The phenotypic and genetic characterization of some virulence factors in MRSA isolated from burn patients

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Abstract. The present study aimed to the phenotypic and genetic detection of some virulence factors in Methicillin resistant Staphylococcus aureus (MRSA) isolated from burn patients. From a total of 38 isolates of Staphylococcus aureus, 15 (39.4%) isolates of MRSA were collected from the patients in Diwaniyah Teaching Hospital during the period from June to October, 2018 in Al-Diwaniyah City. Methicillin resistant Staphylococcus aureus (MRSA) was detected by using chrom agar MRSA and polymerase chain reaction (PCR), 15 isolates (39.4%) reported resistance to methicillin antibiotic. In addition, phenotypic detection of the ability of MRSA to produce the β-lactamase enzymes using rapid iodometric method and its ability to form the biofilm was determined by using the tube method. The results showed 15 (100%) MRSA isolates were able to produce β-lactamase enzymes and 14 (93.3%) MRSA isolates were able to biofilm production. On the other hand, All MRSA isolates were tested by using Polymerase Chain Reaction (PCR) to detection of some virulence factors genes, which include coa gene encoding production of coagulase, icaA gene encoding for biofilm production and blaZ gene encoding for the β-lactamase enzymes. PCR results showed that MRSA isolates were possess blaZ gene (100%), icaA gene (93.3%) and coa gene (86.6%).

Key words: MRSA, PCR, virulence factors, blaZ gene, coa gene, icaA gene.

Introduction

S. aureus is one of the most important human pathogens and it is responsible for a wide range of diseases such as boils, various abscesses, surgical wound abscesses, dermatitis and soft tissue, osteoporosis, joint, bronchial pneumonia, inflammation of the internal parts of the heart and Food Poisoning (1). S. aureus are different from other Staphylococcus sp because they are positive for Coagulase test (2). S. aureus is defined as spherical cells, gram positive, nonmotil, non-sporforming, usually capsulated and aerobic or anaerobic. They have the ability to decompose blood on the blood agar and also they have large yellow colonies on mannitol salt agar (3). S. aureus have many virulence factors, such as Toxins and Enzymes, that help the bacteria to cause infection (4). It has the ability to produce many of the extracellular enzymes and toxins, such as the coagulase enzyme responsible to inhibit the phagocytic process, and it has the ability to produce other enzymes that represent Spreading factors such as protease and lipase. Which contributes to the bacterial invasion of tissues, infection, as well as production of gastrointestinal toxins, and also has the ability to produce exotoxins in cases of squamous skin syndrome (3) and it possess a capsual that helps the bacteria to resist phagocytosis (5). In addition to having the cell wall, which is a protective
compound because of containing peptideoclan and protein A, the cell wall acts as a resistance to the host’s immune system (6).

Some strains of *S. aureus* have the mecA gene responsible for their resistance to the fixed penicillin group as a result of their encoding of penicillin binding proteins (PBPs), which reduce intimacy with the methicillin antibiotic which Called Methicillin-resistant Staphylococcus aureus (MRSA), which is primarily localized in hospitals [HA-MRSA] [Hospital acquired-MRSA]. Its resistance to many antibiotics such as its resistance to all types of β-lactam antibiotics, and many other antibiotics (7). Penicillin has been one of the most effective antibiotics to treatment the staphylococcus aureus but some of strains have begin to resist this antibiotics by producing β - Lactamase, which inhibits penicillin. β-lactamase enzymes are important enzymes responsible for the resistance of the bacteria to β-lactam antibiotics (8).

For all reasons mentioned above, the aim of this study was to isolation and diagnosis of MRSA from burns then the detection for the virulence factors genes (*blaZ*, *icaA*, *coa*) in MRSA by using the PCR.

**Materials and Methods**

**Samples collection**

from June to October 2018, a total of 200 Samples were collected from the burn patients in burn unit of the Diwaniyah Teaching Hospital of AL-Diwaniyah City. All the samples were transferred to the laboratory using cotton swabs.

