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Research Article

Molecular Detection of the *mec*A and some Virulence Determinants in Methicillin-Resistant *Staphylococcus aureus*

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Abstract:

Background: Methicillin Resistant *Staphylococcus aureus* (MRSA) is globally acknowledged as a prominent contributor to both hospital-acquired and community infections. Understanding key virulence factors including coagulase production, hemolysis ability and biofilm formation, is crucial.

Objective: The study aimed to establish a molecular characterization of *mec*A gene and virulence factors genes (*hla*, *icaA*, and *coa)* in clinical isolates of MRSA obtained from two hospitals in Baghdad.

Materials and Methods: A hundred and five isolates were obtained from clinical sources from November 2022 to March 2023 and their antibiotic sensitivity was assessed using the agar diffusion test against seven different antibiotics (Azithromycin, Ciprofloxacin, Nitrofurantoin, Rifampin, Trimethoprim, Ofloxacin and Oxacillin), through Conventional Polymerase Chain Reaction, the presence of virulence factor genes including *mec*A, *hla*, *ica*A, and *coa*, was determined in MRSA isolates.

Results: All MRSA isolates (100%) harbored the *mec*A, *hla*, and *ica*A genes while the *coa* gene was recognized in 50% of the isolates. Regarding antibiotic susceptibility, all MRSA isolates (100%) demonstrated sensitivity to Nitrofurantoin. Additionally, 96.8% of the isolates were sensitive to Oxacillin.

Conclusion: Molecular detection of methicillin resistance genes and virulence genes can be used to diagnose MRSA isolates in hospitals. The presence of these genes may affect their pattern of sensitivity to antibiotics.

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Introduction

Staphylococcus aureus is one of the most developing worldwide public health issues resulting from the emerging resistance to antimicrobial agents and leading to ineffective treatment (1). *S. aureus is* considered one of the most widely spreading bacterial infection in community and hospital settings as it possesses multiple mechanisms of antibiotic resistance, therefore, leading to severe infections (2). The problem is MRSA strains which previously spread in hospital settings but is currently occurring progressively in community settings. MRSA exhibits high infection and virulence (3).

Understanding the prevalence of MRSA is of utmost significance for infection control, the prevention of severe infections and gaining insights into the mechanisms of resistance (4). These bacteria are responsible for widespread diseases spanning from acute skin abscesses to chronic endocarditis and osteomyelitis, affecting both hospital and community settings (5). In recent years, MRSA has become a global concern, with variations in its distribution from one region to another (6). For instance, in Zakho city MRSA frequency is 85% as reported by Hami & Ibrahim (7), while its reported 90% by Hameed *et al.* in Karbala city (8).

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MRSA strains exhibit resistance to *β*-lactam antibiotics through two mechanisms: first, they hydrolyze penicillin *β* lactams by production of penicillinase, and reduce binding affinity for *β* lactams through altered Penicillin-Binding Protein (PBP).

This alteration results in fighting almost all available *β*-lactam antibacterials except for the latest cephalosporins such as ceftaroline (9). The resistance mediated by penicillinase is determined by the *bla*Z gene while the resistance associated with PBP is determined by the *mec*A gene sited in the SCCmec gene (10, 11).

Locally, a study conducted at the Maternity and Children teaching hospital and Al Diwaniya teaching hospital in Iraq, *S. aureus* isolates from different cases were found to be highly resistant to the usually used antibiotics (12). Another Iraqi study, revealed that MRSA strains were resistant to various antibiotics such as azithromycin, methicillin, and ciprofloxacin, but not to ceftaroline (13).

