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# Using of *mecA* Gene as Genetic Marker for *Staphylococcus aureus* Detection Isolated from Atopic Dermatitis Patients

**[1]Nardeen A. Qalawlus, [1]Zainab H. Abood**

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

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**Abstract:** Atopic dermatitis (AD), also identified as atopic eczema, is a long-term type of inflammation of the skin (dermatitis). The symptoms is red, swollen, itchy, and cracked skin, environmental factors such as emotions, sweating and exercise in addition to microbiological factors such as *Staphylococcus aureus* skin colonization which usually wide spread in these patients play an important role in the disease. The aim of the study: The present study was aimed to find the probable microorganism reasons that cause Atopic dermatitis (AD) in Iraqi individuals. During the period of study from beginning December 2022 to March 2023, A total of eighty swab specimens were collected from atopic dermatitis patients attending Medicine City/Baghdad Teaching Hospital and private laboratory. The culture results revealed 20 isolates to the *S. aureus* bacteria depend on culture characteristic, While the conformation by Vitek2 system showed that 20 isolates belonged to the genus *S. aureus.* It was concluded *Staphylococcus aureus* is frequently detected in patients with AD, and *mecA* gene appeared to be useful genetic marker for determination of *S. aureus* and PCR using species-specific primers could be represented rapid, sensitive and specific molecular method for detection of this bacteria in different AD patient.

**Keywords :** *S. aureus* , atopic dermatitis, *mecA* gene

**Corresponding author** (Email: zainabhekmatt@gmail.com, nardeenabdulsattar@gmail .com).

## Introduction

Atopic dermatitis also known as atopic eczema, is a common, familial, chronic inflammatory skin disease, identified by xerosis (increased water loss through the skin), pruritus, erythematous, scaly skin lesions, and high serum IgE levels. It affects between 10 to 20% of children and 1 to 3% of adults worldwide and has adverse medical, financial and social impacts on the patients and their families. 85% of affected children develop the disease before the age of 5 years (60% before the age of 1). Although many eventually grow out of the condition (improvement during puberty is a common phenomenon), it may persist into or manifest for the first time in adulthood (1, 2). There are at least two types of AD: extrinsic and intrinsic, 20- 30% of AD patients represent with normal serum IgE at least initially and are classified as “intrinsic AD” while 70-80% of AD patients represent with a high serum IgE level and classified as “extrinsic AD” (3). AD is part of a family of T-helper-2 (Th-2) driven atopic diseases and is often the first of these disorders to manifest itself. 50% of patients with childhood AD develop asthma and two-thirds develop allergic rhinitis at a later stage. This phenomenon is known as the ‘atopic

march'. Patients with AD are also more likely to develop irritant and allergic contact dermatitis than the general population (4).

The AD is an important skin condition as Significant morbidity may occur in untreated cases such as sleep disorders, scars from picking and scratching, post inflammatory skin changes and secondary skin infections due to bacterial invasion from skin flora particularly *Streptococcus*, *Staphylococcus* and herpes species infections known as eczema herpeticum or Kaposi varicelliform eruption (5).

The word 'atopy' was introduced by Coca and Cooke in 1923, referring to an abnormal level of sensitiveness, the mechanism of which is uncertain. It originates from the Greek word 'atopia' which means 'unusualness or strangeness'. The original definition 'atopy' by Coca and Cooke included only asthma and allergic rhinitis; AD was later added onto the definition. However, the cause of the disease has remained somewhat elusive (6).

The pathogenesis of AD is complicated and still poorly understood, although it is a result of the interaction between genetic predisposition and environmental factors such as immunologic responses, a shift in skin micro-biome, nutritional, and psychological factors (**7**). *Staphylococcus aureus* may be an exacerbating factor of AD .The prevalence of *S. aureus* carriage by patients with AD was 70% on lesional skin. The rate of *S. aureus* colonization was related to disease severity (8).

## Materials and methods

### Samples collection

During the period of study from beginning December 2022 to March 2023, A total of eighty swab specimens were collected from atopic dermatitis patients attending Medicine City/Baghdad Teaching Hospital and private laboratory and transferred to research laboratory**.** All specimen were cultured on ( Blood agar, and mannitol). The culture results revealed 20 isolates to the *S. aureus* bacteria depend on culture characteristic, while the conformation by Vitek2 system showed that 20 isolates belonged to the genus *S. aureus*. Final identification tests included the morphological ,culture characteristics according to colonial morphology, size, shape direct exam by Gram stain under light microscope (40x)followed by biochemical tests and Molecular detection.

