Plant Archives Volume 20 No. 2, 2020 pp. 8851-8862 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

THE PREVALENCE OF SOME VIRULENCE PLASMID GENES IN *STAPHYLOCOCCUS AUREUS* INFECTION

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Abstract

Staphylococcus aureus is a normal flora and opportunistic pathogen. It is the most common in skin and soft tissue. This microbe can cause more diseases, for example: burn inflammation and tonsillitis through the production of virulence factors that are acquired by some plasmid virulence genes. The main research objective explored the prevalence of (*edin-c, sej* and *etb* genes) in each patients and healthy and its relationship with the development of infection in different clinical source. This study was conducted in the Laboratory of Department of the Pathological analysis, Faculty of Science, Thi-Qar University\ Iraq from October 2019 to March 2020, A total of 640 samples were collected from patients and healthy human. The clinical samples include 170 of patients suffered from burns in the department of burns in Al-Hussein Teaching Hospital, also 150 samples of patients were clinically diagnosed as having tonsils infections in the "E.N.T" department in the AL Habboby hospital while human healthy samples were 320 were collected from different parts of the body. These samples were diagnosed phonetically and genetically. Antibiotic susceptibility test was performed by the modified Kirby-Bauer disc diffusion method according to CLSI guidelines. According to the cultural, morphological and biochemical characteristics, Prevalence of *S. aureus* were 17.6% (n=30), 13.3% (n=20) and 5.94% (n=19) to Burn patients, tonsillitis patients and healthy human. The results of phenotypic diagnostic were confirmed by polymerase chain reaction (PCR) technique employing *16SrRNA* gene that showed only 48\50 certain bacterial isolates of this microbe from patients while in healthy humans were in line with phenotypic diagnostic (19\19), results of molecular screening of prevalence *edin-c, sej* and *etb* genes in burn patients had showed 48.27%, 62.06% and 62.06% respectively while in tonsillitis patients was 42.10%, 73.68% and 47.36%. Comparing to healthy humans, there were no results to determine *edin-c, sej* except *etb* (21.05%). In conclusion, Plasmid virulence *sej* gene was most found in *S. aureus* isolates of tonsillitis patients whereas the highest prevalence of plasmid virulence *edin-c* and *etb* gene was in *S. aureus* isolates of burn patients.

Key words: *Staphylococcus aureus*, Plasmid virulence gene, Patients, Healthy humans.

Introduction

Staphylococcus aureus is a normal flora and opportunistic pathogen; typical carriage sites are the anterior nares, pharynx, perineum, and skin. It is the most common pathogen in skin, soft tissue, and musculoskeletal infections. This pathogen occurs in 20-30 percent of people (Troeman *et al*., 2019; Bouvet *et al.*, 2017; Zhang and Burbridge, 2011). *Staphylococcus aureus* has emerged as a major microbe for both nosocomial and community-acquired infections. It may cause types of illnesses such as bacteremia, meningitis, pneumonia, ocular infections, toxic shock syndrome, osteomyelitis, and other systemic infections. This microbe can also cause burn inflammation and other diseases in children and young adults, for example, tonsillitis (Cavalcanti *et al.*, 2019; Ondusko and Nolt, 2018). Burn wounds are a good location for growth and increase of pathogens. They are considered rich sources of persistent infection, The major bacteria isolated from this site are *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes* and different coliform bacilli but it is believed that bacteremia is the best-described manifestation of *S. aureus* infection (Alebachew *et al.*, 2012). Bacterial tonsillitis is an inflammation of the upper respiratory tract that often infects children and teenagers. *Staphylococcus aureus* is considered to be one of the most prevalent organisms in the etiology of tonsillitis and is important due to its antimicrobial resistance and persistence in tonsil internal tissues (Cavalcanti *et al.*, 2019). This bacteria can produce virulence factors that are one of the causes of its pathogenicity. These are: Pantone-valentine (PVL),

