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Detection of *Staphylococcus aureus* Enterotoxins' Genes (Sea and Seg) in a Sample of Iraqi Patients with Psoriasis

Ali N. Umayra[1] *PhD*, Jabbar S. Hassan[2] *PhD*, Hasan N. Mohsen[3] *FICMS*

[1]Dept. of Environmental Health, College of Environmental Sciences, Al-Qasim Green University, Babylon, Iraq, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [3]Dept. of Medicine, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Psoriasis is a common chronic cutaneous disease that seriously affects the life quality of patients. Erythema, papules, and scales are the most common clinical presentations. *Staphylococcus aureus* (*S. aureus*) is an opportunistic pathogen and commensal bacterium. Infection with *S. aureus* exacerbates psoriatic lesions in affected people. Staphylococcal enterotoxins (SEs) are often categorized as superantigens, which can excite significant populations of T cells.

Objective To detect *S. aureus* enterotoxins' genes (sea, and seg) among patients with psoriasis.

Methods Skin swab samples from 225 individuals who enrolled in this case-control study were obtained from February 2021 to February 2022. Those individuals were divided into 3 groups; the first group comprised 75 patients with psoriasis as a patient group. The second group comprises 75 individual burn patients suspected to have a bacterial infection as a positive control. The third group comprised 75 individuals (apparently healthy people) as a negative control. The positive control group was recruited from Burns Specialized Hospital in Medical City, the negative control group was recruited from the blood donation center in Al-Imamein Al Kadhimein Medical City, and the patient group also was recruited from the dermatology consulting department.

Results Out of 225 individuals recruited in this study, there were (112) males while the female was (113) with male to female ratio of (1:1). Females' age ranged from (7-70 years), while males' age ranged from (5-52 years). *S. aureus* isolates were identified in 82 (36.44 %). The results regarding the presence of SEs gene showed that 9 out of 22 isolates of *S. aureus* obtained from the psoriatic patients' group, expressed sea while 19 out of 22 isolates expressed seg.

Conclusion The most common enterotoxin distributed in *S. aureus* isolates of all study groups were seg, which seems to be the most enterotoxin that detect in patients with psoriasis.

Keywords Psoriasis, *S. aureus*, Staphylococcal enterotoxins, Sea gene, Seg gene

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List of abbreviations: D = Desquamation, E = Erythema, I = Infiltration, MHC = Major histocompatibility complex, PASI = Psoriasis area severity index, SEs = Staphylococcal enterotoxins, TCRs = T-cell receptors

Introduction

Psoriasis is a prevalent chronic inflammatory skin condition that affects 2-4% of the general population [(1)]. Psoriasis is clinically characterized by the appearance of erythematous, scaly, and well-defined skin plaques, which typically appear on the external surfaces [(2)]. Although the

pathogenesis of psoriasis is not completely understood, there is substantial evidence that the dysregulation of immune cells in the skin, specifically T lymphocytes, plays a crucial role in psoriasis development [3].

The pathogenesis of psoriasis appears to be driven by the interplay of innate immune cells, adaptive immune cells, and keratinocytes, which is mediated by cytokines (including interleukins (IL)-6, IL-17, and IL-22, interferon, and tumor necrosis factor) and other signaling molecules [4]. Certain pathogens, including bacteria such as *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pyogenes*, viruses such as human papillomavirus, and fungi such as *Candida albicans*, have been reported to induce or worsen psoriasis [5].

Infection with *S. aureus* exacerbates psoriatic lesions in affected people. This bacterium colonizes psoriatic lesions in 60% of psoriasis patients, and 60% of isolates produce Staphylococcal enterotoxins (SEs) [6]. SEs are often categorized as superantigens, which can excite significant populations of T cells (20-30%), resulting in the creation of a cytokine bolus [7]. SEs connect directly to major histocompatibility complex (MHC) class II molecules and cross-linking T-cell receptors (TCRs) via binding to their TCR variable region Beta chains (TCR-Vbs) with extremely high affinity, resulting in extensive T-cell hyperactivation [8]. Some strains of *S. aureus* produce extracellular SEs. However; classical SEs include SEA, SEB, SEC1, SEC2, SEC3, SED, and SEE, while nonclassical SEs include SEG, SEH, SEI, SER, SES, and SET; all have emetic action [9]. This study aims to detect SEs' genes (sea, and seg) among patients with psoriasis.

