Original Article

Prevalence of Extended-spectrum β-Lactamase-Producing Genes among MRSA Isolates from Clinical Samples in Baghdad City

Ali Yaseen Jasim, Jaleel Samanje, Qahtan Adnan Rasheed
College of Health and Medical Technique, Middle Technical University, Baghdad, Iraq

Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an intrusive human pathogen causing community- and hospital-acquired infections worldwide. *S. aureus* was discovered to be one of the leading sources of hospital infections and drug resistance in Iraq, particularly methicillin resistant. **Objective:** The study aims to know the extent of gene prevalence and antibiotic resistance in *S. aureus* isolated from various clinical samples. **Materials and Methods:** A total of 150 clinical samples (urine, wound swabs, ear swabs, burn, and sputum samples) were collected. Disk diffusion method was used for evaluating the antibiotic susceptibility status, while conventional PCR was applied to detect the presence of diagnostic and resistance genes. Microtiter plate method was subjected to study the biofilm formation among the resistance strains. **Results:** The antibiotic susceptibility results of *S. aureus* infections showed full resistance to penicillin and cefoxitin, while full sensitivity against vancomycin was observed. MRSA isolates also showed a resistance rate against some macrolide antibiotics, including azithromycin, clindamycin, and erythromycin. There was no association or correlation between antibiotic resistance and biofilm-producing capacity. All MRSA isolates were screened for *mecA*, *blaZ*, *blaDHA*-1 using conventional PCR. Regarding the *β*-lactamase detection genes among MRSA isolates harbored, only 4/20 (20%) of MRSA isolates carried *blaDHA-1 AmpC* genes, while 20/20 (100%) showed *bla-Z* genes. Only 19/20 (95%) isolates showed positive for *mecA* gene. The *mecA* genes usually circulate in phenotypically-identified MRSA isolate, confirming the phenotypically-resistant strain and explaining the mechanism of resistance. **Conclusion:** The high frequencies of circulating *mecA* genes and *β*-lactam genes demonstrate the necessity of policies for overcoming MRSA problems in clinical specimens, although no isolate showed that vancomycin resistance depends on a phenotypic test. Improper using of antibiotics among patients in Baghdad city may play an essential role in the spread and emergence of antibiotic resistance.

Keywords: MRSA, biofilm, *blaDHA*-1, *blaZ*, *β*-lactamases

Introduction

Staphylococcus aureus is a very successful gram (+ve) bacteria that exhibited resistance to a large number of antibiotics because of environmental selective pressures, medical antimicrobial use, and different genetic mechanisms for horizontal gene transfers. In the health care communities and facilities, penicillin-resistant strains spread, and the narrow-spectrum, semi-synthetic penicillin (methicillin) was shown to overcome infections of the *β*-lactamase producing *S. aureus*. In 1961, identification of the first methicillin-resistant *S. aureus* strains was made, and this strain has the ability to global spread as a community-acquired *S. aureus* and later as a hospital-acquired *S. aureus* in health care communities. In a meta-analysis study by Turlej *et al.*,[1] they showed that 43.0% of the identified *S. aureus* isolates were methicillin-resistant *S. aureus*, suggesting that infections by methicillin-resistant *S. aureus* became

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Address for correspondence: Dr. Jaleel Samanje, College of Health and Medical Technique, Middle Technical University, Baghdad 1011, Iraq.
Email: Jaleel.najah@mtu.edu.iq