### DNA extraction

Genomic DNA was extracted from the *S.aureus* isolates using a commercial wizard genomic DNA purification kit according to manufacturer's instructions (Promega, USA) with some modification. Briefly, 1 ml of an overnight *S.aureus* culture grown at 28°C in nutrient broth (Sigma, USA) was transferred to a 1.5 ml micro centrifuge tube. The microcentrifuge tube was centrifuged at 14,000 rpm for 3 minutes to pellet the cells and the supernatant was removed. 600 µl of nuclei lysis solution (wizard genomic DNA purification kit) was added and gently pipet until the cells is resuspended. The microcentrifuge tube was incubated in water bath at 80°C for 5 minutes to lyse the cells; then cool to room temperature. 3 µl of RNase solution (wizard genomic DNA purification kit) was added to the cell lysate and the microcentrifuge tube was inverted for 5 times to mix. The microcentrifuge tube was incubated at 37°C for 60 minutes and cool to room temperature. 200 µl of protein precipitation solution (wizard genomic DNA purification kit) was added to the RNase-treated cell lysate and vortex

vigorously at high speed for 20 seconds to mix the protein precipitation solution with the cell lysate. The microcentrifuge tube was incubated on ice for 5 minutes and centrifuged at 14,000 rpm for 5 minutes. The supernatant containing the DNA was transferred to a clean 1.5ml microcentrifuge tube containing 600 µl of room temperature isopropanol. The microcentrifuge tube was gently mixed by inversion until the thread-like strands of DNA form a visible mass and centrifuged at 14,000 rpm for 5 minutes.

The supernatant was carefully pour off and the microcentrifuge tube was drained on clean absorbent paper. 600 µl of room temperature 70% ethanol was added and then the microcentrifuge tube was gently inverted several times to wash the DNA pellet. The microcentrifuge tube was centrifuged at 14,000 rpm for 2 minutes and the ethanol was carefully aspirated. The microcentrifuge tube tube was drained on clean absorbent paper and the pellet was allowed to air-dry for 15 minutes. 100 µl of DNA rehydration solution (wizard genomic DNA purification kit) was added to the microcentrifuge tube and the DNA was rehydrated by incubating at 65°C for 1 hour. The solution was periodically mixed by gently tapping the microcentrifuge tube and the DNA sample was stored at - 20°C until use.

**DNA quantification**

The extracted DNA from the *S.aureus* isolates was quantified spectrophotometrically at O.D. 260/ 280 nm with ratios 1.4-1.5. The sensitivity of the *S.aureus* -F and *S.aureus* -R primers was evaluated by PCR amplification for serial diluted concentrations (10-100 ng) of purified genomic DNA isolated from *S.aureus.*

**Primers selection**

The primers for *mecA* gene of *S.aureus* as the target gene for this study were selected according to (9). This set of primers was designed based on the conserved region in *S.aureus*, primers were synthesized by Alpha DNA, Kanda. The primers sequence of *mecA* gene and their size of product are shown in (Table 1).

**Table(1): The primers sequences of *mecA* gene of *S.aureus* and their product size**

| Primer Name | Seq. | Tm | Size of product | Refernce |
|---|---|---|---|---|
| ***mecA*-F** | 5-AAAATCGATGGTAAAGGTTGGC'-3' | **53** | ~500bp | (9) |
| ***mecA*–R** | 5'-AGTTCTGCAGTACCGGATTTGC -3' | | | |

**PCR master mix**

The PCR reaction of *mecA* gene detection of *S.aureus* was performed in 25 µl volumes containing 5.5 µl of nuclease free water, 12.5 µl of GoTaq Green Master Mix 2X containing (GoTaq DNA polymerase supplied in 2X Green GoTaq reaction buffer (pH 8.5), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP, 3 mM MgCl2, yellow and blue dyes which function as loading dyes when reaction products are analyzed by agarose gel electrophoresis), 2.5 µl of 20 pmol *S.aureus* -F primer and 2.5 µl of 20 pmol *S.aureus* -R primer and 2 µl of the genomic DNA sample. The mixes were overlaid with 2 drops of mineral oil (Table 2).

**PCR program**

The PCR was carried out in a thermal cycler (Applied Biosystem, 9902, Singapore) according to the PCR program described, with some modification. Briefly, the amplification of *mec A1* gene of *A S.aureus* was carried out with initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for *S.aureus*

-F and *S.aureus* -R primers for 30 seconds, and extension at 72°C for 60 sec.