Methods

Study design

The present case-control study was conducted on 225 individuals during the period from February 2021 to February 2022 who were divided into three groups; the first group comprised 75 individuals with different types of psoriasis, each of them varying in his/her signs and symptoms (as a patient group), the second group comprised 75 individual of burn patients suspected to have a bacterial infection as a positive control, the third group comprised 75 individuals (apparently healthy people) as a negative control. The patient group was recruited from the Dermatology Consulting Clinic in Al-Imamein Al Kadhimein Medical City, Baghdad, the positive control group was recruited from Burns Specialized Hospital, Baghdad Medical City, and the negative control group was recruited from the Blood Donating Center in Al-Imamein Al Kadhimein Medical City.

All psoriatic Patients, who were from different age groups with different types of psoriasis are diagnosed clinically by dermatologists and included in the current study. Psoriatic patients who received any treatment, topical or systemic for at least 3 weeks before sample collection was excluded from this study. Patients who have other autoimmune diseases also excluded.

The psoriasis area and severity index (PASI) calculations divide the body into the head, trunk, upper extremities, and lower extremities. Psoriasis area, erythema, thickness, and scaliness must be determined for each body region. Body surface area (BSA) percentages vary by body region. The head weighs 0.1, trunk 0.3, upper extremities 0.2, and lower extremities 0.4. PASI calculation involves assessment over 4 body regions (head [h], trunk [t], upper [u] and lower [l] extremities of erythema (E), infiltration (I), and desquamation (D), and body surface area involvement (A). PASI = 0.1 (Eh + Ih + Dh) Ah +0.2 (Eu +Iu +Du) Au +0.3 (Et +It +Dt) At +0.4 (El +Il +Dl) Al [10].

Ethical approval

All Subjects involved in this study were informed and the agreement was obtained verbally from each one before the collection of samples. This project was approved by the Iraqi Ministry of Health and Institutional Review Board (IRB) No. 96 dated 10\01\2021 at the College of Medicine of Al-Nahrain.

S. aureus isolation

All of the swabs taken from the participants were taken from any part of the body with psoriasis or a burn. The samples were then inoculated on Blood agar media and kept at 37°C in an aerobic incubator for one day. Suspected isolates of *S. aureus*, which showed β-hemolysis on blood agar media were further tested using Gram stain. The bacteria that formed characteristic yellow colonies on mannitol salt agar media were then confirmed by using VITEK-2 ID System according to the manufacturer's instructions (bioMérieux).

Molecular assay for detection of SEs

The confirmed *S. aureus* isolates have been used for genomic DNA extraction from a fresh overnight culture by using Geneaid Presto™ Mini gDNA Bacteria Kit (Taiwan). After that DNA template was used to detect SEs A and G genes by using specific primers sequences (Macrogen [Korea]), which are listed in Table 1.

Table 1. Primer sequences of PCR that were used in this study

Genes	Nucleotide sequences (5'⟶3')	Product size (bp)	Reference	Origin
Sea	F: GGGAACAGCTTTAGGCAATC R: ATTTGAATACTGTCCTTGAGC	564	(11)	Macrogen (Korea)
Seg	F: AAGTAGACATTTTTGGCGTTCC R: AGAACCATCAAACTCGTATAGC	287	(12)	Macrogen (Korea)

Conventional polymerase chain reaction (PCR) for screening and amplification of SEs (sea and seg) genes

A conventional monoplex PCR technique was carried out to amplify fragments of sea (564 bp), and seg (287 bp) genes. The Eppendorf tubes were placed in the thermo-cycler (Cleaver Scientific Thermal Cycler TC32/80), which was previously programmed with the following PCR conditions: 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, terminating in 72°C for 5 min. The components of PCR mixture as follow: Forward Primer 1 µl, Reverse primer 1 µl, DNA template 2 µl, (DNAse free) water 16 µl. Total volume: 20 µl. Electrophoresis was performed by subjecting 10 µL of PCR products to each well of 1.5% agarose gel with ethidium bromide (0.5 µg/ml; Sigma). Amplicon was visualized using a UV light transilluminator and then photographed with a digital camera.

Statistical analysis

Graph Pad Prism version 8 software (2018) was used for the statistical analysis of data. The significance between all groups that enrolled in this study was assessed using One-way ANOVA (analysis of variance), which is used to determine if there is a statistically significant difference between the variables. $P < 0.05$ was considered significant.