The pathogenicity of MRSA strains relies on several virulence factors; for example, hemolysins which lead to development of diseases and are categorized into three types: alpha(*α*), beta(*β*) and gamma(*γ*). The alpha hemolysin is a toxin produced by the *Hla* gene of *S. aureus* acting as a virulence factor that

forms pores in cell membranes, disrupting epithelial barriers and leading to cell lysis and death (14).
Biofilms which are multi-cellular bacterial communities embedded in an extracellular matrix pose a major challenge in the setting of non-communicable diseases. These biofilms provide protection to bacteria and increasing their resistance to antibiotics. Antimicrobial resistance is a prominent feature of biofilm-associated bacteria and facilitates their adhesion to infected areas (15). MRSA strains are known to produce biofilms by expressing polysaccharide intracellular adhesion (PIA), which is generated by transcription of the *ica*A gene operon products on chromosomes (16).
Furthermore, a main virulence factor of *S. aureus* is coagulase, which contributes to pathogenic infections such as endocarditis, abscess formation and staphylococcal bacteremia (17). Coagulase, an enzyme-like protein, converts fibrinogen to fibrin and leads to the formation of a plasma clot. Consequently, it enhances the pathogenesis of *S. aureus*, promotes persistent infection, plays a role in immune evasion and facilitates the spread of the bacterium through host tissues (18). Therefore, the current study aims to find the molecular characterization of *mec*A gene and some virulence factors genes (*hla*, *ica*A, and *coa*) in clinical isolates of MRSA obtained from patients.

Materials and Methods

Samples collection: Between November 2022 and March 2023, 105 specimens (nostril, throat, urine, sputum and wound samples) were collected in sterilized transport tube media from Abu Ghraib General Hospital and Al Yarmouk Teaching Hospital laboratories.

Isolation and Identification of *S. aureus* and detection of MRSA isolates: All specimens were inoculated on Mannitol salt agar (MSA) (Accumix, England). The cell culture plates were incubated overnight at 37°C. Biochemical tests for isolated bacteria were identified by oxidase, coagulase, urease and catalase tests (10).
Phenotypic detection of MRSA isolates was done by utilizing a Methicillin disc (10 µg) and measurement of inhibition bacterial zone of growth after cultured on Muller Hinton medium according to the CLSI guidelines (19). Next, the confirmation of identified isolates was carried out via the VITEK 2 identification device.

Antibiotics Susceptibility Test (AST): Antibiotic susceptibility test using seven different antibiotics [Azithromycin (15µg), Rifampin (5µg), Trimethoprim (10µg), Nitrofurantoin (100µg), Ciprofloxacin (10µg), Ofloxacin (5µg) and Oxacillin (5µg)] was performed for MRSA isolates by KB test according to Bauer *et al*. in 1966 (20) and CLSI (19).

Molecular Detection of *mec*A, *ica*A, *coA, and hla* genes
DNA Extraction: Norgen's Blood DNA Isolation Mini Kit (Norgen, Canada), OneTaq DNA Polymerase Kit (NEB, England) and the Qubit Double-stranded DNA high sensitivity Kit (ThermoFisher, USA) were used for extracting pure DNA from Methicillin-Resistant *S. aureus* isolates depending on their manufacturer's guidelines. After that, NanoDrop spectrophotometer was used to accurately determine sample concentration between 10 pg./µl and 100 ng/µl (21).
Polymerase Chain Reaction assay: All Methicillin-Resistant *S. aureus* isolates were checked molecularly for *hla*, *icaA*, *coa* and *mecA* genes using conventional PCR. The amplification procedure for these genes included the initial denaturation phase heated to 94°C for 5 minutes then, at 94°C, 38 cycles of denaturation for 30 seconds, annealing for 45 seconds at 57°C, extension for 45 seconds at 72°C, and final extension for 7 minutes at 72°C. Electrophoresis of the conventional PCR product in a 2% Tris-acetate-EDTA (TAE) agarose gel electrophoresis with 1x TAE buffer for 80 minutes at 80 volts and dyed with RedSafe dye (22). The primer sequences used in conventional PCR for the detection of *mec*A, *ica*A, *coa*, and *hla* genes in MRSA isolates, and the primer sequences are shown in Table 1.

