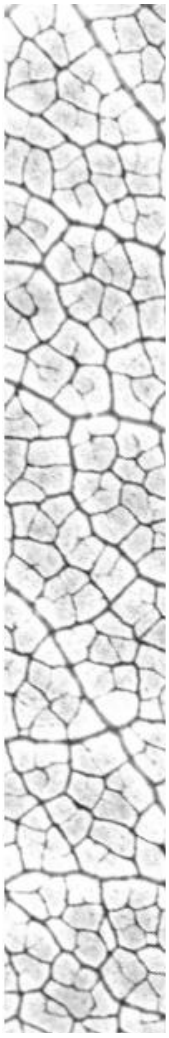

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Molecular detection of staphopain genes (A&B) from staphylococcus aureus isolated from skin lesions

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Abstract

In this study, A total of 100 clinical swabs obtained from patients with skin lesion and also from healthy individuals from aneroid extend from September to December 2019.

Only 20 isolates were identified and diagnosed by using chemical and cultural features as *staphylococcus aureus* where 13 isolates obtained from patients and 7 isolates from healthy skin. Molecular study by using PCR was done to show the presence of positive genes, ScpA, SspB, which encodes for staphopain A and staphopain B respectively. It was found that 13 isolates of *S aureus* (65%) give positive results for the existence of ScpA gene which encodes staphopain A whereas 7 isolates (35%) are free from this gene. Besides, 10 isolates of *S. aureus* (50%) gave positive results for SspB gene which encodes staphopain B.

All the 10 isolates containing SspB gene have the ScpA gene at the same times. Moreover, two genes associated with skin disease are also investigated; these genes are edin-B gene and etd gene. PCR technique by using specific primers for both genes found that only 10 isolates (50%) possessed edin-B gene whereas 15 isolates of *S.aureus* (75%) had etd gene.

Keywords: Staphopain, Staphylococcus. aureus, Skin infections, Extracellular matrix

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INTRODUCTION

Staphylococcus is a genus of Gram positive bacteria in the family Staphylococcaceae. Under the microscope, they appear spherical (cocci), and form in grape-like clusters. *Staphylococcus* species are facultative anaerobic organisms (capable of growth both aerobically and anaerobically).

Staphylococcus aureus, a leading cause of bacterial infections in humans, as a results of it`s ability to produce different virulence factors that contribute to the disease process. Several extracellular proteolytic enzymes, including cysteine proteinases referred to as the staphopains (staphopain A and staphopain B) which have aroles in staphylococcal virulence. *Staphylococcus aureus* is a Gram-positive, non-motile bacterium capable of causing a myriad of bacterial infections. S. aureus frequently colonizes the skin and mucosal surfaces of humans but is most often harbored in the anterior nares (Sakr et al. 2018 & Ataei Moghadam et al., 2020).

These infections range from mild skin and soft tissue infections to potentially fatal infections, such as pneumonia, endocarditis, osteomyelitis, sepsis, and toxic shock syndrome (Nasser et al. 2019). The increasing prevalence of methicillin-resistant *S. aureus* (MRSA) strains in both the hospital and community has exacerbated the problem (Kobayashi et al. 2020).

Moreover, many studies are done in Iraq focused on isolation *S. aureus* from skin and other infections Noticed that most isolates found in nasal passage are related to *S.aureus*. Also, in Najaf city, (Al-Kelaby et al.2016).were found that *S. aureus* is predominant in skin infections. Where compared to other sites of infections.

Avall et al. (2018) observed that S.aureus has multiple virulence factor that made it enable to cause infections as a result of production of specific virulence factors such as proteinase, hyalurinidases, hemolysins, leukocidine and also exofoliative toxins which plays a role in skin diseases. Moreover, many studies are done in Iraq focused on isolation *S.aureus* from skin and other infections (Dhulfiqar et al. 2015)

Besides,the presence of pathogenicity islands in this bacteria which possess many virulence genes also renders it more pathogenic particularly these genes which have ability to attach the skin and soft tissue (Astley et al., 2019). The ability of this bacteria to lyse the blood through production of hemolycinces and degrade hyaluronic acid increase it`s pathogenicity to

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Table 1. Percentages of *S. aureus* and other bacteria in clinical isolates

Bacterial infection	*S. aureas*	Other bacteria	No growth	Total No .
No. of growth	20	67	13	100
Percentage %	20%	67%	13%	100%

include bacteremia and joint infections (guo, et al., 2020).