Results

Demography of participants: baseline characteristics

The distribution of all participants in this study, according to gender and different age groups revealed that out of (225) individuals, there were 112 (49.77%) males while the female was 113 (50.23%) with male to female ratio of (1:1). Female's age ranged from (7-70 years) with a mean age of (31.67) years, while males age ranged from (5-52 years) with a mean age of (25.27) years. Difference between means of age was (6.41±2.2) (Figure 1 & 2).

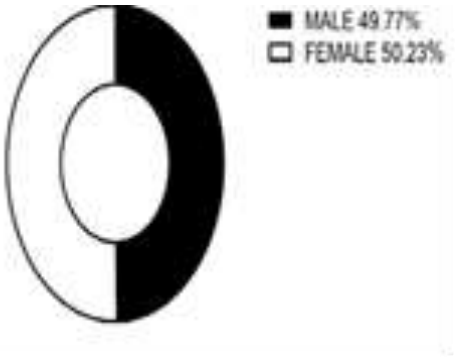

Figure 1. Distribution of participants according to gender [n=225]

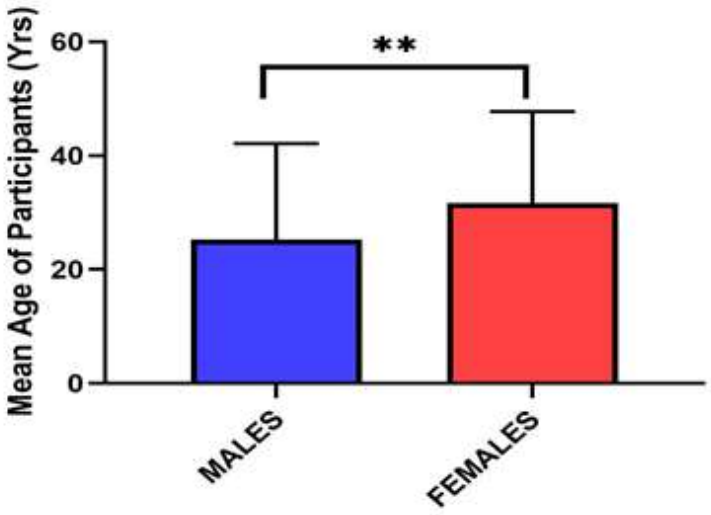

Figure 2. Mean Age of Participants **P<0.01

From other hand, the distribution of psoriatic patient's participants in this study, according to sex revealed that out of (75) individual there were (48) male while the female was (27) (Figure 3).

Distribution of *S. aureus* isolates among the study groups

Out of 225 individuals recruited in this study, *S. aureus* isolates were identified in 82 (36.44%). Most isolates were from the positive control group (35) followed by the negative control group (25), while (22) isolates were from the psoriatic patients' group. The results showed that there was a significant difference (P value <0.05) between the positive control group and the psoriatic patient group. Conversely, there were no significant differences (P >0.05) between the psoriatic patient group and the negative control group (Figure 4).

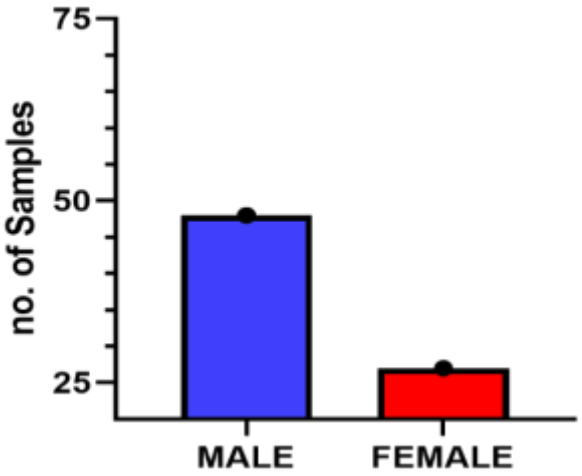

Figure 3. Distribution of psoriatic patient's group according to sex [n=75]

