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Article

Staphylococcus aureus enterotoxin coding genes identified from patients with atopic dermatitis in Iraq by molecular analysis

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Abstract

The present study determined the frequency of Staphylococcus aureus enterotoxins (B, C, Luk-pv) of atopic dermatitis isolated from (AD) cases in Laboratories of Baghdad. 54 Staphylococcus aureus isolates were subjected to primary identification tests using various methods (cultural characteristics, gram staining, biochemical tests, and vitek2 system). This study used antibiotic disc diffusion in fifty-four S. aureus isolates. Multidrug resistance (MDR) against different antimicrobial agents applied to polymerase chain reaction to amplify different genes coding for Staphylococcal enterotoxins, including 3 types (seb, sec and luk-pv), To ensure that the sequences of these genes match NCBI, DNA sequencing was performed for isolate No. (3). As a result of this study, 25 isolates had a multidrug resistance (MDR) percent (46.2%) against different antimicrobial agents—the results of DNA extraction and polymerase chain reaction directed to amplify the specific enterotoxin coding genes. This study showed that the (seb) gene is present in isolates of staph aureus bacteria isolated from patients with atopic dermatitis 12 /25 at a percentage (48 %). Furthermore, the absence of the sec gene in all Staphylococcus aureus isolates isolated from patients with atopic dermatitis—the results of the detection of the luk-pv 23\25 (92%) gene encoded for lukucidin.Polymerase chain reaction using different primers successfully identified Staphylococcus aureus enterotoxins (B, C)and luk-pv, luk-pv gene, which was the most frequent.

Keywords: Staphylococcus aureus enterotoxin genes, polymerase chain reaction, Staphylococcus aureus, atopic dermatitis.

Introduction

TheAtopic dermatitis (AD) is a prevalent, persistent skin disease that affects individuals having an atopic tendency together with bronchial asthma, allergic rhinitis, and food allergies. Atopic dermatitis patients usually complain about itchy skin, especially at night, which is the predominant symptom of dry skin, eczema lesions in flexural areas, and recurrent skin infections[1]. The participation of Staphylococcus aureus in eczematous dermatitis may be an exacerbating factor of (AD)[2]. since patients with AD have a higher susceptibility for microbial colonization and an increased risk of skin infections[3,4] Staphylococcus aureus

produces a group of 21 staphylococcal enterotoxins (SEs).SEs are among the most potent bacterial superantigens associated with atopic dermatitis, asthma, and nasal polyps in humans[5] Our study was planned for the detection of the frequency of genes (sea, seb and sec) that is responsible for enterotoxin excretion in S. aureus isolates by PCR method.

Materials and Methods

Samples collection :

This study included one hundred and fifty clinical samples collected from patients of both sexes and of different ages with atopic dermatitis. The samples were collected from Alzafrania Hospital and private clinics in different areas of Baghdad City from November 2021 to March 2022. The samples were placed in a transport medium and transferred to Al-Madaen General Hospital for transplant in the laboratory for a period not exceeding 24 hours only, followed by bacteriological isolation and identification of S. aureus.

DNA extraction and purification :

Gram stain and biochemical examinations, according6 primarily examined all isolates. DNA Extraction has been achieved using Wizard®'s genomic DNA purification kit (PROMEGA, USA). The determination of DNA purity was done according to Sambrook7. The purity and concentration of extracted staphylococcal DNA were determined by measuring the absorbance ratio at wavelength 260 nm over 280 nm using a scan drop spectrophotometer (analyticajena-Germany). A DNA sample was diluted with TE buffer solution to 1:10, and the optical density was read with a spectrophotometer at wavelengths 260nm and 280nm. The purity of DNA was measured by the equation of A260/A280= 1.8-2.0 (accepted range).

Detection of specific genes by polymerase chain reaction :

The detection of the S. aureus-specific species gene was carried out by the amplification of specific sequences within the target gene using the polymerase chain reaction technique. The experiment was carried out using one of the specific primers designated for each target gene that was mixed with the DNA sample (template) and a master mix reagent that contains (Taq polymerase, PCR buffer, MgCl2 and dNTPs). The final constituent was the deionized water. The reaction mixture was mixed and centrifuged for 3 seconds to collect the drops from walls to ensure the final volume of 25µl and then transferred to a thermal cycler to start the reaction according to the steps of the specific program.