The thermal cycles were terminated by a final extension for 3 minutes at 72°C (Table 3).

**PCR products analysis**

The analysis of PCR products of *mecA* gene of *S.aureus* were performed on 1% agarose gel. The 100 bp DNA ladder (Promega, USA) was used and the gel was run at 100 volt for 45 minutes at room temperature. The PCR products were stained with ethidium bromide and visualized by an image analyzer (ChemiImager 5500, Alpha Innotech, USA).

**Table (2): PCR master mix to detect the *mecA* gene of *S.aureus* isolates.**

| Component | Concentration | Amount (µl ) |
|---|---|---|
| **GoTaq Green Master Mix** | 2X | 12.5 |
| ***mecA* -F primer** | 10 µM/ µl | 1 |
| ***mecA* -R primer** | 10 µM/ µl | 1 |
| **Nuclease free water** | - | 8.5 |
| **DNA sample** | - | 2 |
| **Total volume** | - | 25 |

**Table (3): PCR program to detect the *mecA* gene of *S.aureus* isolates.**

| No. | Step | Temperature | Time | No. of Cycles |
|---|---|---|---|---|
| **1** | **Initial denaturation** | 95°C | 5 min. | 1 |
| **2** | **Denaturation** | 95°C | 30 sec. | 30 |
| **3** | **Annealing** | 58°C | 30 sec. | |
| **4** | **Extension** | 72°C | 60 sec. | |
| **5** | **Final extension** | 72°C | 3 min. | 1 |
| **6** | **Storage** | 4°C | ∞ | - |

## Results and discussion

### Distribution of bacterial Isolates from atopic dermatitis

In the present study the most bacterial 80 isolates was *S.aureus* with percentage at 20(25%) followed by *S.epidermidis* with percentage 22(27.5%), while *E.coli* recorded 10 (12.5%), then *Proteus sp*. documented about 8(10%) , then yeast recorded 15with percentage (18.75%) and 5(6.25%) no. growth as shown in the (Table 4).

**Table (4):Distribution of Bacterial Isolates from Atopic dermatitis**

| No. Isolation | No.% *S. aureus* | No.% *S. epidermis* | No.% *E.coli* | No.% *Proteus* | No.% Fungi | No. % No. growth |
|---|---|---|---|---|---|---|
| 80 | 25 | 17.6 | 12.5 | 10 | 18.75 | 6.25 |

In the study by Al.Fahadawi *et al.*, (10) in Ramadi city-western of Iraq found gram-positive bacteria were the predominant agents companion to Eczema with rate 72.7%, while gram negative bacteria caused secondary infection associated with Eczema with low rate 27.3%.*S. epidermidis* (18, 40.9%) fallowed by *S. aureus* (14, 31.8%), *K. pneumonia* (7, 15.9%), *P. aeruginosa* (4, 9.1%) and *Proteus* spp. (1, 2.3%).The present study revealed that atopic dermatitis skin lesions seem to be specifically prone to *S.aureus* colonization. This comes in accordance with Di Domenico *et al.*, (11) who found 54.3% (44/81) of AD patients were colonized by *S.aureus*. In

addition(12), supported our findings and reported 55(51.8%)out of 106 AD patients harbouring *S. aureus* on their skin lesions. Less than our results, a study in Tehran carried out by Rezaei *et al.* (13) found in 38 AD patients only 18 patients were colonized by *staph aureus* (47%). This could be attributed to different patient's age group included in that study. Also, (14) had found that *staphylococci* were significantly less abundant in one-year-old affected infants in a study that included only 10 AD patients (8) found the most prevalent colonization in AD was (30.8%) followed by *S. epidermidis* (20.5%), and *S. lentus* (10.3%). Analysis of colonization according to AD severity, *S. epidermidis* (30.6%), *S. aureus* (25.5%), *S. lentus* (15.3%), *S. hominis* (15.3%), and Kocuria rosea (10.2%).

In the present study, the detected pathogens other than *S. aureus* colonizing the skin of AD patients; 33.3% of cultures showed fungi growth, 11.3% were *e.coli* ,( *Proteus sp* .were *registries* 4.6% were ,while 6% of cultures gave no growth. This finding goes with the fact declared by Buda *et al.* (15) who stated that there were changes in the microflora of the skin in atopic dermatitis with an increase in the incidence of the presence of CONS, *staph aureus, klebsiella,* and streptococci by Lateef *et al* ., (16) found among 172 specimens from wound swabs, 47(27.33%) isolate were identified as *S.aureus* according to the results of phenotypical assays

The prevalence of microbial colonization of AD is still significant and changed over time. However, the skin is reported to have a large number of normal flora life on the surface and inside the skin tissue like sweat glands and hair follicles. The skin microbiota is well known to have high diversity between young andadult healthy individuals (17).

