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Staphylococcus aureus Incidence in Some Patients with a Topic Dermatitis in Baghdad City

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Abstract: This study was planned to evaluate the incidence of *Staphylococcus aureus* in cases of atopic dermatitis in Iraq. This study included taking 150 samples of Atopic Dermatitis (AD) instances in total. Patients Suspected infected with *Staphylococcus aureus*, all isolates were subjected to primary identification tests by using various methods (cultural characteristic, gram staining, biochemical tests, vitek2 system, and molecular methods (PCR). Only 54 sample (36% isolates) were given the typical biochemical tests and characteristics of morphology that is specific to *staphylococcus aureus*, while the other 96 clinical (64 % isolates) belong to other species of staphylococci or other pathogenic bacteria and fungi. As these (54) samples contained the (*mecA*) gene, which is the diagnostic gene for the *staph aureus* bacteria in this study. It was concluded *Staphylococcus aureus* infection is positively correlated with atopic dermatitis cases.

Key words: *Staphylococcus aureus*, atopic dermatitis.

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory disease causing intense pruritus, and with typical clinical features, eczematous dermatitis affecting 10-20% of children and regarded as a major cause for morbidity, since patients with AD have a higher susceptibility for microbial colonization and an increased risk of skin infections (1). Numerous investigations and studies have shown that in addition to bacteria, particularly S. aureus and fungi may also be significant aggravating factors in AD(2). *Staphylococcus aureus* infection was also found with prevalence of about 1-3% in adults, present in 80-100% of skin from atopic patients and is related to worsening of the disease by the action of enterotoxins (3).

The role of bacteria in AD is largely unknown, however, bacterial flora analysis of the skin has revealed its involvement in clinical conditions. On the skin of children with AD, it has been reported that the diversity of the bacterial flora of the skin decreases in the exacerbation phase, and the proportion of *S. aureus* increases (4).

In addition, eradication or reduction of *S. aureus* colonization on AD patients has demonstrated a positive correlation with decreased severity of atopic eczema (5). Our study planned to detect the spread of *S. aureus* from skin lesions of AD patients.

Materials and methods

Samples collection

This study included a total of one hundred and fifty cotton swab samples

collected from patients of both sexes and of different ages with atopic dermatitis. The samples were collected from Al-Zafrania Hospital and private clinics in different areas of Baghdad City during the period from November 2021 to March 2022. The samples were placed in a transport medium and transferred to Al-Madaen General Hospital for the purpose of transplanting it in the laboratory for a period not exceeding 24 hours only. Followed by bacteriological isolation and identification of *S. aureus*.

Isolation and identification of *S. aureus*

The collection of skin lesion specimens was by the end of wetting sterilized cotton swab with the lesion or discharge of fluid from blisters scrape tissue, then placed in a sterile test tube containing sterilized 2 ml of transport media . All skin swabs were inoculated on 5% human blood agar and mannitol salt agar plates. After incubation at 37Co for 18-24 hr, cultural and morphological features of the colonies were evaluated. These *S. aureus* isolates were further investigated by growth on chromagar specific for the identification and isolation of *S. aureus.*

All isolates were primarily examined by gram stain and by biochemical examinations, according to (Prescott) (6).

- **Coagulase test (7):** To test coagulase enzyme as bound or free coagulase, suspension 0.5ml of bacteria colony is added to 0.5ml plasma of human have been prepared and incubated in human 37C, then the plasma inoculated periodically examine the composition of fibrin formation of coagulase clot within four hours, and this is interpreted as a positive result indicates on *Staphylococcus aureus* strains. The absence of a blood clot after 24 hours incubation is the negative result.
- **Catalase test (8):** This test was used for the detection of catalase enzyme, to differentiate Streptococci (Catalase -ve) from Staphylococci (Catalase +ve). The exposed to hydrogen peroxide, bacteria catalase positive conversion of peroxide to water and oxygen gas, this test was performed by a capillary tube containing hydrogen peroxide solution was carefully dipped into a single colony, and when catalase was represented, oxygen gas was released and the bubbles observed in the capillary tube.
- **Oxidase test (9)**: A piece of filter paper placed in a clean Petri dish and 2-3 drops of Oxidase reagent, were added to the filter paper. Colony from tested organisms was transferred to the filter paper and rubbed onto the reagent with an applicator stick. The positive result was indicated by blue purple color formation within 10-15 seconds.

