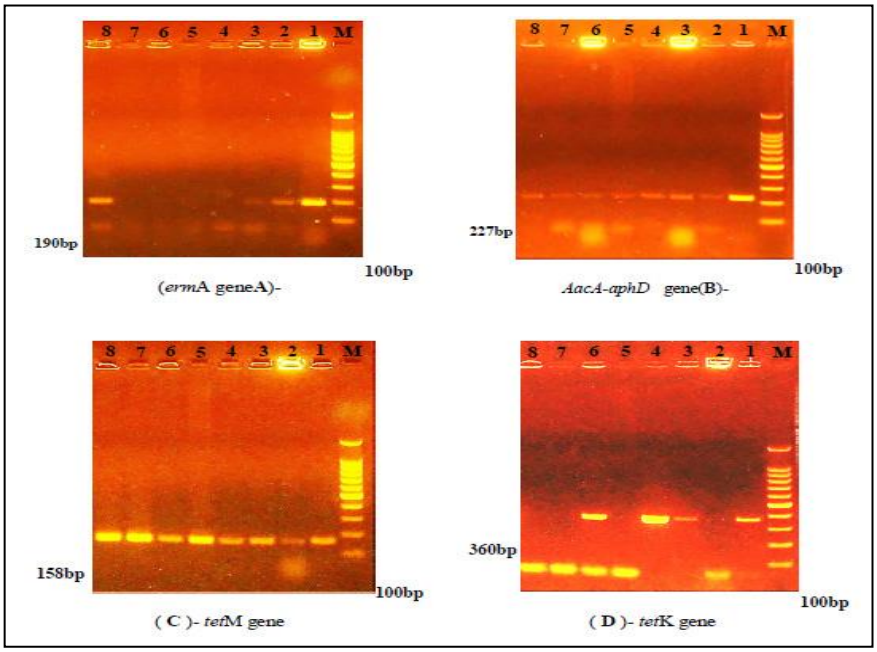

Figure 3. Detection of Erythromycin gene (A), gentamicin gene (B), Tetracycline gene (C and D) for methicillin-resistant *Staphylococcus aureus* isolates responsible for resistance against different antibiotics, M: 100 bp ladder marker, Lines 1-8 positive results

4. Discussion

Despite the fact that these bacteria cause infection in hospital patients and are commonly transmitted from one person to another, 150 samples were collected from people visiting three hospitals in central Iraq between January and September 2020. It was shown that MRSA was prevalent in 53.1% of patient samples, which was consistent with results of research conducted in the United States reporting 54% (18). In our analysis, the presence of MRSA in patients was 8% higher than that in an Indian study (19). The findings of the present study indicated that the incidence of MRSA among patients increased over time as a result of antibiotics abuse during the last year, which was in line with those of a study by Becker and Eiff (20).

The catalase test was used to distinguish between *Staphylococcus* species with a positive result, while the oxidase test was used to identify them from *Micrococcus* bacteria with a positive oxidase result. According to cultural and morphological criteria, all isolates from swabs from male and female patients aged 20-60 years old cultured on MSA demonstrated the development of bacteria colony that looked yellow under microscope cocci in form, clustered, and G+. According to biochemical tests, the isolates tested positive for coagulase and catalase, which were remarkably similar to the results of research in Nigeria (21).

On the MRSA Chromogenic Modified Agar Base combination with Cefoxitin, 34 of 150 isolates previously isolated on MSA emerged as rose to mauve colonies in color, as observed in a prior investigation (Figure 1). This differential and selective MRSA Chromogenic medium was intended to identify MRSA in clinical samples, such as wounds, pus, and skin samples (22), with great sensitivity and specificity. Multi-resistance infections, such as MRSA, are on the rise because of the abuse of antibiotics, the improper administration of antibiotics by caregivers, and the overuse of antibiotics by physicians (23).

Chrome MRSA agar used a chromogenic substrate to distinguish *S. aureus* from other pathogens and selectively develop MRSA in the presence of drugs. After 24 h of incubation, the sensitivity was estimated at 95.4%, which increased to 1005 after 48 h. Hedin and (24) and Kali, Stephen (25) reported a sensitivity of 97.8% for Chrome MRSA. Using chromogenic agars reduces time, confirmatory testing, which can be expensive and time-consuming. In addition, CLSI recommends cefoxitin (12). In another investigation, the sensitivity and specificity were both 100%, and the researchers recommended utilizing cefoxitin as an alternative to PCR because it perfectly matched the PCR approach for detecting MRSA genes (26).

When MRSA isolates were tested for antibiotic resistance via disk diffusion, the findings were compared with those from gene analysis. The phenotypic expression of antibiotic-resistant genes can be affected by a variety of factors, including the temperature and length of incubation, the test agent, the inoculum size, pH, the injected medium, and the NaCl ionic strength (27).

There is an increase in the expenses of therapy and a restriction on the pharmaceuticals that may be used and the usage of alternative antibiotics due to the proliferation of these bacteria. These antibiotics have become less effective as a result of a limited therapeutic range. However, the incidence of resistance to gentamicin (9.1%) and erythromycin (31.8%) was lower than expected.

The ultimate gentamicin gene (*aacA-aphD*) was found in 34 (100%) isolates, which was in agreement with the results of a study conducted in Turkey reporting that 96% of the detected genes were gentamicin-resistant isolates and the *erm*A gene was found in 88% of the isolates. In our investigation, *the erm*A gene was found in 30 of the isolates, which was generally compatible with the results of another study showing the presence of *erm*A in 52.4% of isolates (28).