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protein A, several enzymes, capsule and superficial proteins (Sakr *et al.*, 2018). The genome of *staphylococcus aureus* consists of the core genome for cell survival and accessory genome for adaptation in different ecological conditions. Mobile genetic elements (MGEs) are DNA fragments that encode a number of virulence and transmit horizontal gene transfer (HGT) between cells. *S. aureus* includes many types of MGEs, for example: plasmids, bacteriophages, and staphylococcal cassette chromosomes (You *et al.*, 2018; Segerman, 2012). Plasmids enable genetic material to be transferred; especially antimicrobial genes between bacterial species and genera. *Staphylococci* may have one or more plasmids per cell that are loaded with various gene content. They can be categorized into: small plasmids with a single resistance determinant, larger plasmids with multiple resistance determinants and multi-resistance plasmids with conjugative resistance. Plasmids *of Staphylococci* may encode resistance or virulence to a variety of organic ions, inorganic ions, and toxin genes to help create an appropriate environment for *Staphylococcus aureus* (Al-Oqaili, 2018; Kwong *et al.*, 2017; Peacock, 2010). In Iraq, the prevalence of *Staphylococcus aureus* was considered as a serious indicator, there were local studies had conducted by Mater *et al*., (2019); Taher *et al*., (2019); Rasool *et al*., (2016); Al-Taie, 2014; Alkaabi, 2013 and Jassim *et al*., (2012). These studies had showed increase in the levels of these bacteria; therefore this research would be compare prevalence of pathogen in patients and healthy humans to know the real reasons of prevalence by screening of some plasmidic virulence genes and their effect on the development of infection.

Materials and Methods

Samples Collection and phenotypic analysis

The current study had included 640 samples, 320 samples of healthy humans and 320 samples of patients with burn and tonsillitis, the samples were collected during the period between the first of October 2019 until the first of March 2020. patients' samples were taken from burn unit in Al-Hussein Teaching Hospital and also from those were clinically diagnosed as having tonsils infections in the "E.N.T" department in the AL Habboby hospital in Nassiryia city\Iraq. Samples were immediately transferred by transport media(Cary Blair) to the Pathological Laboratory\Faculty of Science within two hours for cultivation, staining, and sensitivity tests. This conducted following the World health organization standard microbiological diagnosis for *S aureus*.

In the laboratory within aseptic conditions, all swabs were streaked on MacConkey agar to make sure that is a Gram-positive and to ensure free contamination. Also on 5% human blood agar for the activation and detection of bacterial ability to lyses red blood cells and on Mannitol salt agar as well as DNase ager to identify pathological species(Berkowitz and Jerris, 2016; Hall, 2013). *Staphylococcus aureus* has been recognized, according to the morphological characteristics of the bacterial culture and biochemical tests (Greenwood *et al*., 2012). It is used Gram stain to investigate the bacterial morphological characteristics and to discriminate between two broad communities of bacteria - Gram-positive and Gram-negative based on their components in the wall (Murray *et al*., 2015). Several biochemical tests were attained to facilitate identification of *S. aureus* as follows: Catalase test, Oxidase test , Coagulase test and API Staph system (BioMereux, France), (Shell *et al*., 2017; Cobos-Trigueros *et al*., 2017; Berkowitz and Jerris, 2016; Subhash, 2012).

Antibiotics discs

Azithromycin (ATH.15µg), Tetracycline (TE.30µg), Vancomycin (VA.30µg), Ciprofloxacin (CIP.5µg), Gentamycin (GEM10µg), Nitrofurantoin (NIT.300µg), Levofloxacin (LEV.5 µg) from MAST/U.K, Doxycycline (DO.10 µg), Penicillin G (P.10 unit), Ofloxacin (OFX.5µg) from Bioanalys/Turkey, clindamycin (CD.2µg), Erythromycin (E.15µg) from HIMEDIA/India.

Molecular analysis

Primers: Research primers are mentioned in the table 1.