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the most globally prevalent infections.[2] A study carried out in Iraq focused on *S. aureus* isolation from various clinical specimens to determine the antibiotic resistance status of *S. aureus.* This study reached the conclusion that the most resistant group isolates came from burn patients and diabetic foot ulcers, whereas the other group with less resistance was collected from diabetic ulcers at a clinic from those who lived in different sectors,[3] methicillin-resistant *S. aureus* has the ability to produce alpha pore-forming toxin and enterotoxin for host cell and tissue destruction, cause skin infection, urinary tract infection, bloodstream infection, food poisoning, and respiratory infections.[4,5] The mechanism of resistance to methicillin in methicillin-resistant *S. aureus* mainly depends upon *mecA* encoding penicillin-binding proteins, which decreases *β*-lactam antibiotic affinity.[6] Methicillin resistance is the responsibility of *mecA* gene, which is found on the taphylococcal cassette chromosome mec (SCCmec) element. *S. aureus* (MSSA) evolved into MRSA due to the acquisition of a SCCmec element. Following accurate integration and excision mediated by genes of site-specific recombinase ccrAB or/and ccrC, SCCmec was integrated into staphylococcal chromosomes, therefore causing the *β*-lactam antibiotic resistance acquisitions.[7] The augmented penicillin-binding protein (PBP2a/2c/2'/2x) mediates methicillin resistance, and to this protein, the *β*-lactam antibiotic has less binding affinities.[8] Consequently, such isolates resist all antimicrobial drugs from *β*-lactam class (except the ceftobiprole and ceftaroline, named "anti-MRSA" cephalosporin), which largely limits therapeutic options that are effective, safe, and economically advantageous.[9] Resistance to extended-spectrum 3rd generation cephalosporin and monobactam is mediated by the extended-spectrum *β*-lactamase enzyme (ESBL); however, this enzyme has no effect on carbapenem and cephamycin. When an ESBL-producing microorganism is an infectious agent in clinical infections, it can lead to failure in treatment by using 3rd generation cephalosporin or monobactam, and this is explained by the resistance of ESBL-producing strains to all cephalosporins, penicillins, and aztreonams.[10] The extracellular polymeric substance (EPS), called a biofilm, is secreted by *S. aureus,* and this substance assists the bacteria to minimize and resist antibacterial drug effects.[11] Considerable interest was raised by the possibility of an association between antibiotic-resistant phenotypes and capacities of biofilm formation.[9] In one study, an association was found between MDR phenotypes and biofilm formation in clinical isolates of *S. aureus* taken from humans, using different *in vitro* procedures, which concluded no difference in biofilm-forming capacities in isolates of methicillin susceptible and MR, whereas isolates that resisted clindamycin, erythromycin, and rifampicin were related to strong biofilm formation.[12]

Materials and Methods

Samples collection

In the current study, 150 clinical specimens were obtained from different clinical infection sources, including urine, wound, ear, burn, and sputum, from the National Center for Teaching Laboratory (NCTL), Baghdad Teaching Hospital.

Isolation and Identification of *S. aureus* bacteria

Samples were streaked on blood agar and mannitol salt agar (MSA). After overnight incubation, all the suspected colonies, such as *S. aureus,* were subjected to further phenotypic tests, including colony morphology detection, Gram staining, coagulase, and catalase tests.

Antibacterial susceptibility testing with MRSA isolates

The antibacterial susceptibility testing was performed using disk diffusion, and the protocol specified in the instructions of the Clinical and Laboratory Standards Institute was used to interpret the results (CLSI, 2022). The antibacterial agents involved cefoxitin 30 (μg), penicillin 10 (U), vancomycin, doxycycline 30 (μg), azithromycin 15 (μg), clindamycin 2 (μg), and erythromycin 15 (μg) (Bioanalyzed, Turkey).

Biofilm formation detection

Twenty MRSA bacterial isolates were isolated from 150 clinical samples after identification and testing the antibiotic sensitivity profile and were subjected to detection of the ability to form biofilm by the method mentioned below.

Microtiter plate method

Assessment of biofilm formation was performed using the 96-well microtiter plate method. Following an overnight growth in typtic soy broth (TSB), 150 μL of cell suspensions have been adjusted for a 0.5 McFarland standard turbidity and transferred into all microtiter plate wells, then incubated for 36 h at 37°C. Following three short washes with a solution of 10 mM phosphate buffer saline (PBS) and 20 min of fixation with 180 μL methanol, the plates were stained with 180 μL 0.1% v/v crystal violet for 15 min, then washed three times gently with 10 mM PBS. The produced biofilm was then dissolved in 180 μL 33% v/v acetic acid for 30 min. The sterile TSB was used as a negative control. By the use of the microtiter plate reader, the biofilm production was measured at 550 nm optical density. The assays of biofilm measurement were done in triplicate. According to the equation: (the cut-off OD value = the mean OD of the negative control + three standard deviations of the negative control), the biofilms were categorized into four groups: OD ≤ ODc (nonbiofilm producer), ODc < OD ≤ 2 ODc (low biofilm producer), 2 ODc < OD ≤ 4 ODc (medium biofilm producer), and OD > 4 ODc (str6ong biofilm producer).[13]

Genome extraction

All the selected isolates as MRSA (n = 20) were sent for DNA extraction. The genomic DNA of the *S. aureus* isolates was extracted using genomic DNA purification kit purchased from (Intron Biotechnology, Korea), the purity and concentration were tested, and the gel electrophoresis method was used for testing the integrity of DNA samples.

