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Genomic Investigation and Biofilm Characterization of Methicillin-Resistant *Staphylococcus aureus* in Baghdad Province

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Abstract

Background *Staphylococcus aureus* (*S. aureus*) could readily form biofilm, which innervates the drug resistance abilities, resulting in life threatening infections involving different organs. The biofilm formation occurs due to a series of developmental proceeds including bacterial adhesion, aggregation, biofilm maturation, and dispersion, which are controlled by multiple genomic regulatory systems in *S. aureus* strains.

Objective Determination of the biofilm capability with revealing the antimicrobial sensitivity outcomes and their relationship with the genomic content of *S. aureus* including Methicillin resistant S. aureus (MRSA) isolates across Baghdad province.

Methods In this cross-sectional study, 200 variable pathogenic samples were collected from different patients ages across several medical centers in Baghdad, Iraq. Biofilm ability forming examining, antimicrobial susceptibility testing, and polymerase chain reaction (PCR) were applied to determine the adhesion capability, resistance pattern and gene distribution among identified *S. aureus* isolates, as well as biochemical tests to confirm isolates demonstration.

Results Out of 200 collected samples, only 130 coagulase positive cocci were approved, 72 MRSA being identified as verified groups of *S. aureus* isolates. As total, biofilm screening outcomes presents strong biofilm formation in 22 (18.92%) isolates, moderate in 24 (18.46%), weak in 26 (20.00%), and no-biofilm formation in 58 (44.62%) isolates. Across MRSA resistance-associated genes as mecA were detected respectively in 37 (80.4%). Genes within P value have been characterized into true-false ranges and presence-absent count. Antimicrobial screening showed most of isolates were sensitive to Gentamicin. Higher resistances were presented in Oxacillin and Rifampin. SCCmec pattern for MRSA was demonstrated according to PCR outcomes.

Conclusion Results of this study signalized that *S. aureus* including MRSA should be theorized as a serious opportunistic to pathogen to be aware in all people ages and units. Gentamicin and Tigecycline are fairly recommended to treat some of *S. aureus* infections. Biofilm consider an important issue in medication and remediation capabilities and there virulence fairly related with genomic variation and its continuous developments

Keywords *Staphylococcus aureus*; MRSA; biofilm formation capacity; antibiotic resistance; genomic typing

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List of abbreviations: CLSI = Clinical and Laboratory Standards Institute, GEN = Gentamicin, MRSA = Methicillin resistant Staphylococcus aureus, NCBI = National Center for Biotechnology Information, PCR = Polymerase chain reaction, RA = Rifampin, S. aureus = Staphylococcus aureus, TG = Tigecycline

Introduction

Staphylococcus aureus (*S. aureus*) is an opportunistic Gram-positive pathogen, it considers one of the most common agent

of skin and soft tissue infections. It subsists in the nasopharynx, skin, eye, intestine and urogenital tract as normal flora. Moreover, it can breach the skin barriers via wounds or surgical incision and cause an infection. However, it has the ability to adhere and form a biofilm on tissues or medical indwelling devices [(1)].

Biofilm could be defined as a microbially-derived sessile community, typified by cells that are attached to a substratum, interface, or to each other, are embedded in a matrix of extracellular polymeric substance, also exhibit an altered phenotype with regard to growth, gene expression and protein production [(2)].

Biofilms can originate from a single cell, and varying environmental conditions, along with community interactions, can enhance the emergence of diverse subpopulations. Variations in oxygen levels, nutrient availability, and electron acceptors can lead to heterogeneous gene expression within biofilms [(3)].

S. aureus can produce a multilayered biofilm embedded within a glycocalyx or slime layer with heterogeneous protein expression. Investigational studies described the solid component of the glycocalyx as primarily composed of teichoic acids (80%) and staphylococcal host proteins [(4)].

S. aureus represents developmental mechanisms for resistance in aim of using it against a wide range of antibiotics since discovering and using Penicillin as a first effective antibiotic and until nowadays by their continual virulence modifications [(5)].

Methicillin-resistant *S. aureus* (MRSA) is often resistant to many types of antibiotics. Biofilm former isolates can appear reliable resistant than those biofilm non-forming isolates [(6)].