Table 1: The Primers sequences used for detection of *ica*A, *mec*A, *coa*, and *hla* genes

Genes name	Sequence	Size (bp)
*mec*A	F: GTTGTAGAAGGTCCATTATGG R: TAGAACCTTGAGCCTCTTTT	226
Hla	F: TTTTCTTTTCAGGAAGCGAG R: CTTCGATTAATACTGTCCGTC	400
Coa	F: CTGGGAGTAAAAATGGGAAAC R: CAGGTATTGGTCTTCCTCTAA	179
*ica*A	F: GTATTAAGCGAAGTCAGACAC R: CCAGCTTACAAATATGAGTCC	554

Statistical analysis: The R software package was used to analyze the data to determine the sensitivity of the bacteria under study to different antibiotics. The percentages and numbers of resistant and sensitive ones for each type of antibiotic used were determined at a significance level of ($p<0.05$) (23).

Results

Isolation of *Staphylococcus aureus* and MRSA
Only 84 (80%) samples grew on MSA, and based on the primary diagnosis, 47 isolates of these were *S. aureus* (Table 2) and were examined for antibiotic sensitivity tests by the Kirby-Bauer method to identify methicillin sensitivity of *S. aureus*. Out of these 47 isolates, 31 (66.0%) were resistant, 8 (17.0%) were intermediate, and 8 (17.0%)

Table 2: The biochemical tests of *S. aureus* isolates

Test	Result
Gram stain	(+)
Catalase	(+)
Coagulase	(+)
Indole	(-)
Capsule	(-)
Oxidase	(-)

Were sensitive (Table 3). The confirmation of identification was performed by the VITEK2 system. All 31 bacterial isolates were recognized as MRSA based on the primary diagnosis and the VITEK2 system results.

Table 3: Susceptibility of 47 MRSA isolates to Methicillin

Sensitivity	Resistant	Intermediate	Sensitive	P- value
S. aureus	31 (66%)	8 (17%)	8 (17%)	0.0006***
Chi Square test (X^2)				

Antibiotics susceptibility test : A sensitivity test was conducted using seven different antibiotics (Azithromycin, Ciprofloxacin, Nitrofurantoin, Rifampin, Trimethoprim, Ofloxacin and Oxacillin) to determine the sensitivity of MRSA isolates by agar diffusion test.

The results showed that all 31 MRSA isolates (Table 4) were sensitive to Nitrofurantoin at a rate of 100%. However, they exhibited no sensitivity to Ciprofloxacin. Azithromycin, on the other hand, showed a sensitivity rate of 35.5% for MRSA and a resistance rate of 54.8%. Additionally, 29.0% of MRSA isolates were resistant to Trimethoprim, while 9.7% showed resistance to Rifampin and Ofloxacin. Moreover, 96.8% of the isolates were sensitive to Oxacillin, 83.9% were sensitive to Rifampin and 71% were sensitive to Ofloxacin.

Table 4: Antibiotics susceptibility test of MRSA isolates against seven different antibiotics

Antibiotics	% of isolates			
	sensitive	intermediate	resistant	P - Value
Azithromycin	11 (35.5)	2 (6.5)	18 (58.1)	0.01**
Ciprofloxacin	0	14 (45.2)	17 (54.8)	0.354
Nitrofurantoin	31 (100)	0	0	0.00***
Rifampin	26 (83.9)	2 (6.5)	3 (9.7)	0.0216*
Trimethoprim	1 (3.2)	21 (67.7)	9 (29.0)	0.865
Ofloxacin	22 (71.0)	6 (19.4)	3 (9.7)	0.0453*
Oxacillin	30 (96.8)	0	1 (3.2)	0.004**
Chi square test (x^2)				

Identification of *mecA* gene and virulence factor genes: The conventional PCR test was used to identify the main virulence factor genes including *mec*A, *ica*A, *coa*, and *hla* genes that are responsible for methicillin resistance, polysaccharide production, coagulase production, and blood lysis, respectively. Ten MRSA isolates were used for determining these genes. The results detected that all samples contained *mec*A, *hla*, and *icaA* genes (100%), the *coa* gene was identified in 50% of the isolates. as shown in Figure 1.