Primers

Three types of primer were selected to study the properties of MRSA isolates, and these primers included *mecA* genes, which are used as diagnostic as described by Rahimipour *et al.*,[14] and the other primer was used as a virulence gene for the detection of the ESBLs genes, including *bla-DHA1* and *bla-Z*.[15]

Condition of PCR

The optimization of PCR reaction was done by the application of temperature and concentration gradients. Eventually, 25 µL reaction composed of 1.5 µL templates DNA, 5 U/µL DNA polymerase, 10 pmol/µL primer, 5 U/µL dNTPs, 2 µL $MgCl_2$ (1.5 mM), 5 µL PCR buffers (10×), and 16.5 µL DDW. The final PCR program was determined by the use of gradient PCR optimizations. The following conditions were used to set the final program: at 94°C for 5 min for the initial denaturations, then by 35 cycles composed of denaturations at 94°C for 30 s for *mec-A*, *blaZ* and *blaDHA-1*, annealing at 55°C for 1 min for *mec-A* and *bla-Z* and at 50°C for 1 min for *bla-DHA1*, and extension at 72°C for 1 min for *mecA* and *bla-Z* and72°C for 1 min for *bla-DHA1* and at 72°C for 30 s, with the final extension step at 72°C for 7 min. Then, the PCR products were subjected to electrophoresis by 1% agar gel. The UviDoc system was used to stain and visualize the gel.

Statistical analysis

Fisher's exact test or Pearson's chi-square test was used to compare categorical variables and percentages. A level of P = 0.05 was considered significant in the test. The data in the study were analyzed by using the SPSS v.23 program.

Ethical Approval

This research was certified by the ethical commission in the medical city health directorate in Iraqi Ministry of Health. Health (45/804 on August 1,2023) Administration.

Results

Results of our study showed that most MRSA isolate was identified from urine samples 7/20 (35.0%) while fewer cases of MRSA isolate were from sputum samples 2/20 (10.0%) [Table 1]. In regard to antibiotic susceptibility of MRSA isolates, resistance was shown to penicillin and cefoxitin with absolute percentages 100% and 100%, while there was absolute action of vancomycin against MRSA isolates with a sensitive rate of 100%, whereas

Table 1: Frequency and percentage of MRSA isolates from various clinical specimens

Clinical samples	*N* and %
Urine	7 (35.0%)
Wound swabs	5 (25.0%)
Ear swabs	3 (15.0%)
Burn	3 (15.0%)
Sputum	2 (10.0%)
Total	20 (100.0%)

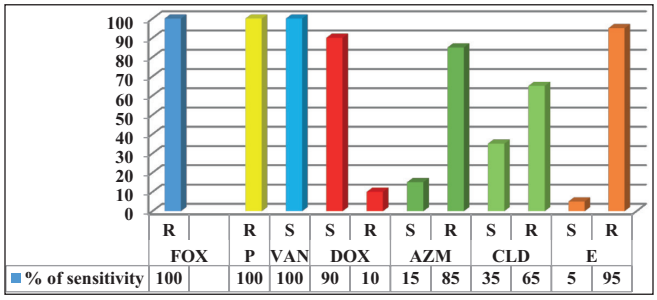

Figure 1: Antibiotic susceptibility profile of MRSA isolates (n = 20)