Molecular identification of *Staphlococcus aureus*

The detection of methicillin resistance is important not only in the clinical management and treatment of *S. aureus* infection (10), but also it was based on the amplification of the genes responsible for methicillin resistance and *S. aureus* genes and Coding toxins in diagnosing *Staphylococcus aureus* and distinguishing it from other species (11).

Results and discussion

The results presented in this chapter are based on 150 Atopic Dermatitis

(AD) instances in total. Patients Suspected infected with *Staphylococcus aureus*, Dermatologists and criteria are used to confirm the clinical diagnosis of AD patients of Hanifan and Rajka (12), Total of 150 clinical samples were collected, only 54 sample (36 % isolates) were given the typical biochemical tests and characteristics of morphology that is specific to *staphylococcus aureus*, while the other 96 clinical (64 %) isolates belong to other species of staphylococci (8.6) or other pathogenic bacteria and fungi (33.3). Figure (1).

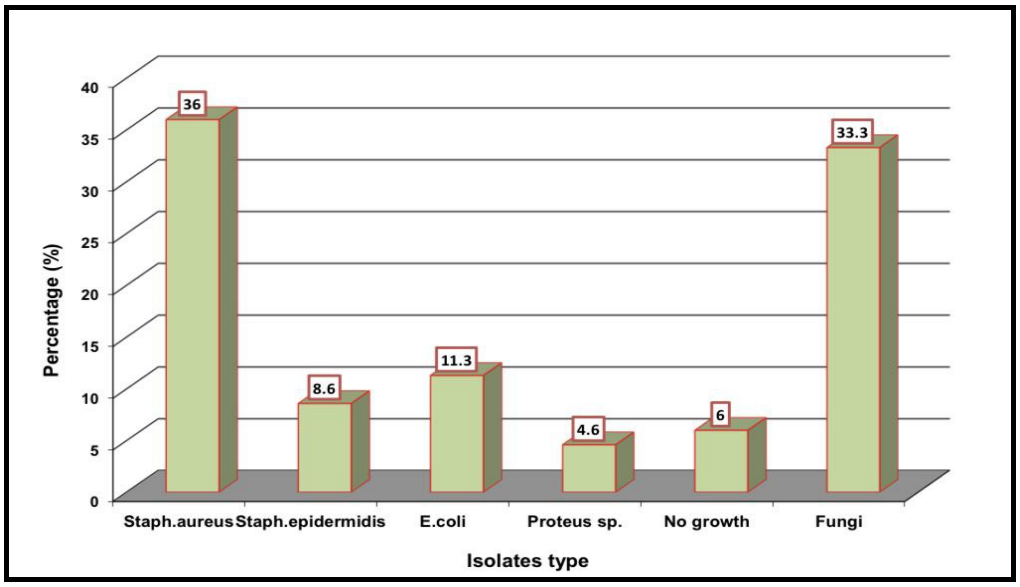

Figure (1): Bacterial types and fungi isolated from the skin of (AD) patients.

The Colonies of clinical specimens were cultured on blood agar media. The results found that bacteria have smooth, slightly and spherical shape. In addition, these colonies are able to form β hemolysis when grown on blood agar (13) (figure 2).

Figure (2): Growing of *S. aureus* colonies on blood agar at 37 °C for 24 hrs.

Fifty-four of clinical isolates form yellow (golden) colonies due to fermenting the mannitol salt change the phenol red to golden and resist high salts amount of MSA (Mannitol salt agar) selective medium. These gave the typical morphological characteristics of that specific to *S. aureus* (14,15). It was

a differential culture medium specific for *S. aureus* prevent the growth of other bacteria (figure 3) (16,17,18).

Figure (3): Growing of *S. aureus* colonies on MSA at 37 °C for 24 hrs.