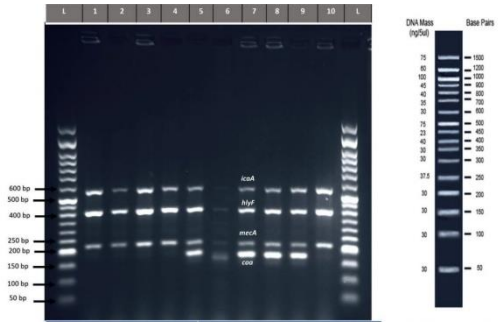

Figure 1: Multiplex Conventional PCR of amplified PCR products for *mecA* (226bp), *hla* (400bp), *ica*A (554bp), and *coa* (179bp) from MRSA isolates

Discussion

S. aureus is a main contributor to the hospital-acquired and community contagions globally. The transformation of *S. aureus* from methicillin-sensitive to methicillin-resistant is primarily due to the acquirement of the *mecA* gene (24, 25).

The prevalence for MRSA in the present study differs from other studies which may be due to various factors, including poor hygiene, antibiotic excessive use, regional conflicts and close contact, such as through religious visits.

The majority of MRSA isolates showed Azithromycin and Ciprofloxacin resistance which agrees with other studies (26), such as Bastidas *et al.* at 61.8% (27) and Ponvelil *et al.* at 16% (28). additionally, studies in Jordan showed a 15% resistance for Azithromycin and a 28.6% resistance to Ciprofloxacin in Pakistan (29). In this study, Nitrofurantoin demonstrated sensitivity among MRSA isolates aligning with previous findings (30). Consequently, Nitrofurantoin is considered an effective antibiotic for treating MRSA infections, as no resistance was observed among the tested isolates.

Resistance rates for Rifampin (9.7%) and Trimethoprim (29.0%) were seen in this study, showing some agreement with findings by Bai *et al.* for Rifampin (5.9%) (31), but showing a higher rate (11.8%) for Trimethoprim than in the study by Ibrahiem *et al.* (32).

The frequency of MRSA isolates resistance to Oxacillin was 3.2%, in contradiction to the results of Khasawneh *et al.* (33), who found a resistance rate of 42.1% for MRSA isolates in Jordan. The 9.7% resistance to Ofloxacin disagrees with Sohail *et al.* (34), who found a resistance rate of 98%.

In the current study, Ofloxacin exhibited a sensitivity rate of 71% for MRSA isolates, which is close to the results of Ndedy *et al.* (35), who reported a sensitivity of 50% for Ofloxacin. Hense, the most effective antibiotics for treating MRSA as found in the current study were Ofloxacin, Rifampin

and Oxacillin since only a small number of MRSA isolates exhibited resistance to these drugs.

The molecular detection method of the *ica*A, *mecA*, *coa*, and *hla* genes was achieved using multiplex PCR. Vieira *et al*. found that 100% of MRSA isolates from clinical sources were *mecA* producers (36). MRSA is a primary human pathogen causing significant morbidity worldwide and is closely monitored by the World Health Organization. This pathogen contributes to biofilm formation, acute human infections and the development of antibiotic resistance (37).

A study by Bayirli *et al*. reported that 92.6% of isolates exhibited alpha-hemolysis, while 1.6% displayed beta-hemolysis (38). Additionally, the current study reveals that MRSA isolates which possess the *mecA* gene were phenotypically susceptible to Nitrofurantoin. The expression of the *icaA* gene through the study revealed all isolates had positive results and expressing the gene, which is similar to the findings of Azmi *et al*. (39), who reported a prevalence of 100% for the *icaA* gene in MRSA.

Regarding the *coa* gene, the current study reported that it was found in 50% of MRSA isolates, which was lower than that reported by Hezam (86.6%) (40), which may be due to differences in sample treatment, geographical locations, and the dynamic nature of bacterial evolution.