**Isolation and identification**

*S. aureus* were isolated and identified according to (9),(10). include by cultivated on blood agar and mannitol salt agar. The growth of the colonies is observed after incubated at 37ºC for 24-48 hours. Colonies of *S. aureus* were diagnosed on mannitol salt agar as large yellow colonies. The identification was confirmed by Coagulase test (11).

**Phenotypic investigation of MRSA**

MRSA was detected by using the CHROMagar MRSA, which is an selective media for isolating and identification MRAS strains based on the color.

**Production of β - Lactamase enzymes**

The rapid iodometric method was used to detect the ability of MRSA to produce the β - Lactamase enzymes according to the following steps:

Transfer the MRSA colonies that grew in Brain-Heart infusion broth to the microplate containing 100 μl of penicillin G solution in each hole, mix well then incubated at 37º C for 30 minutes. add 50 μl of starch solution then add 20 μl of iodine solution. Blue is obtained as a result of iodine interaction with starch and rapid color change from blue to white within 5 minutes is a positive result (10).

**Biofilm formation test**

MRSA are grown in Tryptic soy broth+ 1% glucose, then incubated at 37 º C for 24 hours. Remove the suspension, wash the tubes with the normal saline solution, and staining with the crystal violet for 3 minutes, then wash with distilled water, then inverted the tubes and leave to dry. Note the formation of the Biofilm in the bottom and the inner walls of the tube as violet layer (12).

**Molecular Identification**

Polymerase chain reaction was performed to confirm the identification of MRSA (13).PCR was conducted to detect of *MecA* gene and also to detect of virulence factors genes in MRSA by using specific primers were provided by Bioneer Company, Korea ( table 1).
Table (1): DNA primers

| genes     | Primer Sequences (3'-5') | Length |
|-----------|--------------------------|--------|
| blaZ gene | ACAGTTCACATGCCAAAGAGTF   | 477 bp |
|           | TACCGAAAGCAGCAGGTGGTR    |        |
| icaA gene | TCGTTGATCAAGATGCACCAGTF  | 305 bp |
|           | GCGTTGCTTCAAAGACCTGTR    |        |
| Coa gene  | GCTTGGTGCGGATAAACTGTF    | 280 bp |
|           | AGCAGTTAAGAGCAGACGTR     |        |
| MecA      | TCTTGGGTTGGTACACAGTF     | 541 bp |
|           | ACCACCCAATTCTGCTGCCA     |        |

Genomic DNA was extracted from MRSA isolates by using Genomic DNA Mini Kit. PCR master mix reaction was prepared according to kit instructions (AccuPower® PCR PreMix kit. Bioneer. Korea) in 20 μl total volume by added 5 μl of purified genomic DNA and 1.5 μl of 10 pmole of forward primer and 1.5 μl of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20 μl. The reaction was performed in a thermocycler by set up the following thermocycler conditions; initial denaturation temperature of 95 °C for 5 min; followed by 30 cycles at denaturation 95 °C for 30 s, annealing 58°C for 30 s, and extension 72 °C for 1min and then final extension at 72 °C for 5 min. The PCR products were tested by electrophoresis in a 1.5% agarose gel, and visualized under UV illumination.

Results and discussion

Isolation and diagnosis of S. aureus

From a total of 200 burn specimens, 38 (19%) S. aureus isolates were identified by bacteriological and biochemical tests. S. aureus appeared on mannitol salt agar with yellow colonies. The results of the biochemical tests are summarized in Table (2) Where they identical to the results of (10),(11),(15),(16).

The higher percentage of S. aureus in burns patients is due to a many of reasons, including the lack defense mechanisms of patients and as a result of the presence of S. aureus normal flora in the body and this is confirmed by (17), as well as being one of the main causes of Nosocomial infection.