The present study aims to Detection of staphopain enzyme at the molecular level and traditional techniques in a clinical isolate of *Staphylococcus aureus.*

MATERIALS AND METHODS

1. Isolation of S. aureus from healthy skin and skin lesion.
2. Investigation of staphopain enzymes at the molecular level.
3. Detection of enzyme extracellularly in traditional media.
4. Study of staphopain on fibrinogen level.

A total of 100 swabs obtained from patient with skin lesion and also from healthy skin. Only 20 isolates (20 %) of staphylococcus are isolated and identified by using culturing and biochemical tests, whereas (67) samples diagnosis for other bacteria and (13) samples with no growth. Samples were collected during this study which obtained from patient Suffering different infection such as skin infections, vaginitis, pus and wound.

RESULTS AND DISCUSSION

The samples are included nasal passages for healthy individual and skin lesions for patients. Only (7) isolates are isolated from healthy individual and (13) isolates from skin lesions. The rate of the isolation of this study is closed to that obtained by (Linda et al, 2006) observed that (29 %) of the isolates from skin related to *S areus* whereas recent study found the infection of skin increased when the rate of infection become (35.8 %) or more (Ansari et al. 2019).

S.aureus is present at distinct sites such as the nose, ear, pharynx and conductive and can produce different types of virulence factors. In addition to those sites, skin is also the main source of infections in human body where the number of this bacteria is high in the skin when be compared to other sites of infections (Nvick et al., 2001).

Yamaguchi, et al 2002, Indicated that S.aureus had been isolated at high rate with the presence of different virulence factors associated with skin infections. However, (Ajayi et al., 2017) found that most swabs obtained from skin lesion particularly from acne is related to Staphylococcus aureus and other staphylococcus such as S.epidermidis.

In Iraq, at Najaf city, a study done by (Alkaabi et al.2016), it was observed that only 50 isolates of *S.aureus* were isolated (20 %) from a total of 250 clinical

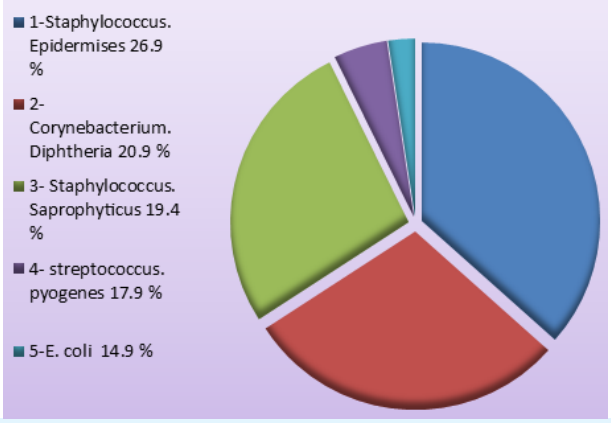

Fig. 1. Other types of bacteria associated with skin infections

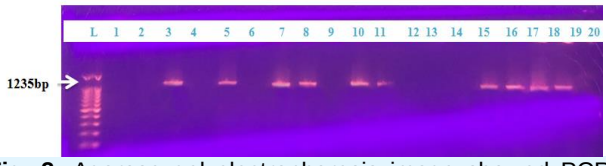

Fig. 2. Agarose gel electrophoresis image showed PCR product analysis for ScpA gene in Staphylococcus aureus isolates. (Marker ladder 100-1500 bp). (1,2,3,5,7,8,10,11,15,16, 17,18, 19) Positive isolates (ScpA) gene, (4,6,9,12,13,14,20) negative isolates gene. *Staphylococcus aureus* isolates at (1235 bp) product size

samples and this rate is close to that obtained at this study.

In this study we found a number of bacteria that grown on the patient's skin (healthy skin & skin lesions) in different characteristics, some of which are pathological and others are not pathogenic. These types of bacteria may grow due to the availability of suitable conditions for growth. **Fig. 1** shows these types of bacteria and their proportions.