Quorum sensing is a mechanism, known as bacterial cells, communicate and coordinate their behaviors depend on population density. Accessory gene regulator (agr) of quorum sensing system plays a key role in *S. aureus* pathogenesis. Agr quorum sensing genes mainly reported in several studies with regulation of S. aureus biofilm formation, as example four polymorphic agr types (agrI, agrII, agrIII, and agrIV) have been studied with documented researches [(7)].

This study aimed to isolate and identify MRSA strains from clinical samples in Baghdad province, to investigate the presence of antibiotic resistance genes, especially mecA, to analyze genetic diversity relationships among MRSA isolates, to evaluate biofilm formation in MRSA strains and correlate with genomic data, to assess the impact of biofilm and resistance on MRSA infections and to provide recommendations for infection prevention based on research findings.

Methods

In this cross-sectional study, 130 coagulase-positive staphylococci were isolated from the several type of sample cultures for outpatient and inpatients hospitalized in different substantial Baghdad governate hospitals and medical laboratories from July 2023 to January 2024. The characterization of *S. aureus* was conducted through standard microbiological examinations and biochemical confirmational procedures [(8)].

For biofilm assay determination, initially, *S. aureus* isolates were incubated in brain heart broth for 24 hr at 37°C. Subsequently, *S. aureus* biofilms were prepared by transferring 100 µl of adjusted inoculums into sterile 96-well polystyrene microtiter plates. As a negative control, broth without bacteria was prepared. The incubation was done at 37°C for 7 days. Media were then removed by slightly tapping the plate. Plate was washed three times with sterile distilled water to remove free-floating planktonic bacteria and was then drained off by inverting to allow it to air dry. The biofilms were stained with 100 µl 0.1% (w/v) crystal violet for 10 min. For removing the crystal violet, plate washed three times with phosphate-buffered saline. To detach the biofilms, 100 µl of 95% ethanol was added into each well. The solubilized biofilm formations were finally measured by the micro plate

reader at the wavelength of 570 nm. The experiments were performed in triplicate [9].
Following formulas were determined to classify the biofilm formation.
Non-adherent [NA = OD ≤ ODC)].
Weak adherent [WA = ODC < OD ≤2 x ODC].
Moderate adherent [MA = 2 x ODC < OD ≤4 x ODC].
Strong adherent [SA = 4 x ODC < OD].
*OD = Optical density
*ODC = Optical density cut-off value
*MA = Moderate adherent
*NA = Non-adherent
*SA = Stronge adherent
Antibiotic susceptibility of *S. aureus* isolates was conducted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [10] using the commercially available discs (Liofilchem® S.r.l.Via Scozia, Italy) of 17 antimicrobial agents belonged to14 different classes by the Kirby–Bauer disk diffusion technique. The antibiotic panel include: Gentamicin (GEN:10 µg/disk), Rifampin (RA:5 µg/disk), Ceftaroline (CPT:30 µg/disk), Oxacillin (OX:5 µg/disk), Ciprofloxacin(CIP:5 µg/disk), Moxifloxacin (MFX:5 µg/disk), Trimethoprim-sulphamethoxazole (TMP/ SMX:1.25/23.75 µg/disk), Fusidic acid (FU:10 µg/disk), Vancomycin (Van:30 µg/disk), Teicoplanin (TEI:30 µg/disk), Clindamycin (DA:2 µg/disk), Erythromycin (E:15 µg/disk), Linezolid (LZD:30 µg/disk), Chloramphenicol (C:30 µg/disk), Tetracycline (TE:30 µg/disk), Doxycycline (DO:30 µg/disk), Tigecycline (TG:15 µg/disk), Quality control was maintained using *S. aureus* ATCC 25923, test and antibiogram analysis results were done and explicated as the Clinical and Laboratory Standards Institute particulars (CLSI 2022) [10].
The genomic DNA of *S. aureus* was extracted by boiling method [11]. DNA was extracted after centrifugation for 5 min within 12,000 rpm. Presence of *S. aureus* resistance-related genes as (MecA) and other virulence genes was detected by polymerase chain reaction (PCR) within suitable annealing gradient. Specific primers of *S. aureus* were designed using bioinformatic software as Geneious Prime. Agr (I, II, III, IV) (55°C) and SCCmec (55°C) systems gene, Pvl (53°C) and MecA (53°C) were relied according National Center for Biotechnology Information (NCBI) references [12]. In order, to obtain a specific primer sequence, the sequence of desired genes downloaded from NCBI, aligned first with multiple alignment on Geneious Prime.
The total volume of the PCR reaction mixture was 25 µl. Thermal cycling was performed using the following reaction conditions: 95°C for 5 min followed by 30 sequential cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 1 min and a final elongation step at 72°C for 10 min, electrophoresis of products was performed using 1.5 % agarose gels and the bands visualized under ultraviolet light, the conditions of annealing optimization vary according each gene [13,14].