Members of the normal mucous membrane flora, candida yeasts primarily interact with the immune system through the gastrointestinal tract and, in women, even the vagina (18).

**Identification of *staphylococcus aureus***

*Staphylococcus aureus* colonies were identified by a golden yellow color when culturing on mannitol salt agar media at 37˚C for 24 hrs as (Figure1), because fermentation mannitol aerobically and changed the color of media from red to yellow (19).

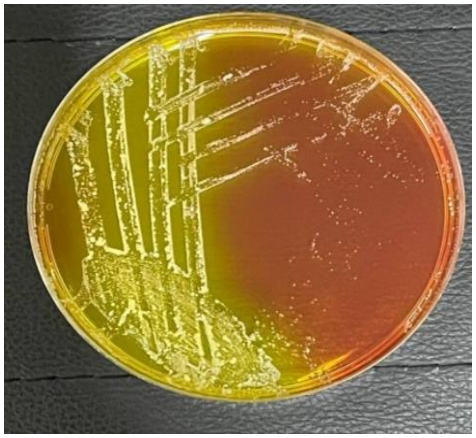

**Figure (1):Colonies morphology of *S.aureus* culture on mannitol salt agar medium at 37ºC for 24hrs.**

Whereas colonies appear when cultured on blood agar, as clear zone surrounding the colonies give a sign of blood cell lysis as (Figure2). Blood agar plates also have beta hemolysis. Beta hemolysis is a full red cell lysis in and under the media of a colony: the region becomes lightened (yellow) and clear, with the hemolysis being at times called complete hemolysis (20)

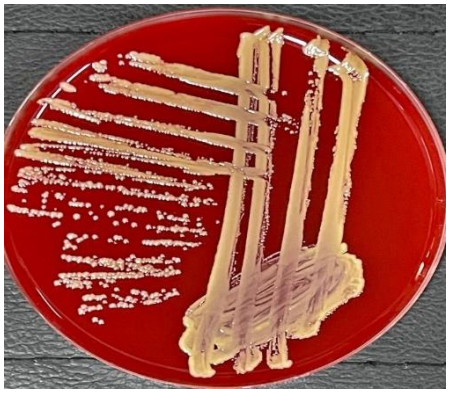

**Figure (2): Colonies morphology of *S.aureus* culture on blood agar medium at 37ºC for 24hrs.**

## Microscopic characteristics

*Staphylococcus* was examined microscopically and showed that it was Gram positive, round shaped bacterium, and form in grape like clusters as (Figure3), In a Gram stain test, bacteria retain the crystal violet stain, coloring all the Gram positive bacteria with a purpule color. This is because of theexistence of thick layer of peptidoglycan in the cell wall (21)

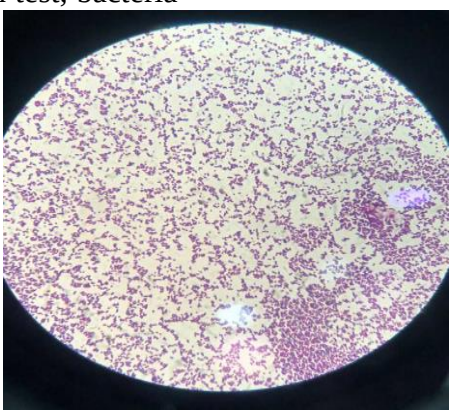

**Figure (3): Microscopic examination of *S.aureus***

## Biochemical tests

Biochemical tests were performed for the isolates, results indicated in table (5) showed positive result for coagulase (which convert fibrinogen in plasma to fibrin and cause clot formation and give positive results for catalase due to the ability to produce catalase enzyme as (catalase reduce hydrogen peroxide to water and oxygen gas bubbles).

The clinical isolates have positive results for urease, the enzyme was breakdown the urea (by hydrolysis) was alkaline and converted into pink red to supply ammonia or carbon dioxy with ammonia release. As well as *S. aureus* were negative for oxidase, which differentiates them from others *Micrococcus* genus as shown in table (5) (22).