Molecular detection of staphopain

Detection of (ScpA gene)

In this study ScpA gene which express on staphopain A protein was detected by using PCR technique. It was noticed and shown in **Fig. 2**. That only (13) isolates (65 %) give positive amplicon for this gene at molecular size 1235 pb.

It is known that ScpA gene express staphopain protease with assistance of staphopain gene B (SspB). The presence of (13 isolates) of S.aureus isolated from skin lesion may increase the virulence of these isolates where this photolytic enzyme has many activities where in addition of it is being cysteine proteinase, it have fibrinolytic activity and elastinolytic activity, moreover, the ScpA gene is located inside specific operon which is controlled by a repressor gene called ScpB (Magro, G. 2018). and is also regulated by specific regulatory gene called SarA gene (Hume *et al.*, 2020).

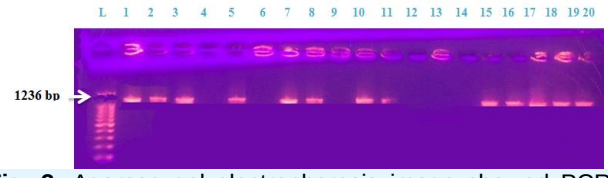

Fig. 3. Agarose gel electrophoresis image showed PCR product analysis for etd gene in Staphylococcus aureus isolates. (Marker ladder 100-1500 bp). (3, 5, 7, 8, 10, 11, 15, 16, 17, 18) Positive isolates (etd) gene (1, 2, 4, 6, 9, 12, 13, 14, 19, 20) negative isolates (etd) gene. *Staphylococcus aureus* isolates at (1236) bp product size

Staphopain A gene is seen only in (13 isolates) but other isolates (7 isolates) free from this gene. It means that these isolates may contain other gene that may give rise to produce the staphopain A or there is mutation at the site of this gene that PCR primer fails to bind to it.

However, *S. aureus* may cause a variety of infections. These include relatively harmless superficial skin infections but also life-threatening conditions, such as pneumonia, endocarditis, osteomyelitis, and sepsis (Lowy, 1998; Miyata et al. 2007). *Staphylococcus aureus* secretes the zinc-dependent metalloprotease aureolysin (Aur), two cysteine proteases, staphopain A (ScpA) and staphopain B (SspB), and several serine proteases, including V8 protease (SspA) and epidermolytic toxins (ETs).

On the other hand, staphopain A is expressed as inactive zymogen not as other protolytic enzyme, however, it will undergo rapid autocatalytic activation (Tam and Torres, 2019). Also, (Golonka et al, 2009) indicate that ScpA gene is very important gene for encoding staphopain consistent with other Scp gene located in specific operon in most coagulase positive staphylococcus. Although some genes related to the *S. aureu* are specialized for skin infections, are located within pathogenicity islands (Yamaguchi et al. 2002) but ScpA gene is not arranged with this specialized loci, it means that this gene is organized and regulated by some genes located so far from pathogenicity islands.

Detection of sspB gene

The presence of SspB gene was also detected in 20 isolates of *S.aureus* isolated from skin lesion and healthy skin. Specific primer at molecular size 1236 bp was used for PCR technique, it was found that only 10 isolates (50 %) give positive amplicons for this gene and the other isolates are free from this gene as shown in **Fig. 3**.

This gene is very important in increment the pathogenicity of *S aureus* where it has v8 protease activity in *S aureus* strains (Gimza et al. 2019)

Also, the presence of such gene as single copy gene not present in operon made strongly conserved in pathogenic *staphylococcus aureus* (Lehman et al. 2019) but in this study only 50% of isolates are positive to it, that means that not all isolates found in this study is pathogenic.

Littlie studies indicate the existence of sspB protein and its importance but the study but (Rice et al. 2004) explain that sspB gene has a role in binding of extracellular matrix (ECM) such as collagen, fibrinectin, fibrinogen and lectin (Lindsay et al. 2017), sspB gene play a role in regulation of Scp gene located inside the operon.

However, Fageland et al. (2009) indicate that SspB protein is one of the most important competence induced pneumococcal protein produce by coagulase +ve staphylococci.

CONCLUSION

Only 10 isolates (50%) possessed edin-B gene whereas 15 isolates of *S.aureus* (75%) had etd gene from 100 clinical swabs obtained from patients with skin lesion and from healthy individuals from aneroid extend.

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