Original Article

Sequence Characterization of CA-MRSA Isolates Recovered from Clinical Infections

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Abstract

Background: *Staphylococcus aureus* is a significant and widespread human bacterial pathogen that is associated with both hospital- and community-acquired illnesses globally. **Objectives:** The purpose of the study was to characterize the sequences of the *SCCmecA* type IV and *pvl* genes in community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) isolates from various cutaneous infections. **Materials and Methods:** Intensive care unit burns, private clinics, and Al-Hilla Teaching Hospital provided samples from 100 different skin infections for our study. *S. aureus* was recognized using normal culture media and microscopic inspection. Utilizing the Vitek-2 susceptibility system, the antibiotic sensitivity test was conducted. Polymerase chain reaction (PCR) screening of the *16sRNA*, *mecA*, *SCCmecA* type IV, and *pvl* genes was carried out. **Results:** Phenotypic detection of MRSA was detected by cefoxitin and oxacillin screening test using the Vitek2 system. The results of this study demonstrated that all of the isolates 20 (100%) were resistant to cefoxitin and oxacillin. Conventional PCR showed that 83.33%, 80%, 50%, 55% of *S. aureus* were positive for the*16sRNA*, *mecA*, *SCCmecA* type IV, and *pvl* genes, respectively. **Conclusion:** The current study revealed high *S. aureus* isolates in burns, followed by impetigo, wounds, and boils, respectively. In contrast, there were no bacterial isolates in acne or folliculitis. In addition, the genotypic method is more accurate than the phenotypic one for detecting MRSA and is considered the gold standard method. Previously. This extremely important CA-MRSA, hitherto believed to be exclusive to the hospital setting, is now also being isolated from community settings. The majority of CA-MRSA carry the *pvl* gene and have the easily transferable tiny mobile *SCCmec* elements IV.

Keywords: CA-MRSA, *mecA* gene, *SCCmecA* type IV gene, sequence analysis

Introduction

Globally, hospital—acquired (HA) and community-acquired illnesses have been related to *Staphylococcus aureus*, a significant and widespread human pathogen.[1] *S. aureus* can be found on the skin and mucous membranes of healthy persons, most frequently in the nasal area. It can also cause infections of different severity, from minor illnesses to potentially fatal situations. *S. aureus* most frequently manifests clinically as skin and soft tissue infections (SSTI).[2]

Nearly half of all deaths attributed to antibiotic-resistant organisms are caused by the most hazardous microbe, methicillin-resistant *S. aureus* (MRSA).[3] The two main causes are the frequent use of broad-spectrum antibiotics and prior nasal surgeries.[4]

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Penicillin-binding protein, a modified protein that confers resistance, is encoded by the *mecA* gene and is found in mobile genetic elements on the staphylococcal cassette chromosome (SCC).[3]

A range of virulence factors and resistance determinants are encoded on this cassette (SCC). Characteristic DR sequences that characterize the transferable unit are located on either side of this cassette. SCC composite islands (SCC-CIs) are created when two or more SCCs are

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introduced side by side in specific strains.[5] The cassette chromosomal recombinase gene (CCR), which is present on each SCC, catalyzes the excision and integration of both individual SCCs and/or SCC-CIs.

The best SCC to describe is SCCmec. It has β-lactam antibiotic resistance and carries the *mecA* gene. The SCCmec components are very different. There are presently 13 recognized SCCmec types (I–XIII), according to the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements, based on the combinations of five mec complexes (A, B, C1, C2, and E) and nine *CCR* gene complexes (types 1–9).[6]

Additionally, *S. aureus* is capable of producing a variety of virulence factors, such as toxins like panton–valentine leucocidin (PVL), a pore-forming toxin consisting of two components that cause leukocyte destruction, tissue necrosis, and apoptosis.[7] This gene is carried by a temperate bacteriophage, and it can spread horizontally and from one isolate to another through phage transduction. These isolates typically contain this toxin, which is a persistent hallmark of community-acquired MRSA and infrequently appears in hospital isolates.[8]

The diagnosis was made in the outpatient setting or by a positive culture within 48 h of admission to the hospital/clinic, as per the Centers for Disease Control and Prevention.[9,10] These community isolates were separated from hospital strains. The patient has neither a previous history of MRSA infection nor colonization, nor has there been any hospitalization within the last year.