Table (2): Biochemical tests to the diagnosis of S. aureus.

| Biochemical tests         | Results |
|---------------------------|---------|
| Catalase test             | +       |
| Oxidase test              | -       |
| Coagulase test            | +       |
| Clumping Factor test      | +       |
| DNase test                | +       |
| Voges Proskauer test      | +       |
| Gelatinase test           | +       |
| Urease test               | +       |
| Mannitol Fermentation test| +       |

Phenotypic and Molecular detection of MRSA

The phenotypic investigation of MRSA was performed through cultured 38 isolates of S.aureus on CHROM agar MRSA, 15 (39.4%) MRSA isolates were showed their ability the resistance to Methicillin and growth on CHROM agar MRSA and it was characterized by pink colonies, while CHROMagar MRSA inhibits the growth of Methicillin Susceptible Staphylococcus aureus (MSSA).
CHROMagar MRSA is an selective media for isolating and differentiate MRSA strains (18). All isolates of S.aureus were tested to confirm the presence of mecA (resistance to methicillin) using PCR. The results demonstrated that 15 isolates (39.4%) have mecA (Figure 1), therefore the Bacteria were considered as MRSA. The prevalence of MRSA in present study different with other studies in Iraq, which recorded percentages of (65.3%), (88%) and (75%) (19,20,21) respectively.

![Agarose gel electrophoretic results](image1)

**Figure (1):** Agarose gel electrophoresis, which show the PCR product results for S.aureus of mecA gene at 541 bp PCR product size, where M: Marker(100 -2000) bp, Lane (1-5,7-8,10-11, 14-17) are positive samples.

**Molecular and phenotypic detection of some virulence factors in MRSA**

The rapid iodometric method was used to phenotypic investigation of the ability 15 MRSA isolates to produce of β - Lactamase enzymes, The results obtained reported that 15 (100%) isolates have β - Lactamase enzymes( Figure 2) , Production of the β - Lactamase enzymes in S. aureus due to use of β-lactam antibiotics which may promote the production of β - Lactamase enzymes (22). The tube method was also used to investigate the MRSA's ability to Biofilm formation, where 14(93.3%) isolates demonstrated their ability to form the Biofilm. The formation of Biofilm is one of the virulence factors, which act as bacterial protect against host defenses and treatment with antibiotics and also increases the ability of Bacteria to adhesion to living surfaces (23). The ability of MRSA to secrete coagulase was diagnosed using the slide method, 13(86.6%) isolates showed their ability to produce the enzyme.

![Bar chart](image2)

**Figure 2:** Percentage of some virulence factors in MRSA isolated from burns
On the other hand, PCR technique was used to investigate some of the virulence factors genes in 15 isolates of MRSA (24). They included coa gene responsible for the production of coagulase enzyme, icaA gene responsible for producing the Biofilm and blaZ gene responsible for the production of β-Lactamase enzymes. All isolates were showed positive results to the blaZ gene (Figure 3), icaA gene(Figure 4) and coa gene(Figure 5), where Percentage to each gene is (100%,93.3%,86.6%) respectively. Current study results agreed with study(25) , who showed that all MRSA (100%) have blaZ gene.

![Figure 3](image1.png)
**Figure (3)**: Agarose gel electrophosis, which show the PCR product results for MRSA of **blaZ** gene at 477 bp PCR product size, where M: Marker(100 -2000) bp, Lane (1-13) are positive samples.

![Figure 4](image2.png)
**Figure (4)**: Agarose gel electrophosis, which show the PCR product results for MRSA of **icaA** gene at 305 bp PCR product size, where M: Marker(100 -2000) bp, Lane (1-14) are positive samples.

![Figure 5](image3.png)
**Figure (5)**: Agarose gel electrophosis, which show the PCR product results for MRSA of **coa** gene at 280 bp PCR product size, where M: Marker(100 -2000) bp, Lane (1-13) are positive samples.

**Conclusions:**
The present study demonstrated prevalence of MRSA strains in burns, their resistance to antibiotics, especially β-lactam antibiotics compared with MSSA and dominance of blaZ, coa and icaA genes in all MRSA isolates.
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