Extraction of the bacterial DNA:

Genomic DNA and Plasmid DNA was extracted and purified according to the instructions of the company Favorgen/China the instructions.

Concentration and purity estimation of DNA

The obtained nucleic acids were measured using the Nanodrop spectrophotometer, which evaluates the concentration of DNA (ng/µl) and regulates. Pure DNA & absorbance were at (260/280 nm).

Statistical analysis

The Chi square test had conducted of all variables by IBM SPSS, version 20 and Microsoft Office Excel 2010. The P - value < 0.05 was considered significant.

Results and Discussion

Isolation and identification of *Staphylococcus aureus*

From 170 and 150 burn and tonsillitis samples, only 30(17.6%) and 20(13.3%) were *Staph.aureus* respectively and 15(8.80%) and 2(1.30%) were others

Table 1: Sequences of research primers.

Gene	Primer (5'-3')		Amplicon size (bp)	Annea-ling(c)	Reference
etb	F	CAGATAAAGAGCTTTATACACACATTAC	612 bp	59	(Xie *et al.*, 2011)
	R	AGTGAACTTATCTTTCTATTGAAAAACACTC			
sej	F	CAGCGATAGCAAAAATGAAACA	426 bp	59	
	R	TCTAGCGGAACAACAGTTCTGA			
16s rRNA	F	TCAACCGTGGAGGGTCATTG	556 bp	59	(Shakir, 2018)
	R	GTTTGTCACCGGCAGTCAAC			
edin-C	F	TATTAAGCATTCATTCAA	629 bp	44.5	(Maleki *et al.*, 2019)
	R	AGTGTAGTCTGTTCCTCT			

Staph.spp. respectively, in comparison to 320 samples healthy human (control), only 19(5.94%) were *Staph.aureus* while the others *Staph.* spp were 10(3.12%).

The isolates that could grow on Mannitol salt agar (MSA) considered the genus *Staphylococci* because MSA is selective and differential medium. The high sodium chloride concentration (7.5 %) makes toxic medium and destroys most bacteria except for *Staphylococci*, which can withstand and survive in the medium largely due to its tolerance to salty environments such as human skin. If the species can ferment mannitol sugar and create acid, then the medium phenol red would change the color from red to yellow. This made the medium differential (Devisscher *et al*., 2013).

All the positive 67(20.93%) of patients with burn and tonsillitis, also 29(9.06%) of healthy humans were considered as *Staphylococci* because it were yellow colonies, the high proportion of salt encourages *Staphylococci* to develop carotenoid pigments and can turn the medium around the colony into yellow due to mannitol fermentation whereas others white colonies were non-pathogenic *Staphylococci* would not ferment mannitol, Fig. 1 and table 2 (Thakur *et al*., 2017).

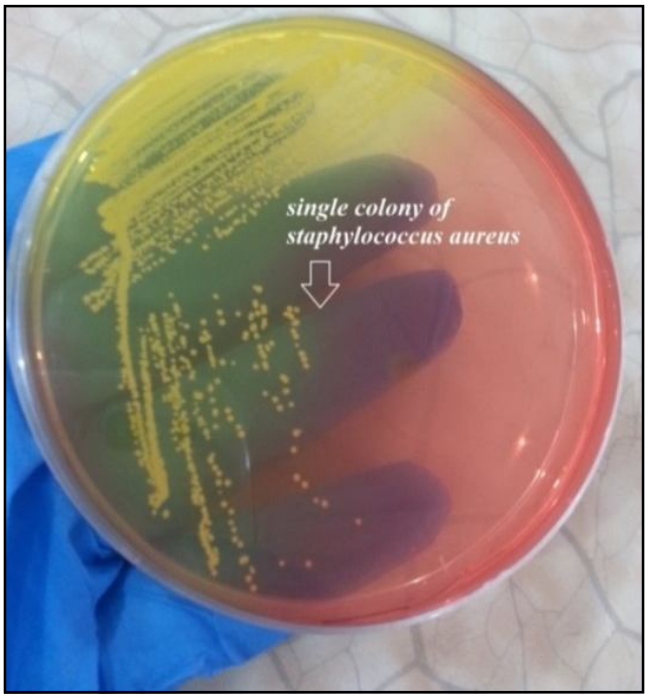

Fig. 1: *Staphylococcus aureus* colonies on Mannitol-Salt agar under 37°C for 24 hours.