Statistical analysis

The R studio ggplot2 package was utilized to perform the correlation and display the results. The Chart Builder tool in R studio with the ggplot2 package is used to create stacked charts [15].

Ethical Statement

This study was approved by the Ethics Committee of Mustansiriyah University college of Sciences (Ref.: BCSMU/0923/0007B).

Results

In total of this study, out of 130 coagulase positive cocci, 58 isolate shows (44.62%) negative phenotyping biofilm formation, weak formation percentage was (20.00%) for 26 isolate, moderate shows (18.46%) for 24 isolate and strong biofilm distribution appeared in percentage of (16.92%) for 22 Staphylococci, P value of Chi-Square test = 0.766, which means there is no significant association between Biofilm and group.
The calculations illustrate that female patients were about (87) sample whom *S. aureus* bacteria were isolated from it, female elucidate

for multidrug (43) isolate, extended resistance (14) isolate and multidrug sensitivity (30) isolate categorization within P value = 0.0006, for male patient calculation were about (43) sample, moreover, state appear for multidrug (23) isolate, extended resistance(4) isolate and multidrug sensitivity (16) isolate categorization within P value = 0.0015, as final reckoning, total P value for patient's gender characterization were 0.5734, which means there is no significance relation between gender and antibiotics resistance.

Depending on age, the highest frequency of antibiotics resistance was counted in ages within 20-70 years old, that has been show a maximum numeral with (70) isolate, while ages <20 years old presented (11) isolate, furthermore, ages >71 years old displayed minimum number for resistance within (4) isolates, total P value were 0.9836, no significance relation between age and antibiotics resistance.

According to sample types of clinical isolates, wound swab has the majority count within (53) isolates with significance P value = 0.0075, but as total calculation for all type of sample with P value = 0.0798, there is no significance relation between types of sample isolation and antibiotics resistance. The total significance P value was presented in the variable sources of hospital's isolates in Baghdad, the predominant hospital was Al-Kindy hospital within (50) isolate, total P value was 0.0028.

MRSA antimicrobial susceptibility analysis showed a high resistance for RA antibiotic, Fusidic acid and Linezolid were fairly closed in their resistance, minimal resistance was shown in Moxifloxacin and GEN (Figure 1).