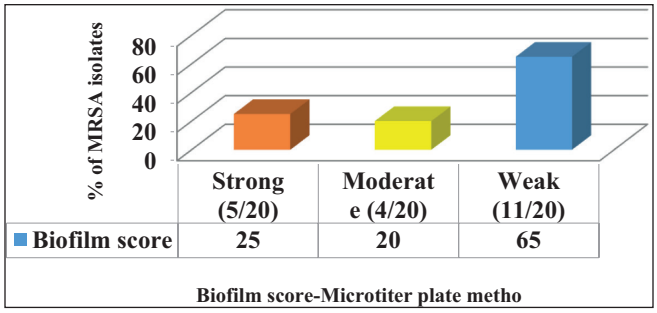

Figure 2: Percentages of biofilm score of MRSA isolates by using microtiter plate method

the resistance was reported against macrolide antibiotics groups including, azithromycin, clindamycin and erythromycin with 85%, 65%, and 95% resistance rate, respectively. MRSA isolates exhibited less resistance rate (10%) against doxycycline [Figure 1]. Our findings revealed that isolates of MRSA produced biofilm with weak scores more than strong and moderate scores 11/20 (65%), 5/25 (29%), and 4/20 (20%), respectively [Figure 2]. In regard to the association between the number and percentages of antibiotic resistance with biofilm formation among MRSA isolates, there was a nonsignificant association between the resistance and biofilm scores (P value ≥ 0.05) with inverse correlations between the azithromycin and erythromycin antibiotic resistance status with biofilm formation score with most MRSA isolates, while there was a weak positive correlation between doxycycline and clindamycin resistance status with biofilm formation score [Tables 2 and 3].

Table 2: Comparison between antibiotic susceptibility status and biofilm production among MRSA isolates

Antibiotic	Susceptibility profile	Biofilm scores			Total	*P* value
		Strong	Moderate	Weak		
Cefoxitin	R	5 (100.0%)	4 (100.0%)	11 (100.0%)	20 (100.0%)	–
Penicillin	R	5 (100.0%)	4 (100.0%)	11 (100.0%)	20 (100.0%)	–
Vancomycin	S	5 (100.0%)	4 (100.0%)	11 (100.0%)	20 (100.0%)	–
Doxycycline	S	5 (100.0%)	3 (75.0%)	10 (90.9%)	18 (90.0%)	0.45
	R	0 (0.0%)	1 (25.0%)	1 (9.1%)	2 (10.0%)	
Azithromycin	S	1 (20.0%)	1 (25.0%)	1 (9.1%)	3 (15.0%)	0.7
	R	4 (80.0%)	3 (75.0%)	10 (90.9%)	17 (85.0%)	
Clindamycin	S	3 (60.0%)	0 (0.0%)	4 (36.4%)	7 (35.0%)	0.1
	R	2 (40.0%)	4 (100.0%)	7 (63.6%)	13 (65.0%)	
Erythromycin	S	1 (20.0%)	0 (0.0%)	0 (0.0%)	1 (5.0%)	0.2
	R	4 (80.0%)	4 (100.0%)	11 (100.0%)	19 (95.0%)	

Table 3: Correlation between biofilm concentration and antibiotic resistance of MRSA isolates ($n = 20$)

Antibiotic	Statistic test	Results	Status
Cefoxitin	ρ	0	No correlation
	P value	0	
Penicillin	ρ	0	No correlation
	P value	0	
Vancomycin	ρ	0	No correlation
	P value	0	
Doxycycline	ρ	0.24	Weak positive correlation
	P value	0.3 (NS)	
Azithromycin	ρ	−0.27	Weak negative correlation
	P value	0.2 (NS)	
Clindamycin	ρ	0.33	Weak positive correlation
	P value	0.1 (NS)	
Erythromycin	ρ	−0.07	Weak negative correlation
	P value	0.7 (NS)	