The study revealed that Oxacillin and Nitrofurantoin as effective treatments for MRSA infections but with controlled use to prevent antibiotic resistance.

Conclusion

Molecular detection of methicillin resistance genes and virulence genes can be used to diagnose MRSA isolates in hospitals. The presence of these genes may affect their pattern of sensitivity to antibiotics, especially with the increasing antibiotic resistance due to antibiotic misuse, and person-to-person transmission.

Author contributions:

The manuscript should mention the contribution of each author to the research done: Study conception & design: (Huda S.A. Al-Hayanni). Literature search: (Wed L. Khalil). Data acquisition: (Wed L. Khalil). Data analysis & interpretation: (Wed L. Khalil & Huda S.A. Al-Hayanni). Manuscript preparation: (Wed L. Khalil). Manuscript editing & review: (Wed L. Khalil & Huda S.A. Al-Hayanni).

Authors' declaration:

Conflicts of Interest: The authors declare no conflict of interest.

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad according to the code number (5296/22) on (18/ 10/ 2022).

Limitations: The study was based on only two hospitals (Abu Ghraib General Hospital and Al Yarmouk Teaching Hospital) hence, the findings don't represent the whole population.

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الكشف الجزيئي عن جين *mecA* وبعض محددات الفوعة في المكورات العنقودية الذهبية المقاومة للميثيسيلين

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الخلاصة
خلفية البحث: تُعد المكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA) بأنها أحد الممرضات المساهمة في العدوى المكتسبة من المستشفيات والمجتمعات عالمياً. إن فهم عوامل الفوعة الرئيسية، مثل تكوين الأغشية الحيوية، والقدرة على إنحلال الدم، وإنتاج إنزيم التخثر، أمرٌ بالغ الأهمية.
الهدف: هدفت الدراسة الحالية إلى تحديد التوصيف الجزيئي لجين *mecA* وبعض جينات عوامل الضراوة (*hla*، *icaA*، و*coa*) بين العزلات السريرية للـ MRSA التي تم الحصول عليها من مستشفيين في بغداد.
المنهجية: تم الحصول على 105 عزلة من مصادر سريرية ما بين نوفمبر 2022 الى مارس 2023، وتم فحص حساسيتها للمضادات الحيوية باستخدام طريقة كيربي باور ضد سبعة مضادات حيوية مختلفة (أزيثروميسين، سيبروفلوكساسين، نيتروفورانتوين، ريفامبين، تريميثوبريم، أوفلوكساسين، وأوكساسيلين). بالإضافة إلى ذلك، تم تحديد وجود جينات عامل الفوعة، بما في ذلك *mecA* و*hla* و*icaA* و*coa*، في عزلات الـ MRSA بواسطة تقنية تفاعل انزيم البلمرة المتسلسل.
النتائج: أظهرت نتائج الدراسة الحالية أن جميع عزلات MRSA (100%) تحتوي على جين الـ *mecA* وجين الـ *hla* وجين الـ *icaA*، في حين تم اكتشاف جين الـ *coa* في 50% من العزلات. فيما يتعلق بالحساسية للمضادات الحيوية، أظهرت جميع عزلات الـ MRSA (100%) حساسة للنيتروفورانتوين. بالإضافة إلى ذلك، وجد أن 96.8% من العزلات حساسة للأوكساسيللين.
الاستنتاج: لخصت نتائج الدراسة الحالية الى ان الكشف الجزيئي لجينات مقاومة الميثيسيلين وجينات الفوعة يمكن استخدامه لتشخيص الـ MRSA في المستشفيات وان وجود هذه الجينات قد يؤثر على نمط حساسيتها للمضادات الحيوية.
الكلمات المفتاحية: المكورات العنقودية الذهبية المقاومة للميثيسيلين، جين *mecA*، جين *hla*. جين *icaA*، جين *coa*.