Primers Preparation:

The DNA Company (Promeg) supplied the primers as a lyophilized product of 100 pmol/µl in concentrations. DNA company protocol was adopted for primer resuspension by bringing the final concentration of primers to 10 pmol/µl of TE buffer and storing at -20Co until being used. The sequences of primers used in the study are in Table 1.

No.	Primer names	Sequence 5'- 3'	product (bp)	Ref No.
1	Luk-pv-For	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	McClure et al., 2006
	luk-pv-Rev	GCATCAAGTGTATTGGATAGCAAAAGC		
2	Seb-For	TCGCATCAAACTGACAAACG	478	A. Leke et al. 2017
	Seb-Rev	GCAGGTACTCTATAAGTGCC		
3	Sec- For	GACATAAAAGCTAGGAATTT	257	A. Leke et al. 2017
	Sec- Rev	AAATCGGATTAACATTATCC		

Table 1. The sequences of primers used in the study.

Working Solution:

PCR Pre Mix was accomplished after several trials. Thus, the following mixture was adopted Table).

Item	Mastermix	Target DNA	Forward Primer (10 pm/ µl)	Reverse Primer (10pm/ µl)	Nuclease free water	Total volume
Volume	12.5 µl	3 µl	1 µl	1 µl	7.5 µl	25 µl

Table 2. The 25 µl PCR mix.

The amplification conditions were: an initial denaturing step of 5 min at 94°C, following 30 cycles, each consisting of 1min at 94°C, annealing 1min at (50,53 and 55°C) respectively—furthermore, extension at 72°C for 1min and final extension at 72°C for 10min. As in Table (3), PCR products were analyzed by electrophoresis on a 1.2% agarose gel. After electrophoresis, gels were stained with ethidium bromide (5 ng/ml) and photographed under a UV trans-illuminator. A 75-bp DNA Ladder was used as a molecular size marker.

No. of cycles	Time	Temperature	Steps
1	5 min	94 C°	Initial denaturation
30	60 sec	95 C°	Denaturation
	60 sec	62C°for***seb***	Annealing
		Optimization for *sec*	
		55C°for***luk-pv***	

	60 sec	72 C°	Extension
1	10 min	72 C°	Final extension
	10	4C°	Hold

Table 3. The thermocycling conditions program (seb, sec and luk-pv) genes.

Identify Sequences of PCR Product :

After confirmation of the presence of beams, send 25µl from reaction products with forward and reverse primers to Macrogen company in the USA to identify sequences of product PCR**.**

Statistical Analysis:

The Statistical Analysis System [8] program was used to detect the effect of different factors on study parameters. The chi-square test was used to significantly compare between percentages (0.05 and 0.01 probability) in this study.

Results:

The results of this study showed that the (seb) gene is present in isolates of *staph aureus* bacteria isolated from patients with atopic dermatitis 12 /25 at a percentage (48 %), which is higher than the results of researcher [9], where the results showed the emergence of 34 \110 (30.9 %)seb gene from all isolates. The results of [10] showed 26/85 (30.5 %)of the seb gene isolated from the *staph aureus* bacterium, where the number of seb genes is 27/74 (36.4%) Table 4 and Figure 1.

Gene	Description	+ ve *staph. Aureus*	%	-ve *staph. aureus*	%	Total	P-value
Seb	*Staphylococcus* enterotoxin b	12	48 %	13	52 %	25	0.781 NS
Sec	*Staphylococcus* enterotoxin C	0	0 %	25	100%	25	0.0001 **
P-value		--	0.0001 **	--	0.0001 **	--	--
** **(P≤0.01).**							

Table 4. SE (Seb–Sec) genes were distributed in *Staphylococcus aureus* isolated from atopic dermatitis patients.