Discussion

Methicillin-resistant *S. aureus* is a bacteria that resists many antibiotics, including methicillin, which makes it difficult to treat infections caused by this bacterium. MRSA infections can happen in both community and healthcare settings and may range from mild skin infections to severe, life-threatening diseases like pneumonia, bloodstream infections, and surgical site infections. MRSA is a significant public health concern worldwide due to its high incidence, morbidity, and mortality rates and ability to spread rapidly and efficiently. Therefore, understanding the epidemiology, risk factors, and mechanisms of MRSA resistance is crucial for preventing and controlling MRSA infections.[16] As a result, *S. aureus* was the second most common bacterium (24.05%) in infected burn wounds.[17] In our current study, MRSA isolates are found in different clinical samples. The percentage of MRSA isolates was identified from urine, blood, sputum, wound, and other clinical samples. The findings indicate that the highest rate of MRSA isolates was identified in urine samples (35%), followed by wound swabs (25%), ear swabs (15%), burns (15%), and sputum samples (10%). Results of the current study demonstrated that the majority of the MRSA samples had low biofilm scores (65%), with fewer samples having moderate (29%) and strong (25%) biofilm scores. The findings are consistent with a study in Iran by Ahmadrajab *et al.* in 2017,[18] who showed that 9% of isolates were strong, 26% were moderate, 48% were weak, and 17% were nonbiofilm producing. Another study in Japan showed that 6.97% were strong, 27.90% moderate, and 34.88% were weak biofilm producers.[19] According to our study, there is a connection between the development of biofilm and the level of antibiotic resistance. MRSA isolates with low biofilm formation were found to have high antibiotic resistance, whereas those with moderate biofilm formation displayed lower resistance. On the other hand, MRSA isolates that strongly produced biofilms showed less resistance to antibiotics. The study also showed no association between biofilm production and antibiotic resistance. This study agreed with other research carried out recently in 2022.[8] Researchers have found no correlation between the ability to form biofilm and antibiotic resistance in *Staphylococcus* spp. isolates. The study's authors observed a nonsignificant variation in the capacity of biofilm formation among methicillin-resistant strains.[8] Our study found that MRSA isolates showed complete resistance to cefoxitin and penicillin, with a 100% resistance rate. However, there was 100% sensitivity to vancomycin. These results agree with another Irani study by Moosavian *et al.*[6] The isolates revealed high resistance rates to macrolide antibiotics like erythromycin, with a 95% resistance rate. A 95% resistance rate to erythromycin was discovered, far higher than documented before.[20] The rate of resistance to the antibiotic azithromycin was found to be 85%, which is less comparable with what was obtained at 57%.[21] The 64% clindamycin resistance rates were greater than the 19.8% rate found by Assefa[22] but lower than the 68.8% rate found by Khodabandeh *et al.*[23] However, there was a lower

resistance rate of 10% against doxycycline for MRSA isolates. The proportion of MRSA isolates in this research showed that resistance to doxycycline was 10%, and it was reported that the resistance rate of MRSA isolates was 30% and 20.6%, respectively, which is less comparable to the findings of Fri *et al.*[24] and Khoramrooz *et al.*[25] Our study revealed that out of 20 MRSA isolates, 19 (95%) were positive for the *mecA* genes, indicating the existence of methicillin resistance [Figure 3]. The *mecA* gene is commonly found in MRSA isolates and is associated with methicillin resistance. This suggests that the *mecA* gene is prevalent among MRSA strains that have been identified. In 2016, a study was conducted on 45 bacterial isolates and found that out of them, 42 isolates were found to carry the *mecA* gene, indicating a rate of 92% consistent with the results.[14] Locally, the results of the current study were harmonized with the results of all 20 isolates tested positive for *blaz-β* genes, indicating the existence of *β*-lactamase enzymes [Figure 4]. The presence of the *blaZ* gene in MRSA strains is one of the factors that contribute to their resistance to *β*-lactam antibiotics. Our study was consistent with another finding of the *mecA* gene in all six tested samples. In this study, it was found that 4 out of 20 (20%) of the MRSA isolates carried the *blaDHA-1 AmpC* genes. These genes are a type of *β*-lactamase detection gene commonly found among MRSA isolates. *blaDHA-1-β* genes in MRSA isolates can limit the effectiveness of *β*-lactam antibiotics [Figure 5].