Materials and Methods

Specimen collections

A total of 100 samples were collected from different skin infections, burns, wounds, impetigo, boils, acne, abscesses, folliculitis, and infected atopic dermatitis, from patients who attended Al-Imam Al-Sadiq Hospital, Al-Hilla Teaching Hospital, and a private clinic during the period from July to November 2022. The collected swabs were streaked on mannitol salt agar and then incubated at 37C°for 24 h. The suspected colonies, depending on the morphological bases, were selected for identification by conventional methods and the Vitek 2 system.

Phenotypic detection of methicillin-resistant *S. aureus*

Before testing, all isolates (24) were grown on blood agar. After 18–24 h of incubation on a blood agar plate, a bacterial suspension equal to a 0.5 McFarland standard was created. As directed by the manufacturer, Vitek 2-AST-P592 cards (bioMe´rieux, Marcy l'Etoile, France) were inoculated. The VITEK-2 system software was used to interpret the isolates' antibiotic susceptibility pattern, screening for isolates with cefoxitin and oxacillin was carried out, and MIC for oxacillin ≥4 μg/mL was regarded as MRSA Clinical and Laboratory Standards Institute (CLSI) 2022 Guidelines.[11] If cefoxitin screening was negative and oxacillin MIC ≤2 μg/mL, it was regarded as MSSA CLSI 2022.[11]

Molecular Detection of Community-Acquired Methicillin-Resistant Staphylococcus Aureus Isolates

Identification of *SCCmecIV* gene by polymerase chain reaction

Molecular identification of *S .aureus* and MRSA isolates by polymerase chain reaction (PCR) using *16sRNA* and *mec*A was described previously.[12] In this research, the *SCCmec* type IV gene of MRSA was detected to confirm community-acquired methicillin-resistant *S. aureus* (CA-MRSA) isolates using a rapid PCR technique. The GoTaq® Green master Mix (2×) Promega, 12.5 μL, 7 μL of DNA template, 2 μL from forward and reverse primers, and 1.5 μL of nuclease-free water were added to create the PCR mixture. The gene *pvl* and *SCCmec* type IV genes were used for molecular verification as CA-MRSA[13] with the primer F-TTTGAATGCCCTCCATGAATAAAAT and R-AGAAAAGATAGAAGTTCGAAAGA, which amplify at 458 base pairs. The thermocycler programme conditions, initial denaturation at 94°C for 5 min; 35 cycles of amplification, denaturation at 94°C for 1 min; annealing at 55°C for 1 min; extension at 72°C for 1.5 min; and a final extension at 72°C for 1.5 min. Gel electrophoresis was utilized to identify the product by running a 100 bp ladder in 1% agarose gel for 1 h at 50 V.

Sequence analysis

For DNA sequencing, next-generation sequencing has been used. Specific *pvl* and SCC*mecA* genes PCR products were confirmed by sequencing. Specimens were sent to Macrogen Company in Korea for DNA sequencing.

Ethical approval

The Declaration of Helsinki's ethical guidelines, which guided the study's conduct, were followed. Before the sample was taken, it was done with the patient's verbal and written consent. According to the document number, a local ethics commission evaluated and approved the study protocol, subject information, and permission form on June 22, 2022.

Results

A total of 100 MRSA isolates from various skin infections were included in this study. Twenty (100%) recovered *S. aureus* isolates, of which 6 (30%) isolates came from impetigo, 9 (45%) isolates from burn, 4 (20%) isolates from wound, 1 (5%) isolate from boil, 0 (0%) isolate from acne, 0 (0%) isolates from secondary infection, 0 (0%) isolate from folliculitis, 0 (0%) isolate from abscess, and 0 (0%) isolate from infected atopic

dermatitis. The percentage of bacterial growth, which was isolated [Table 1].