Growth on chromagar, a medium specifically designed for the detection and isolation of *S. aureus*, was used to further analyze these *S. aureus* isolates figure (4).

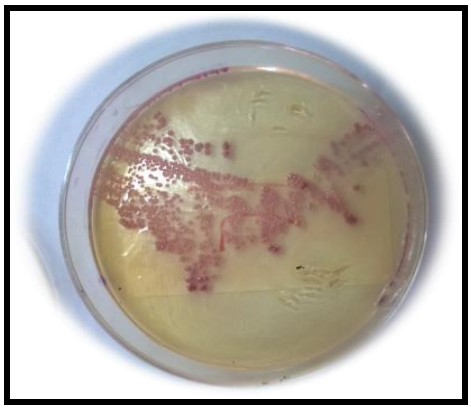

Figure (4): *Staphylococcus aureus* colonies on ChrmoagarTM.

The clinical specimens of diagnosed based examination of microscope. The Gram staining show the Staphylococci spp. properties when appear Gram positive reaction, non-spore-forming, arranged like irregular clusters and cocci shape under the microscope as shown in the Figure (5) (19).

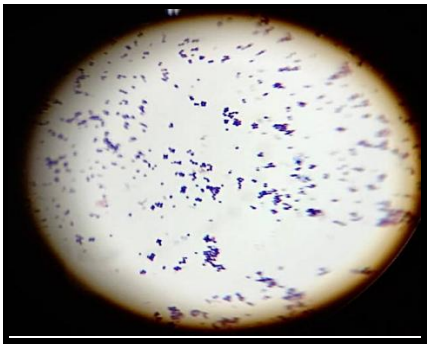

Figure (5): Microscopic examination of *Staphylococci* isolates (10x).

Biochemical identification of *S. aureus* isolates

The basic biochemical tests were used to specimens shown a positive reaction for catalase, coagulase. But, it was a negative reaction for oxidase tests.

They are subjected to coagulase reactions that show the bacterial ability to form coagulase enzyme which differentiates between *S. aureus* species (positive coagulase) and other Staphylococcus species (negative coagulase) due to reaction coagulase enzymes of bacteria with prothrombin of human blood and form staphylo-thrombin (clot) that convert the fibrinogen into fibrin Figure (6)(20).

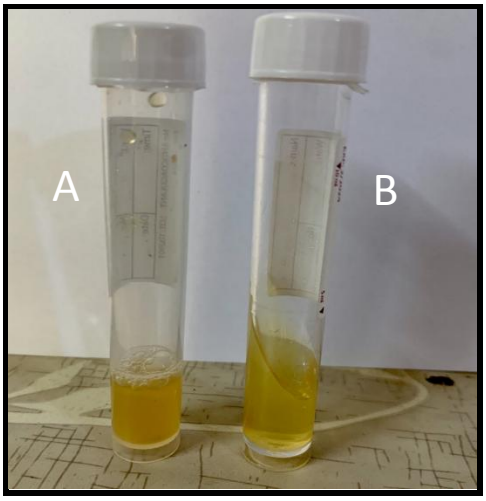

Figure (6): Coagulase test for *S. aureus*. A) negative result B) positive result.

Catalase was an enzyme that decomposes H2O2 (hydrogen peroxide) to oxygen and water, it prevented the toxic metabolites accumulation (21). The *S. aureus* isolates were positive Catalase genus differentiates from others *Streptococcus* genus (Figure 7). *S. aureus* were negative for oxidase, which differentiates them from others Micrococcus genus (22, 23) Figure (8).