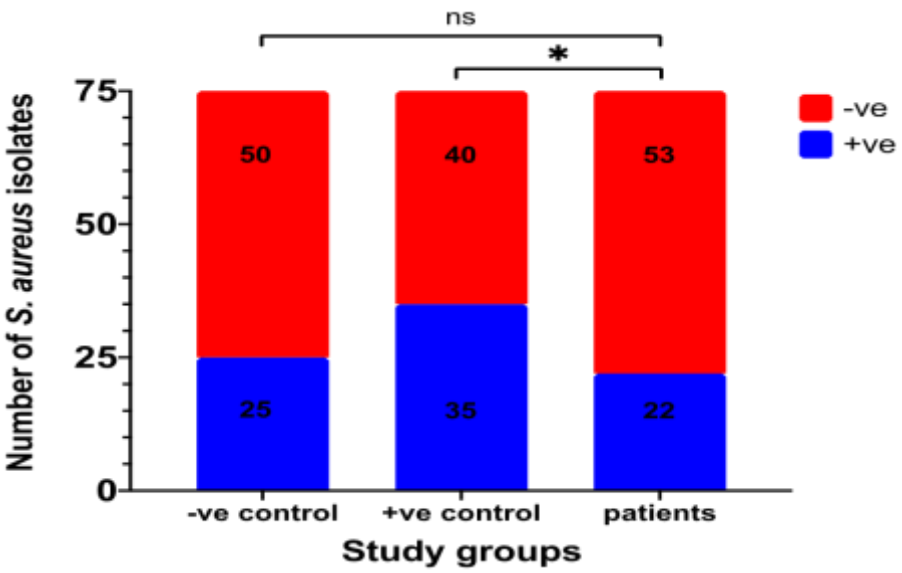

Figure 4. Distribution of *S. aureus* isolates among study groups (* P value <0.05)

Patients' distribution according to psoriasis disease severity

According to PASI, out of 75 psoriatic patients enrolled in this study, as many as 28 (37.33%) of cases presented with mild disease activity, and 32 (42.66%) of cases presented with moderate disease activity and 15 (20.01%) of cases presented with severe disease activity (Figure 5).

Conventional PCR screening for Staph. aureus enterotoxin genes (sea and seg)

A. S. aureus enterotoxin gene A (sea)

The study revealed that 9 out of 22 (40.9%) *S. aureus* isolates from the psoriatic patient's group, expressed sea and 19 out of 35 (54.28%) isolates from the positive control group expressed sea, while 16 out of 25 (64%) isolates from the negative control group, expressed sea. The enterotoxin gene amplicon

was produced in size 564bp of sea, as shown in figure 6.

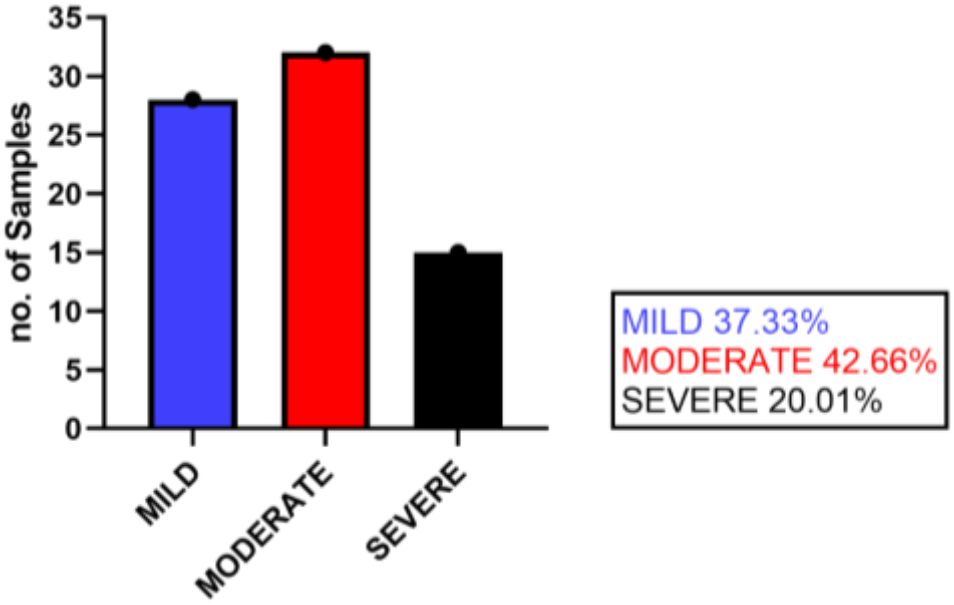

Figure 5. Distribution of psoriatic patients according to severity of the disease (n=75)