Detection of methicillin-resistant *S. aureus* phenotypically

Phenotypic detection of MRSA was performed using the cefoxitin and oxacillin screening test through the Viteck2 system. The results of this study demonstrated that all of the isolates, 20 (100%), were resistant to cefoxitin and oxacillin.

Table 1: Number and percentage of *Staphylococcus aureus* isolated from skin infection

Sample source	Total no.	*S. aureus* isolates no.	Percentage (%)
Impetigo	25	6	30
Burn	30	9	45
Wound	21	4	20
Boil	12	1	5
Acne	2	0	0
Abscess	2	0	0
Secondary infection	2	0	0
Infected atopic dermatitis	3	0	0
Folliculitis	3	0	0
Total number	100	20	100

Polymerase chain reaction assay for detection of the *16s rRNA* gene

From the total of 24 *S. aureus* isolates of the patients, only 20 isolates (83.33%) were found to be positive for the *16sRNA* gene is shown in Figure 1.

Detection of *mecA* and *SCCmec* type IV genes by polymerase chain reaction

In this study, monoplex PCR was used for the *mec*A gene determination from a total of 20 *S. aureus* isolates of the patient. The results showed that 16 isolates were MRSA (80%) and 4 isolates were MSSA (20%), as shown in Figure 2.

According to *SCCmec* type IV genes in this study, the results showed that 11 (55%) out of 20 isolates harbored *SCCmec* type IV genes, as shown in Figure 3.

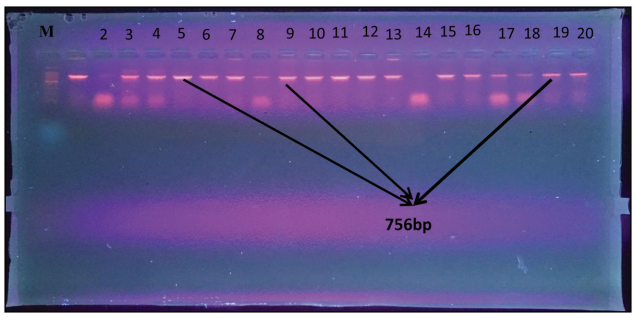

Figure 1: Agarose gel electrophoresis (1% agarose, 50 V/cm, for 1 h) for *16sRNA* gene (amplified size 756 bp) compared with (100 bp) DNA ladder. Lanes 1–13 and 15–20 gave positive results for the *16sRNA* gene, whereas lanes 2 and 14 gave negative results for the *16sRNA* gene

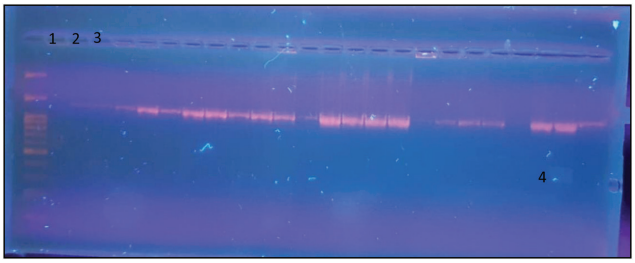

Figure 2: Agarose gel electrophoresis (1% agarose, 50 V/cm, for 1 h) for the *mecA* gene (amplified size 1333 bp) compared with a 100 bp DNA ladder. Lanes 3–11, 13, and 15–20 gave positive results for the *mecA* gene, whereas lanes 1, 2, 14, 19, and 20 gave negative results for the *mecA* gene.