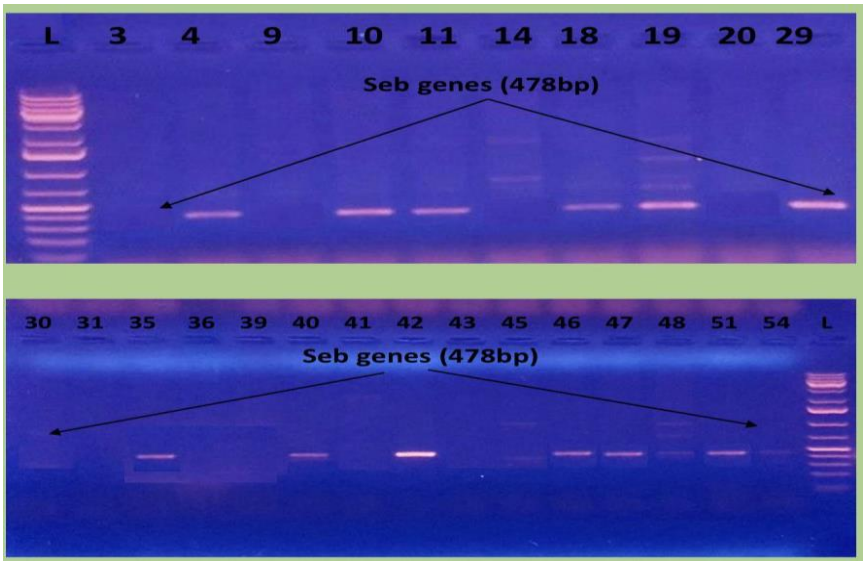


Figure 1. Agarose gel electrophoresis of PCR products for *Seb*genes(1%Agarose, 70 v/ 120 min).

An explanation for this diversity in superantigen production by S. aureus in AD patients is the severity of the disease and the site of the skin involved or where the swab was taken from. There is research reported the detection rate of S. aureus that produces superantigen from different skin areas in AD patients and found the following: 40.7% in the non-lesional area, 61.7% in the dry-lesional area, and 75.3% in the exudative-lesional area. The results of the detection of the luk-pv 23\25 (92%) gene encoded for lukucidin gave a positive result of the genetic table 5, Figure 2. The study showed a relatively high prevalence of luk-pv in Staphylococcus aureus in India.

Gene	Description	+ ve *staph. aureus*	%	-ve *staph. aureus*	%	Total
Luk-pv	Panton-Valentine Leukocidin	23	92 %	2	8 %	25
Chi-Square -χ^2 :(P-value)		17.64 **, (0.0001)				--
** **(P≤0.01).**						

Table 5. Distribution of (luk-pv) genes in *Staphylococcus aureus* isolated from atopic dermatitis patients.

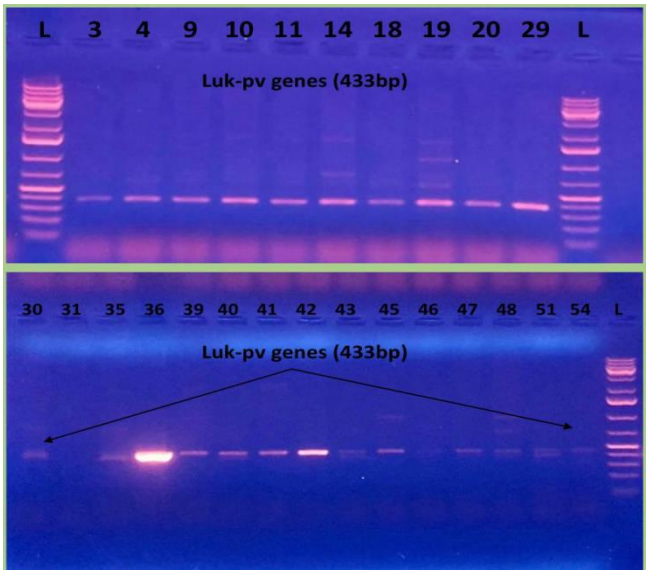


Figure 2. Agarose gel electrophoresis of PCR products for *Luk-pv*genes(1%Agarose, 70 v/ 120 min).

The results available on the global website of the gene bank (National Center for Biotechnology Information) (NCBI) found that (Luk-pv) was 100% back to the Staphylococcus aureus bacteria, as it is found in the international website (NCBI), as in the figures(3).

Staphylococcus aureus strain S230 Panton-Valentine leukocidinLukS-PV (lukS-PV) and Panton-Valentine leukocidinLukF-PV (lukF-PV) genes, complete cds

Sequence ID: MK902786.1Length: 1930Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
614 bits(680)	2e-171	340/340(100%)	0/340(0%)	Plus/Plus

Table 6: Range 1: 658 to 997GenBankGraphics. Next MatchPrevious Match.