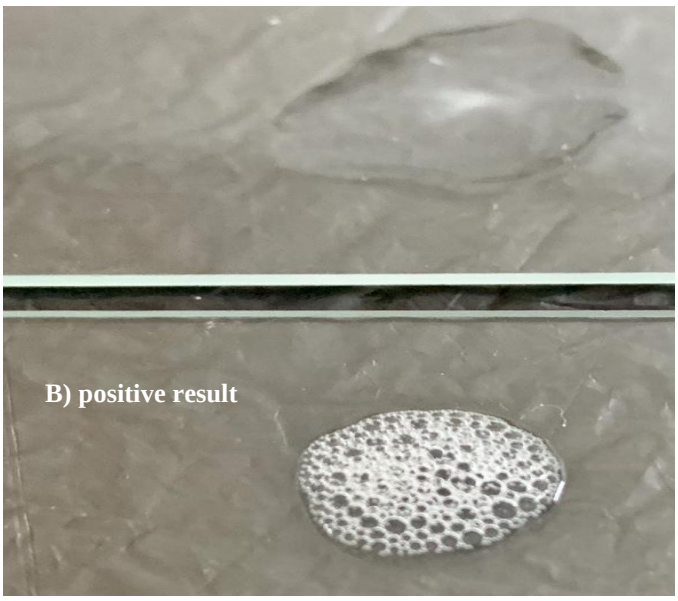

Figure (7): Catalase test for *S. aureus*.

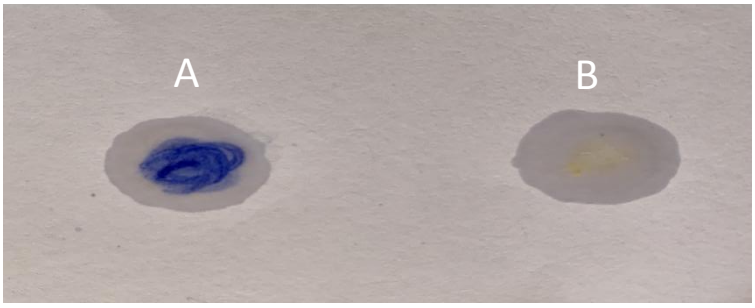

Figure (8): Oxidase test for *S. aureus*; A) Positive result B) Negative result.

Molecular identification of *Staphlococcus aureus*:

Detection of *mecA* gene in *S. aureus* isolates, the bacterial DNA amplified for this gene used PCR technique in a monoplex pattern by used specific primers, and the optimum condition to amplify this gene in PCR. The *mecA* gene confirmed by agarose gel electrophoresis and photographed under ultraviolet (UV) transilluminator shown in Figure (9)) were the amplification revealed a product of 147bp. The rate of clinical *S. aureus* isolates that gave a positive result for the *mecA* gene was 100%, The result consistent with the research of Jamil et al., (2017) (24) and Ghaznavi-Rad and Ekrami, (2018) (25) which was the rate of clinical *S. aureus* isolates. The one that gave a positive result for the *mecA* gene is 100% as well.

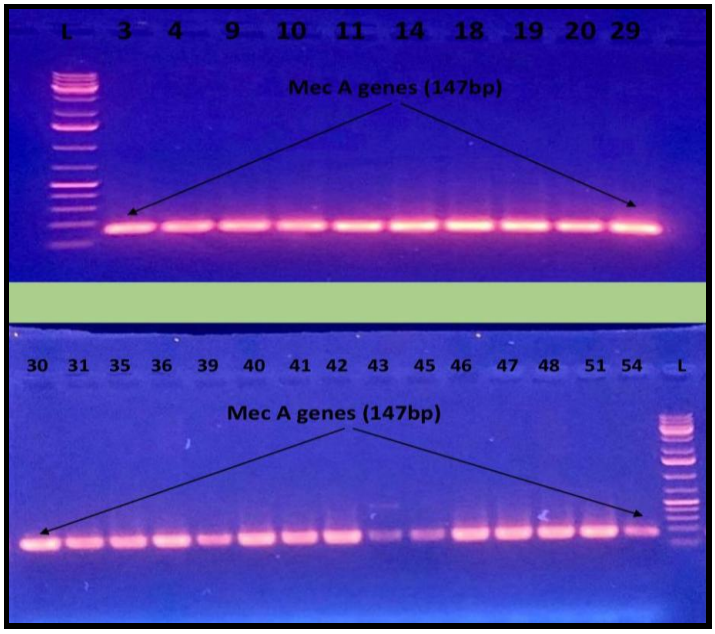

Figure (9): Gel electrophoresis of PCR products of *mec*A gene at 1%Agarose, 70 V and for 1 hr.

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