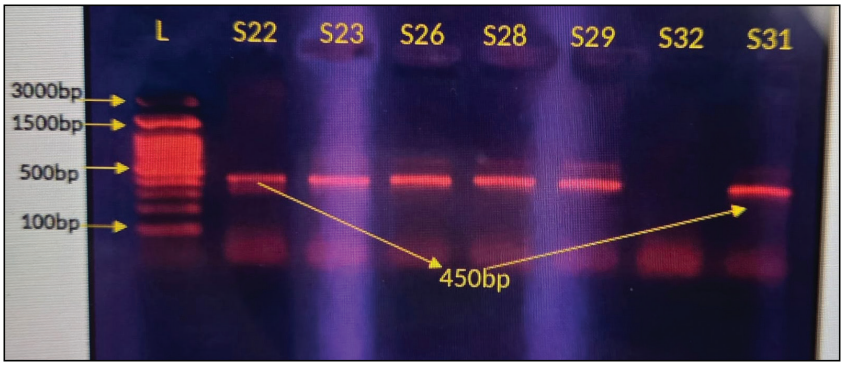

Figure 3: Gel electrophoresis of PCR of SCCmec IV amplicon product: Lane L: Ladder (3000-bp ladder), Lane (22, 23, 26, 28, 29, 31) no. of positive isolates from skin, Lane (S32) shows negative result

Molecular identification of the *pvl* gene in MRSA using polymerase chain reaction

All 20 MRSA isolates were tested, and the leukocidin *pvl* gene was identified. However, 11 (55%) isolates showed the *pvl* gene. About 9 (45%) isolates lacked this gene, as shown in Figure 4.

Sequence analysis of *pvl* and *SCCmecA* type IV genes of community-acquired methicillin-resistant *S. aureus* isolates

The results of PCR were subjected to DNA sequencing, and the nucleotide sequences and their close relations with other worldwide strains were analyzed. The test castoff to approve was using National Center for Biotechnology Information (NCBI)-Blast-query nucleotide-online, which was a program that produced the exact percentage of identified results, extended from 98% to 99%. In this study, genotypic variations in *pvl* and *SCCmecA* type IV within 10 isolates [Figures 5 and 6] were studied for the first time in Iraq. CA-MRSA evolutionary genetic relationships were examined by comparative sequence analysis using the NCBI database.

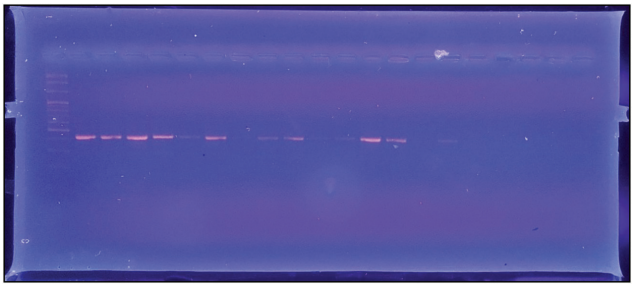

Figure 4: 1% Agarose gel electrophoresis (50 V/cm, for 1 h) for the *pvl* gene (433 bp) compared with 100 bp DNA ladder. Lanes 3, 4, 5, 6, 8, 9, 13, and 15 gave positive results for the *pvl* gene, whereas lanes 5, 7, 10, 11, 14, 16, 17, 18, 19, and 20 gave negative results for the *pvl* gene.

Discussion

By using culture traits, colony morphology, Gram's stain, and the Vitek2 method (cefoxitin and oxacillin screening test) of study results, 20 out of 100 isolates were identified as *S. aureus*. Modern molecular detection offers good MRSA detection results. The presence of 16sRNA in 20 isolates with genotypically verified MRSA status. The previous study showed approximately the same resistance results at 95.4%, 77.1%, and 92.7%, respectively, according to Hasan and Ismael.[11] The study by Fayyadh[14] reported low resistance rates toward cefoxitin (57%) and oxacillin (59.1), respectively, but Ahrabi *et al.*[15] reported a low resistance rate (18.3%) against cefoxitin. Regarding the resistance to oxacillin result, it was consistent with a study conducted by Al-Musawi *et al.*[16] who reported that 100% resistance to oxacillin by *S. aureus* clinical isolates from hospitals in Babylon, Iraq, but more than the result obtained by Al-khudheiri[17] and Iqbal *et al.*[13] determined that the oxacillin resistance was 32.2% and 22.5%, respectively.