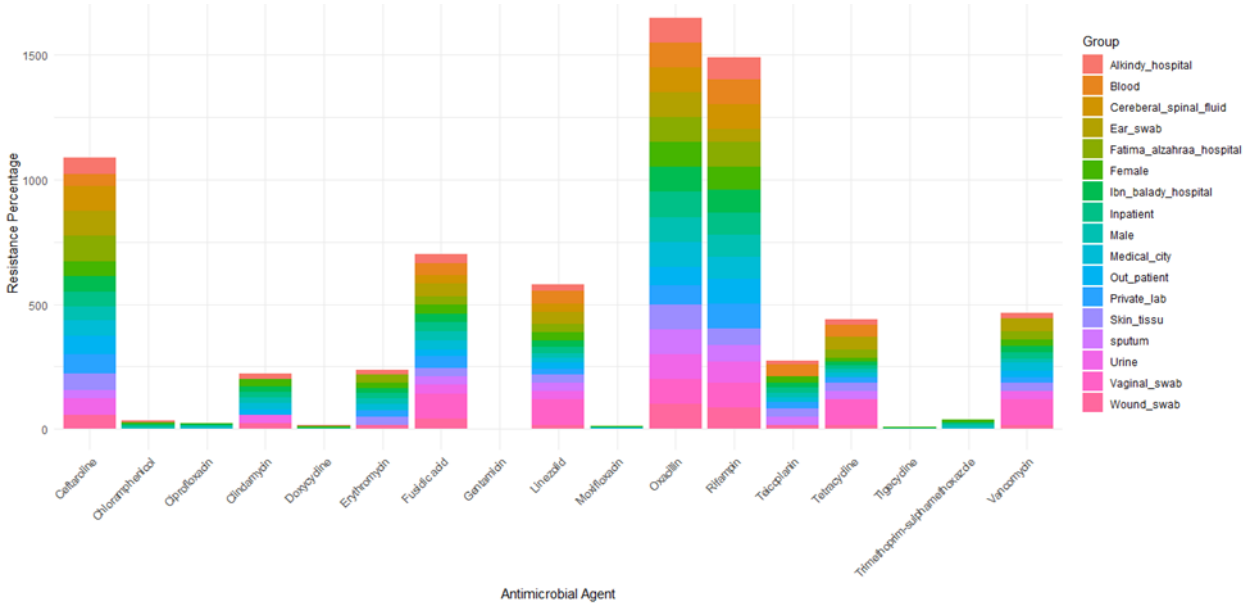

Figure 1. MRSA's stacked bar plot of antimicrobial resistance with source grouping

MRSA's presence counting of PCR results analysis outcomes revealed as 5 (10.9%) for AgrI, AgrII 6 (13%), AgrIII 4 (8.7%) of Agr genes system. SCCmec genes demonstration appeared as 2 (4.3%) for ccrA2-B, ccrC 10 (21.7%), IS1272 5 (10.9%) and mecA-IS431 19 (41.3%). MecA gene presented a highest presence percentage of 37 (80.4%) for the total MRSA isolates counts while PVL gene was totally absent in whole MRSA isolates genomic typing. Furthermore, additional absence counts rates have been detected as AgrIV as illustrated in figure (2).

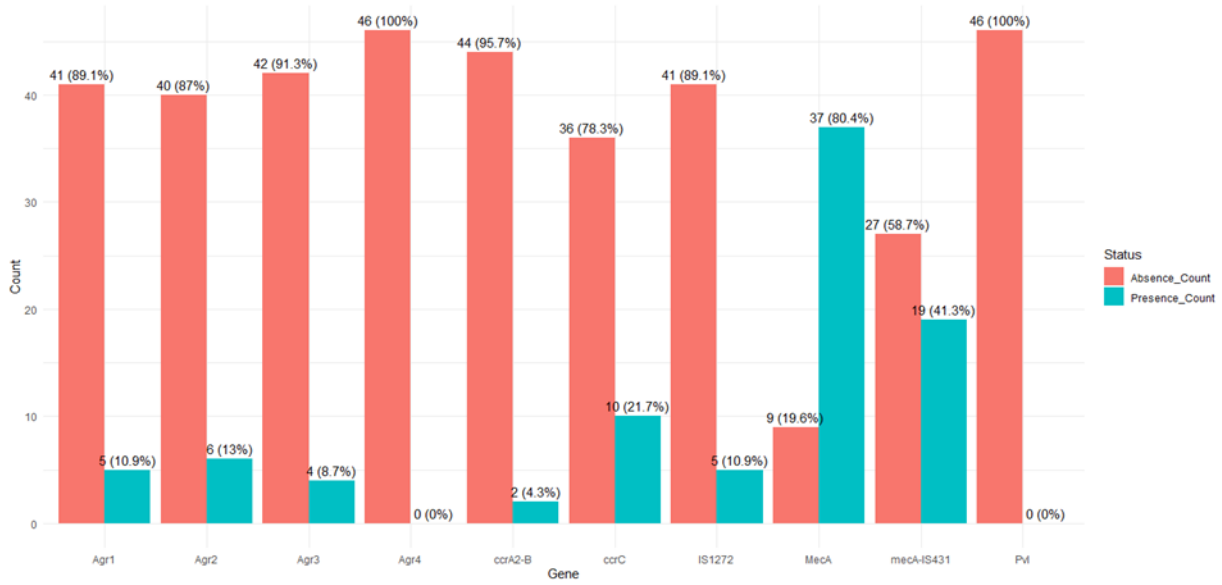

Figure 2. MRSA's typing genes presence and absence counts with percentages

PCR analysis SCCmec pattern revealed peculiar types and percentages in MRSA isolates, which were divided into 4 common types in addition to unknown one (Figure 3).