Query 1 TTTGTGCCAGACAATGAATTACCCCCATTAGTACACAGTGGTTTCAATCCTTCATTTATT 60

Sbjct 658 717

Query 61 GCAACTGTTTCTCATGAAAAAGGCTCAGGAGATACAAGTGAATTTGAAATAACGTATGGC 120

Sbjct 718 777

Query 121 AGAAATATGGATGTTACTCATGCTACTAGAAGAACAACACACTATGGCAATAGTTATTTA 180

Sbjct 778 837

Query 181 GAAGGATCTAGAATACACAACGCATTTGTAAACAGAAATTACACAGTTAAATATGAAGTG 240

Sbjct 838 897

Query 241AACTGGAAAACTCATGAAATTAAAGTGAAAGGACATAATTGATATGaaaaaaTAGTCAA 300

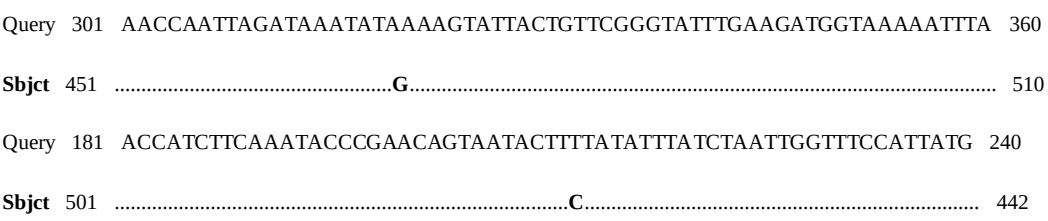
Sbjct 898 957

Query 301 ATCATCAGTTGTTACATCAATTGCATTGCTTTTGCTATCC 340

Sbjct 958 997

Figures 3. The DNA sequence of the Luk-pv gene from Staphylococcus aureus.

The Gene Bank found that part of the (Seb) gene having 99 % compatibility with the subject of the (sub) gene in NCBI under sequence ID: **KX168628.1**as seen in Figure (4), have two Transition(A\G) and (T\C) in location(470) for both, with Silent effect.



Figures 4. DNA sequence of the Seb gene from Staphylococcus aureus.

Discussion

The results of this study showed that the (seb) gene is present in isolates of *staph aureus* bacteria isolated from patients with atopic dermatitis 12 /25, which is higher than the results of the researchers (Asaad, A. M., Jehan et al., 2010) in Egypt. However, the results [10] showed the presence of (30.5 %) of the seb gene isolated from the *staph aureus* bacterium, which is different from the results of the researcher [11]. The results of the current study showed the absence of the sec gene in all isolates of Staphylococcus aureus isolated from patients with atopic dermatitis, which are similar results to what was stated in the research of (Asaad et al., 2000) and Slightly similar to the results of the researcher [11]. Whereas the number of sec genes is 2/74 (2.7%), it is in agreement with the results of (Lo, W. T., et al., 2010), as they showed that the (sec) gene did not appear in any of the isolates. [15'16] reported the detection rate of S. aureus that produces superantigens from different skin areas in AD patients.

Moreover, Wongboot et al. suggested that the differences in the geographic distribution of S. aureus SEs genes may be explained if SEs are located on mobile genetic elements that may be exchanged between bacteria of the same or different species [17]. Luk-pv was the gene carried by Staphylococcus aureus. It is a convergent result with [18]. In a study of [19], it was found that (87.59%) out of 137 Staphylococcus aureus isolates contain the luk-pv gene, which is comparable to the current results. Another study conducted in Greece [20] showed that the number of isolates was only 10/260 of Staphylococcus aureus. It was positive for the PVL gene, which differs from the results of this study.

Conclusions

This study showed that the (seb) gene is present in isolates of staph aureus bacteria isolated from patients with atopic dermatitis 12 /25 at a percentage (48 %). Furthermore, the absence of the sec gene in all Staphylococcus aureus isolates isolated from patients with atopic dermatitis—the results of the detection of the luk-pv 23\25 (92%) gene encoded for lukucidin.Polymerase chain reaction using different primers successfully identified Staphylococcus aureus enterotoxins (B, C)and luk-pv, luk-pv gene, which was the most frequent.

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