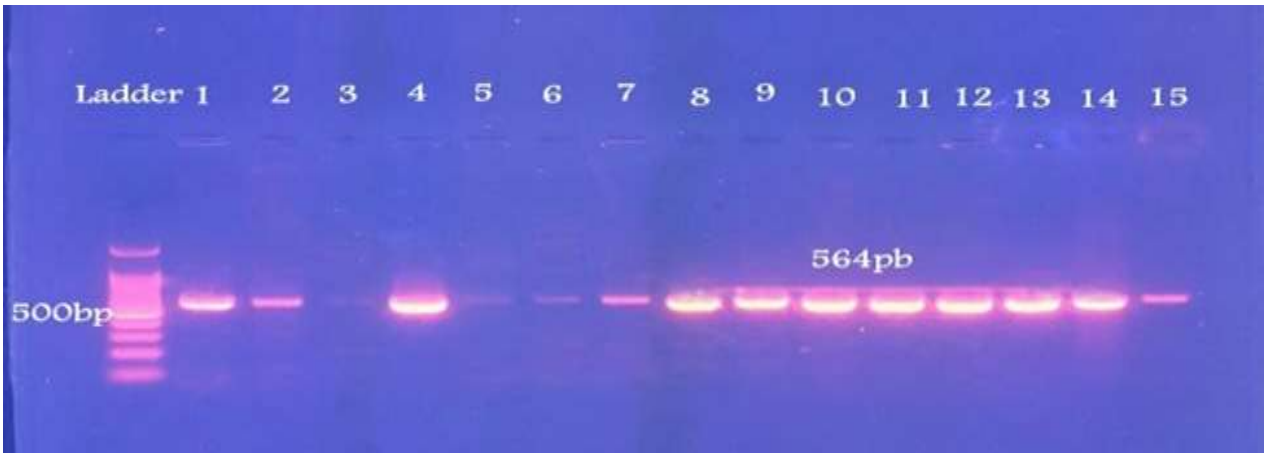

Figure 6. Gel electrophoresis (1.5 % agarose, 75 v/cm^2, 45 min.) of amplified PCR product (564 bp) for sea gene, which was visualized under UV light at 280 nm after staining with ethidium bromide concentration 0.4 µg/ml. Lane 0: 100 bp ladder. Lane 1-5 shows negative control. Lanes 6– 10 show positive control. Lanes 11-15 show the psoriatic patient group

B. S. aureus enterotoxin gene G (seg)

The current study revealed that 19 out of 22 (86.36%) isolates of *S. aureus* from the psoriatic patient's group, expressed seg, and 32 out of 35 (91.42%) isolates from the positive control group expressed seg, while 21 out of 25 (84%) isolates from the negative control group, expressed seg. The enterotoxin gene amplicon was produced in size 287bp of seg, as shown in figure 7.