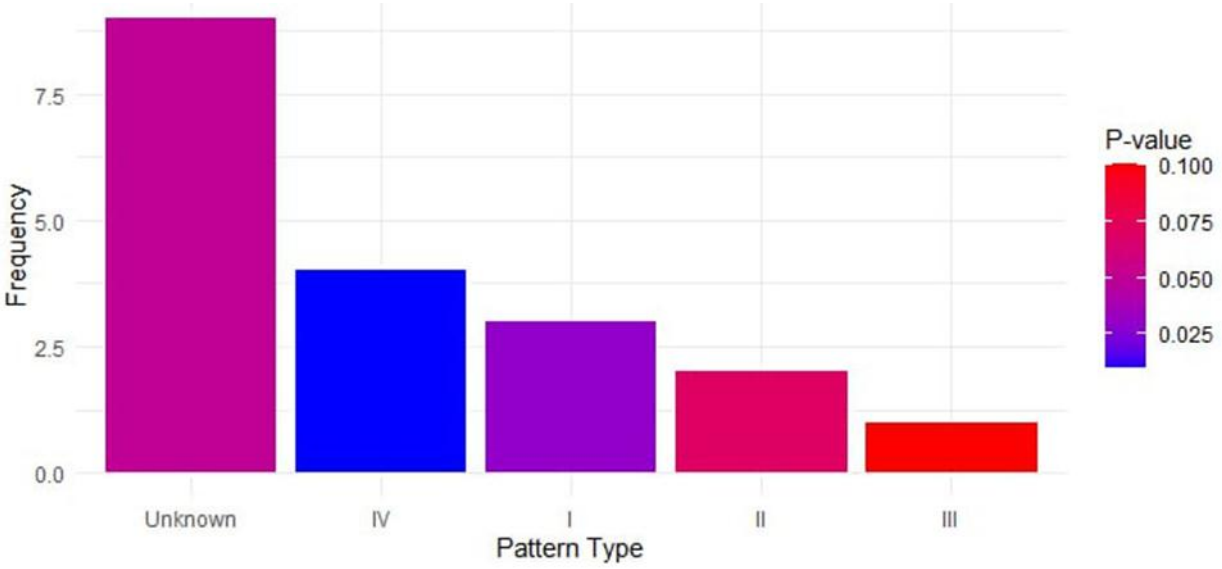

Figure 3. MRSA's SCCmec typing pattern

Genomic differences between isolates were variable, indicating that there are main related factors to the differences in biofilm formation strength and antibiotic resistance ability and the origin of isolates.

Discussion

In this present study, the genetic profiles of *S. aureus* isolate from various central hospitals in the Baghdad governorate exhibited variations when compared to the findings of other studies based on the reviewed medical literature. Although the prevalence of these isolates may be lower in comparison to other hospital-acquired bacteria, their significance is underscored by the pathogen's inherent resistance to commonly used antibiotics, the presence of virulence genes, and its capacity to form biofilms.

Various reports declare about distribution of *S. aureus* in Baghdad province, as comparison clinical sample of isolate collection and distribution were variable higher than a previous study in 2015, while presented results shows that dispersal of *S. aureus* were counted from samples as follow: urine (n=44), blood [9], cerebral spinal fluid (n=7), skin tissues (n=6), sputum (n=5), vaginal swab (n=2), wound swab (n=53), ear swab (n=4) and these could be compared as relational views with [16] study outcomes.

The study's investigations were fairly inconstant with [17] results who appeared out of the positive isolates were 46.15% strong biofilm producers, 46.15% moderate ability to produce biofilm, and 7.70% were weak producers for the biofilm.

Across study's outcomes, TMP/SMX and vancomycin considered effective antibiotics to treat *S. aureus* infections. Moreover, several of demonstrated articles of resistance *S. aureus* induced infections raised attends over its continued efficiency. An alternative antibiotic, such as GEN and linezolid, had reported as functional agents across invasive *S. aureus* related infections [18,19].