**Table (5): The biochemical test and their results for *S.aureus* isolates.**

| Biochemical tests | Results |
|---|---|
| Gram stain | + |
| Catalase | + |
| Oxidase | - |
| Coagulase | + |
| Urease | + |

## Molecular diagnosis of *S.aureus*

### Extraction of genomic DNA

The DNA of *S.aureus* isolates were extracted by a commercial wizard genomic DNA purification kit (Promega,USA) and then analyzed by using gel electrophoresis by Abood *et al.*, (23).The DNA concentration and purity were measured by nanodrop,all the isolates had DNA concentration between (50-100 ng/µl) and purity of the DNA were (1.4- 2). The results of DNA extraction as figure (4).


**Figure (4): Gel electrophoresis of extracted genomic DNA of *S.aureus* isolates using 1% agarose gel at 7volt/cm for 1 hour. Lane 1-10: Extracted genomic DNA.**

## Molecular identification of *S.aureus* by detection *mecA* gene

In order to detect the presence of *mecA* and determination the prevalence of gene in *S.aureus* clinical isolates, polymerase chain reaction (PCR) for each DNA extracted sample have been used. The PCR reaction included 25 isolates for detection the *mecA* gene. The PCR products have been confirmed by analysis of the bands on gel electrophoresis. PCR products have been confirmed by comparing their molecular weight with 1500 bp DNA Ladder. The results of PCR reaction for *mecA gene* carrier in all isolates as (Figure 5).

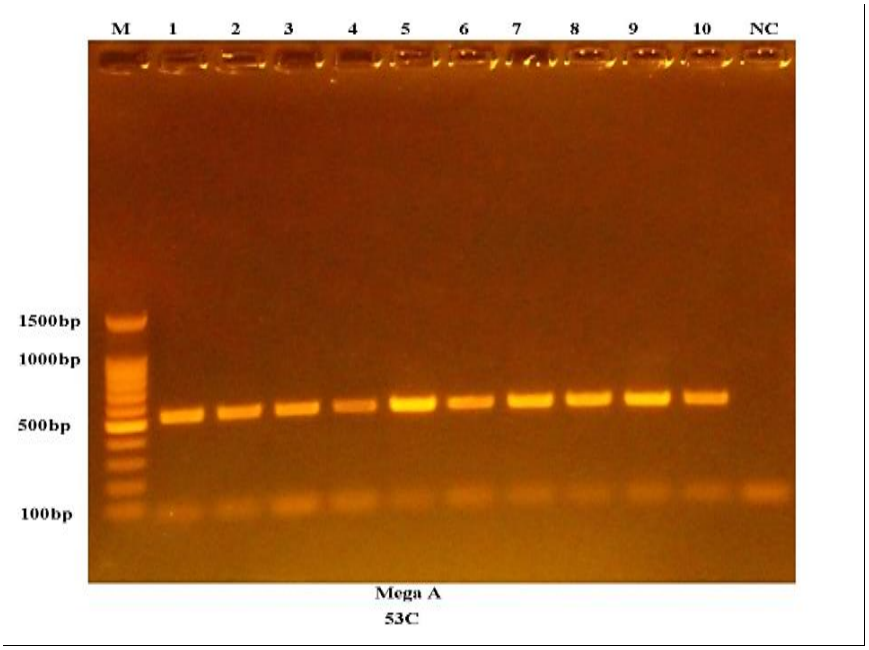


**Figure (5):Gel electrophoresis of PCR product of *S. aureus* isolates using 1.5% agarose gel at 7volt /cm for 1 hour. Lane 1: 100 bp DNA ladder, lane 2-10: PCR product of *mecA* gene**

All the positive isolates (137) that were characterized as MRSA by the (methicillin disc test) were subjected to PCR to detect the presence of *mecA* gene by Alagely *et al.,* (24). found that 38 (27.73%) isolates of MRSA out of 137 contained SCC*mec*I (23) who found that the *mecA* gene presented (45.1%) of *S. aureus* isolates, while another studies by Pournajaf *et al.,* (25) found this gene presented (90.1%) in *S. aureus* isolates and in 50.6 % by Schlingloff *et al* .,(26), in 56% by Neuroscience *et al.*(27), in (28), (29) and (30) reported the percentage were 44%, 55.5% and 42% respectively by Sherfi *et al.*, (31).The *mecA* gene is located within a large mobile genetic element known as the staphylococcal chromosomal cassette mec (SCCmec). Various SCCmec types are likely found in both community associated and hospital-acquired MRSA (32, 33).

## Conclusion

It was concluded *Staphylococcus aureus* is frequently detected in patients with AD, and *mecA* gene appeared to be useful genetic marker for determination of *S. aureus* and PCR using species-specific primers could be represented rapid, sensitive and specific molecular method for detection of this bacteria in different AD patient.

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