MRSA strains are no longer just found in hospitals; they can also be found in apparently healthy communities. This new pathogen, community-acquired MRSA, mostly

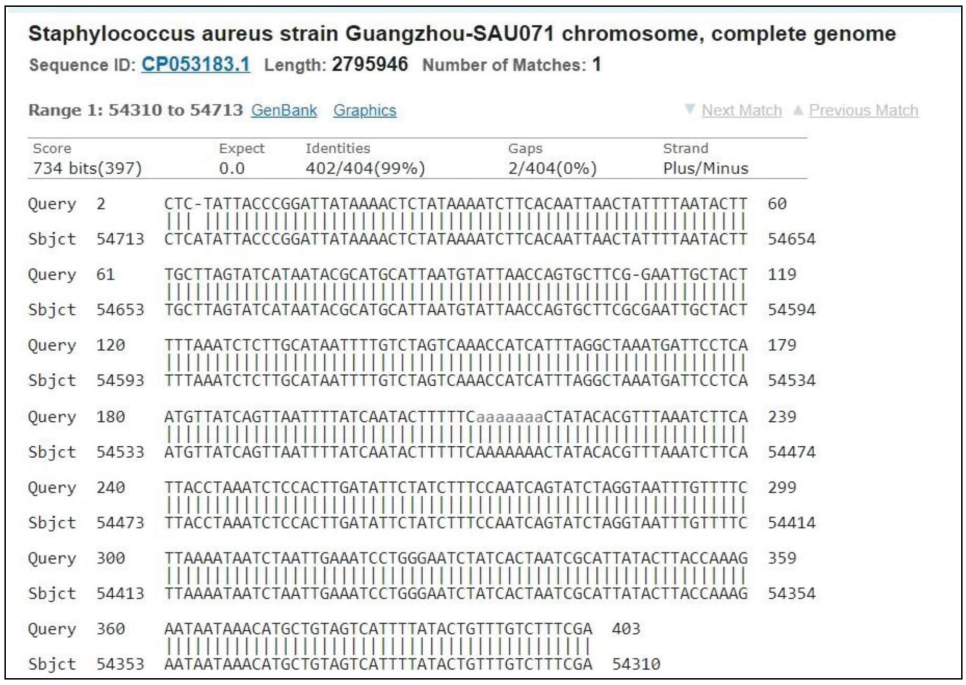

Figure 5: Multiple sequence alignment analysis of *SCCmecA* type IV gene partial sequence for local *S. aureus* (Guangzhou-SAU071). With NCBI-Blast of *S. aureus* isolates. The Clustalw alignment tool in MEGA 6.0 version was used to show that the nucleotide alignment similarity was * with different *S. aureus* isolates