This study investigates the effectiveness of GEN and TG against *S. aureus*, while indicating that RA has lower effectivity in antimicrobial sensitivity testing. Additionally, the efficacy of quinupristin-dalfopristin was not evaluated due to its limited availability in Iraqi hospitals and its general lack of prescription in the region. Various antimicrobial susceptibility results differing from those presented in this study have been reported from Iraq and elsewhere.

Inconsistent with these following results, a study in Baghdad university present 40 (69%) *S. aureus* isolates exhibited resistance to GEN. The results also clarified that 40 (69%) of *S. aureus* isolates were resistant to cefoxitin. Results of Vancomycin susceptibility was tested by Vitek-2 compact system for *S. aureus* isolates. The result showed that 57 (98%) of *S. aureus* isolates were sensitive to vancomycin and only in one isolate (2%) an intermediate resistant was noticed. Fifty-eight isolates identified as *S. aureus* were isolated locally from different clinical specimens [20].

As correspondence between presented result and several other reported studies shows a proportionality range of resistance to RA. RA-resistant arises from a chromosome mutation. Moreover, may this antibiotic have been inappropriate against S. aureus in advanced hospitals infections [21,22].

MRSA is an important hospital pathogen, the incidence of which is increasing every year especially in high-risk groups. Another unique characteristic of MRSA is that it's a disease-causing pathogen in the condition of nosocomial infection and also community-onset infections which has shown a widespread distribution in the last 40 years [23].

In this related paper, MRSA has been revealed by known principal microbiological methods. Genetic diversity of MRSA isolates verification was demonstrated unexpensively and quickly utilizing PCR tool with using a specific designed primer as described in previous study parts. Verified 46 MRSA isolate's PCR result classifiable into different SCCmec types as described previously which consider a discriminative pattern.

Accessory gene regulator (agr) locus of *S. aureus* is a quorum-sensing virulence regulator. The effect of dysfunctional agr on the outcome of invasive *S. aureus* infection may vary depending on various conditions, such as Oxacillin susceptibility and the site of infection. Dysfunctional agr was generally associated with unfavorable clinical outcomes and its effect was prominent in MRSA and pneumonia; these could be reliable considerations for our study's genomic typing for agr genes [24].

The study findings corroborate with others published substations proposed that SCCmec categories I, II, and III are prevalent and variable among hospital acquired MRSA isolates in addition for the genetic diversity for MRSA isolates depends on socio-demographic and additional related characteristics, as well as the considerable countenance of community acquired MRSA are SCCmec types IV and V. This could perform as investigational and epidemiological signs for hospital and community acquired-MRSA discrimination [25].

For this study findings, there is no presence for Pvl gene in MRSA isolates, and this was disagreement with the outputs of [26] study in Babylon province who demonstrated that 19 (79%) out of 24 isolates had positive result for pvl toxin gene but there is an agreement with same study in presence of mecA genes percentages.

The use of different polymerase chain reaction-based approaches has been successfully utilized for the rapid genomic content detection of S. aureus, including MRSA directly from various clinical specimens, standred tool of the PCR with its sensitivity and specificity precisely needed for accurate results appearance and making a relational correlation between phenotypic and genotypic aspects.

In conclusion, MRSA consider an important matter to study in addition to the displayed biofilm formation capabilities, especially manifesting strong biofilm capacity and antimicrobial resistance situations. Subsidiary, PCR recognition approved that the plurality of *S. aureus* isolates belongs to variable agr and SCCmec types. Moreover, analysis of virulence genes revealed that MRSA isolates presented a varied array of virulence genes. Genomic and molecular typing of Staphylococcus aureus in the context of human related infections is conclusive for progressing infections administration and evolving effective treatment delineations.

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Author contribution

Qader: data acquistion and writing the manuscirpt. Dr. Ali: was mainly responsible for software and visualization. Dr. Alsakini: took the lead on methodology and administration. All authors were involved in conceptualization, analysis, investigation, and validation.

Conflict of interest

Authors declare that no conflicts of interest.

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