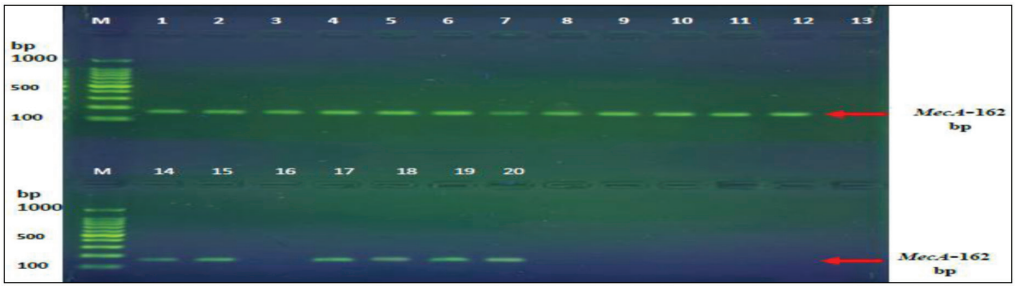

Figure 3: Gel electrophoresis of PCR products of *mecA* genes among MRSA isolates, M = DNA molecular marker size (1000 bp), 1 to 20 represents MRSA isolates

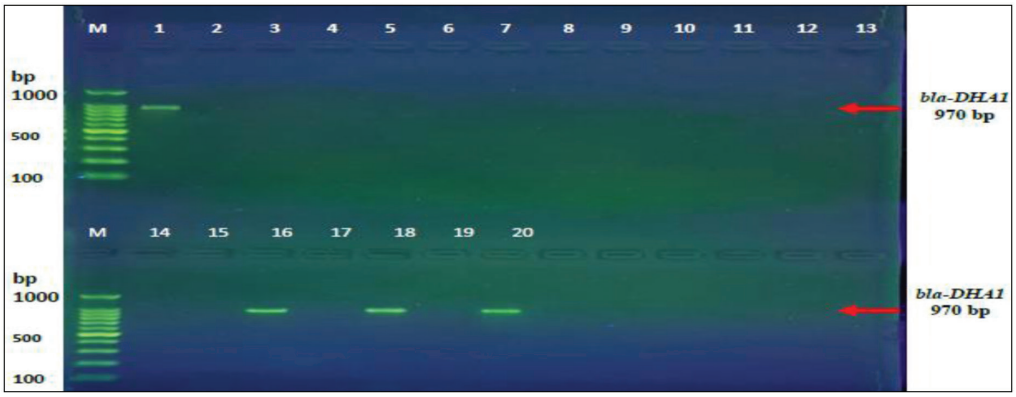

Figure 4: Gel electrophoresis of PCR products of blaZ genes among MRSA isolates, M = DNA molecular marker size (1000 bp), 1 to 20 represents MRSA isolates

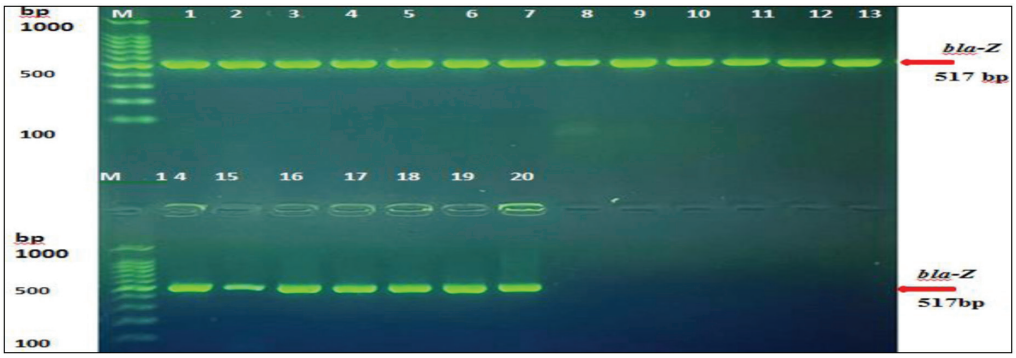

Figure 5: Gel electrophoresis of PCR products of blaz-*DHA1* genes among MRSA isolates, M = DNA molecular marker size (1000 bp),1 to 20 represents MRSA isolates

According to the current study and previously published studies, the mec-A genes are widely found in the strains of S. aureus,[26-29] which may result in high treatments with macrolides antibiotic groups and ultimately can lead to resistant strain emergence.

Conclusion

Together, our study demonstrated no relationship between biofilm production and resistance to many antibiotics against the isolates of MRSA. There is a need for precise protocols for appropriate antibiotic usage in all clinical facilities, and agents like macrolides antibiotic groups and vancomycin should also be used in particular conditions when necessary.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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