The most frequent genotype among erythromycin-resistant MRSA isolates was found to be *erm*A (29). Target-site modification after that ribosome makes methylation by an enzyme encoded by the *erm*A gene

erythromycin ribosome methylase is the major method of resistance in staphylococci (30). In this study, there were 88% and 11.7% connection rates between phenotypic tetracycline resistance and the presence of the *tet*M and *tet*K genes in clinical MRSA isolates, respectively. In general, the results were consistent with those of a study performed in Algeria, which indicated that the *tet*M and *tet*K genes were present in 72.2% and 66.7% of the population, respectively (31). The disparity between phenotypic tetracycline resistance and determinants could be due to a number of reasons. The acquisition of the *tet*K gene on plasmid results in the active efflux of *tet*M determinants on the transposon or the chromosome mediate ribosome protection. This may justify the high rate of resistance to tetracycline in this study.

Isolates showed a low rate of resistance to ciprofloxacin in this study that was in line with the results of a study by Dormanesh, Siroosbakhat (32), while it was inconsistent with those of other research that showed a 78.1% increased rate of resistance because of the mutation, overuse of antibiotics in the wrong way (33).

In this study, antibiotic resistance rates were low against quinupristin-dalfopristin, rifampicin, linezolid, and clindamycin, which were in agreement with the results found in Iran (34). However, the rate of resistance was greater in a study conducted by Jung, Chung (35) in Korea than in our findings. The isolates in this investigation had a high resistance rate to cefoxitin and penicillin (100%), which was in line with the findings of a study by Kouhsari, Hosseini (15). This explains the widespread usage of these antibiotics in the treatment of various illnesses (36).

Considering clindamycin resistance, isolates showed 47% resistance. In a research carried out in Iran, the prevalence of MRSA resistance to clindamycin was greater than that in our research (86.5%) (37). According to the results of other studies, the majority of MRSA isolates obtained in this study (67.6%) belonged to type III of SCC*mec*, whereas the MRSA SCC*mec* types II and I isolates were found from infection inside the hospital, which was in agreement with the findings of a study by Monecke, Coombs (2). Similar results were obtained in research in south Iran reporting that the majority of MRSA isolates belonged to the third type (10). According to research conducted in the southwest of Iran, multidrug resistance is more common in type III than in other types of SCC*mec* (38). Approximately all MRSA isolates that belonged to type III, had multi-drug resistance, and were highly rated in hospital isolation came in the first rank. While the second rank in prevalence is related to type IV with a rate of 20.5%, which was close to the results of a study reporting a rate of 33.3% (39). In the current study, five sequences (ST) were found from local MRSA, including ST45, ST585, ST22, ST239, and ST240. The first rank, ST239, had types III and I of SCC*mec* that achieved the rate of 43% in Iran (36), which was close to our result: however, it was lower than those in other studies at the rate of 17.1% in clinical isolates and spreading rate of more than 79% (38). Moreover, these isolates were found to be multi-resistant more prevalently in Europe (39). In the present study, ST22 was reported to be the second most common MRSA strain, which was in line with the results of previous studies (40). ST22 has type III of SCC*mec* that was stated in Iran with multiple genetic descriptions for MRSA, whereas ST585 has type III of SCC*mec* that was mentioned in Iran with several genetic descriptions for MRSA (41). It was revealed that ST45 had SCC*mec* types I and II and ST240 had SCC*mec* type III, and the majority of strains were resistant to gentamicin, ciprofloxacin, tetracycline, and erythromycin, which was consistent with the findings of a study by Goudarzi, Seyedjavadi (36). However, the results of research conducted in the north of Iran reported that there was increased resistance to a wide spectrum of antibiotics (15).

The findings of our study indicated the strain of MRSA isolates belonged to ST22, ST45, ST239, ST240, and ST585. Finally, these isolates showed

several genetic descriptions from hospitals in middle Iraq. The spreading of MRSA was dependent on the found area that described the different rates of multidrug resistance of these isolates (90%), which was in agreement with the findings of a study carried out in Taiwan (39). The emergence of the five types of different sequences in this study requires strict measures to prevent its spread, leading to dangerous and uncontrollable results in the future in Iraqi hospitals.

The current study aimed to investigate MRSA dissemination. According to our findings, MRSA was found in 53.1% of clinical infection samples. This could be related to the high prevalence of methicillin prescriptions in Iraqi health clinics and hospitals. Furthermore, this discovery revealed that the hospital environments are extremely contaminated in Iraq. In addition, our findings showed that antimicrobials were used in Iraqi hospitals in a highly unequal manner. Considering the low rates of resistance for MRSA locally isolates against 4 antibiotics, namely vancomycin, linezolid, quinupristin-dalfopristin, and rifampicin, it is suggested to use them as a drug of choice for the treatment of complicated infections by MRSA.

Authors' Contribution

All authors made a direct contribution to the study and its publication.

Ethics

This study was approved by the Ministry of Health of Iraq and involved only the collection of samples from the patients after obtaining the written consent of the doctors in the hospitals through monitoring cases of patients who were presented inside the units of the three hospitals in the middle of Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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None.

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Data Availability

All datasets analyzed during this study are included in the manuscript.

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