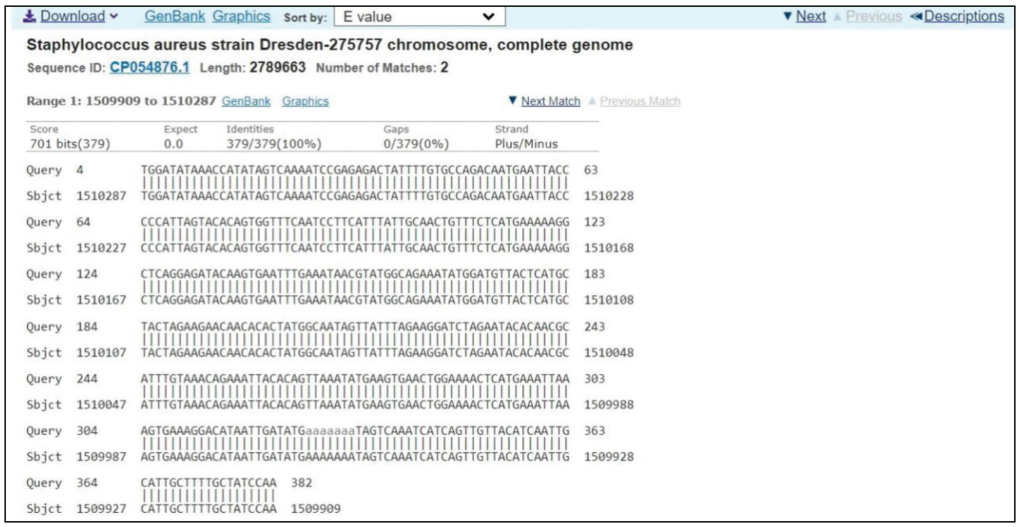

Figure 6: Multiple sequence alignment analysis of *the pvl* gene partial sequence for local *S. aureus* (Dresden-275757), with NCBI-Blast of *S. aureus* (isolates NCBI BLAST Online). The multiple alignment analysis was constructed using the Clustalw alignment tool in MEGA 6.0 version, which showed that the nucleotide alignment similarity was * with different *S. aureus* isolates

causes SSTI. According to the report, 25.0% of urinary tract infections were caused by CA-MRSA, which was found in the community.[18]

Molecular detection pattern revealed from a total of 20 *S. aureus* isolates of patients, the results showed 16 isolates were MRSA (80%), whereas 4 isolates were MSSA (20%). The results of this study are contradictory to those of Sawsan *et al.*[19] found that 21.62% of *S. aureus* isolates in the local investigation were MRSA isolates; these findings were consistent with Al-Hassnawi,[20] who found that 74.32% of *S. aureus* isolates were MRSA. The high frequency of MRSA among Iraqi hospital patients showed a growth in the prevalence and outbreak of MRSA isolates, which is one of the key factors contributing to the number of patients exceeding the hospital's capacity.

Out of the 20 isolates, the *SCCmec* type IV element was detected in 11 (55%) isolates. The results of this study is low the results obtained by Milheiriço *et al.*[21] revealed that the percentage of *SCCmec* type IV is 95%. The smallest structural kind of *SCCmec* type IV is thought to be the most migratory type and is connected to CA-MRSA infections.[2,22] Many researchers have made the PVL toxin's involvement in *S. aureus*' virulence famous, as one of the causes of the strains' high pathogenicity in the population is one of the key traits of CA-MRSA and a clinically practical virulence marker.[23] In recent years, nosocomial PVL-positive MRSA transmissions and outbreaks have become more frequent in Europe,[24,25] showing that the boundary between MRSA associated with hospitals and the community has already been crossed. Despite the association between PVL presence and illness severity. Explain the origin of the high pvl gene rate due to the inclusion of purulent skin infections (boils and abscesses) in the current study, given the strong link between this gene and suppurative infections. The present discovery (55%) was consistent with findings of Al-Mohana *et al.*,[26] who found the *pvl* gene in 27.2%26 of CA-MRSA isolates. However, there is a significant association (85%) between the *pvl* gene and cutaneous infections, as disclosed in a study by Lina *et al.*[27]

S. aureus (including drug-resistant strains such as MRSA) is found on the skin and mucous membranes, and humans are the major reservoir for these organisms. It is estimated that up to half of all adults are colonized, and approximately 15% of the population persistently carry *S. aureus* in the anterior nares.[28] Some populations tend to have higher rates of *S. aureus* colonization (up to 80%), such as healthcare workers, persons who use needles regularly (i.e., diabetics and intravenous [IV] drug users), hospitalized patients, and immunocompromised individuals. *S. aureus* can be transmitted person-to-person by direct contact or by fomites.[29]

Conclusion

This study revealed high *S. aureus* isolates in burns, followed by impetigo, wounds, and boils, respectively. This extremely important virus, CA-MRSA, hitherto believed to be exclusive to the hospital setting, is now also being isolated from community settings. The majority of the CA-MRSA carry the *pvl* gene and have the easily transferable tiny mobile *SCCmec* elements IV. This study emphasizes *mecA*, *pvl*, and *SCCmecA* type IV of *S. aureus* isolated from skin infection appropriate PCR-based strategy (identification of *pvl*, *mecA*, and *SCCmec* elements) to be used in epidemiology, is necessary for determining MRSA isolates and controlling HA-MRSA and CA-MRSA infections.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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