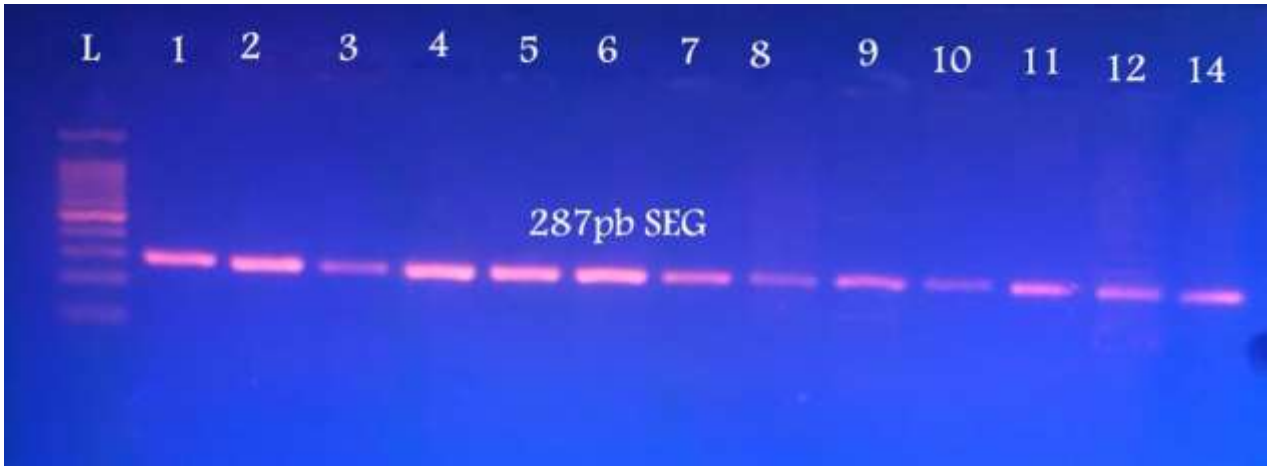

Figure 7. Gel electrophoresis (1.5 % agarose, 75 v/cm^2, 45 min.) of amplified PCR product (287 bp) for seg gene, which was visualized under U.V light at 280 nm after staining with ethidium bromide concentration 0.4 µg/ml. Lane 0: 100 bp ladder. Lane 1-5 shows positive control. Lanes 6-10 show the psoriatic patient group. Lanes 11-15 show negative control

Discussion

Demographic of participants

In this study, regarding sex distribution there is no significant differences between males and female enrolled in this study with male to female ratio (1:1), while there was statistical difference between means age of participants (**P<0.01**). This may be due to sex and age bias estimates for three different groups (healthy, burns and psoriatic groups) with two disease categories (burns and psoriatic).

Based on the current data, out of (75) psoriatic patients, there were 48 (64%) males, while the female was 27 (36%) with male to female ratio of (1:1), this observation in agreement with a study done in Iraq by Al-Jebory (2012), who reported that male dominance on female [13]. The current study disagrees with a study carried out by Mallbris (2005), who found female dominance (approaching 1.3:1) as compared with male [14]. However, Guillet et al. mentioned that the incidence, prevalence, and manifestation of psoriasis are similar between males and females [15]. The discrepancy in such results may reflect the fact that genetic and hormonal factors that consider risk factors in psoriasis are not equally relevant or apparent in women and men [16].

Distribution of *S. aureus* isolates among the study groups

This study showed that many *S. aureus* were isolated from all groups. However, 82 out of 225 (36.44%) participants had *S. aureus* isolates.

Nearly 20-50% of the population are nasal carriers and colonization of the skin in the anterior nares is a major source of endogenous *S. aureus* infections and transmission [17,18].

S. aureus nasal carriers are at risk of infection, according to many studies. Humans' noses are *S. aureus*' main ecological niche, but the carrier state's determinants are unknown. *S. aureus* eradication from nasal carriers prevents infection in hemodialysis and general surgery patients [19]. Stensen et al. [20] reported that *S. aureus* prevalence in the nasal carriage was 32% in male out of 752 males and in another study by the same authors related to females they found that the prevalence of S. aureus in the nasal carriage was 22% out of 724.

Reducing the acidic pH of human perspiration by producing ammonia (NH_4^+) from L-arginine catabolism enhances *S. aureus* skin colonization. To survive, it can quickly adapt to host damage. The bacteria are known for their ability to infect the skin and other soft tissues,

by adding genes and making changes to the regulatory [21].

Distribution of psoriatic patients that showed positive S. aureus according to severity

In this study, 12 out of 22 (54.54%) *S. aureus*-infected psoriatic patients were mildly diseased. Such a result is intriguing because it suggests that patients with a severe condition are more likely to be colonized by *S. aureus*. This finding may be due to the fact that severe psoriasis patients receive more medical attention and practice better hygiene. Current findings support other studies that found bacterial pathogens in mild to moderate psoriasis cases [22,23]. Besides that, 10 out of 22 patients with S. aureus were in moderate and severe disease activity 5 patients for each activity.

PCR detection of *S. aureus* enterotoxins genes

The most prevalent enterotoxin gene in all study groups was seg gene toxin, which was detected in psoriatic patients in 19 isolates (86.3%), in the positive control group (burns patients) in 32 isolates (91.4%), and in the negative control group in 21 isolates (84%) by PCR technique. The present results were in disagreement with the study done by Atefi et al. [24] who found that the most prevalent toxin was sea gene in psoriatic patients.

In conclusion: the seg gene was the enterotoxin that was most frequently found in S. aureus isolates. According to PASI in current study, the moderate disease activity more prevalent than mild and severe disease activity.

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Author contribution

Dr. Umayra: conducted the sampling, bacterial isolation, and diagnosis. Dr. Hassan: the molecular work and writing the manuscript. Dr. Mohsen supervised the work, edit and finalize the writing of the study.

Conflict of interest

There are no conflicts of interest.

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Correspondence to Dr. Ali N. Umayra
E-mail: skyoneskytwomef@gmail.com
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