Distribution of *Staph.aureus* depend on type of source

The presented study showed 17.60%, 13.30% and

Table 2: Microscopic and biochemical identification of *S. aureus.*

Culture & Biochemical tests	Isolates patients		Control	
	Staph.spp	***Staph.aureus***	***Staph.spp***	***Staph.aureus***
Mannitol-Salt agar	67(20.93%) yellow colonies		29(9.06%) yellow colonies	
Catalase	67(20.93%) bubbles		29(9.06%) bubbles	
Oxidase test	67(20.93%)without color		29(9.06%) without color	
Gram stain	67(20.93%)clusters		29(9.06%) clusters	
MacConkey agar	67(20.93%)No growth		67(20.93%)No growth	
Coagulase	17(5.3%)	50(15.6%)	10(3.12%)	19(5.94%)
DNase agar	17(5.3%)	50(15.6%)	10(3.12%)	19(5.94%)
API Saph	17(5.3%)	50(15.6%)	10(3.12%)	19(5.94%)
Blood agar(Hemolysin test)	-	50(15.6%)	-	19 (5.94%)
Urease production test	-	50(15.6%)	-	19 (5.94%)
Proteases production	-	50(15.6%)	-	19 (5.94%)

5.94% of *staph aureus* to each burn and tonsillitis patients as well as healthy human, Fig. 2.

from170 samples, 17.60%(30) *staph.aureus* was documented in clinical specimens of burn patients, this finding very line with Li *et al*., (2018) who recorded 19% in Southeast China, furthermore, there was an agreement with a study in Pakistan which by Saaiq *et al*., (2015) who noted 18.62%. In Nepal, it recorded 18% by Lamichhane *et al*., (2019), our findings were close to other local studies in Iraq, 20.6% by Aljanaby (2018). There were other studies pointed out a low level of prevalence *staph aureus*, these were in Ghana, 2.3% by Forson *et al*., (2017), in Iraq (Sulaimani City), 10% by Rashid *et al*., (2017), in Turkey, 11.2% by Bayram *et al*., (2013).

From 150 samples, Prevalence of *staph. aureus* was 13.3% (20) in tonsillitis patients, these outcomes were very similar to Kalaiarasi *et al*., (2018) who indicated 12.62% also approaching of the results of Abd El Galil *et al*., (2014) who mentioned 17.1%, but dissimilar with other local studies in Iraq by Fahad (2018) and Mater *et al*., (2019) who recorded 6.84% and 26.66%, respectively. Other studies pointed out different percentages, for example, were 45.1%, 40.7% and 56.75% in Poland, Brazil, and India, respectively (Katkowska *et al*., 2020; Cavalcanti *et al*., 2019; Moirangthem and Gurung, 2013).

The current results showed a high prevalence in anterior nose samples 22.5% and low prevalence in Skin chest and Axilla samples (1.25% and 0% respectively), as well as the following percentage, was recorded: 8.33%, 4% and 2.85% for Hands, Forearm and Throat samples, respectively, table 3.

These results were consistent with local previous study by Amna *et al.,* (2012) who found that 22% of carrier *S.aureus* in the anterior nares as well as other local studies by Rasheed and Hussein (2020); Dogramachy (2018); AL-Janabi (2014); Al-Dahbi and Al-Mathkhury (2013) and Abdulrahman and Taher (2019) recoded close ratio 15.65%; 24.5%; 28.8%, 30% and 34%,

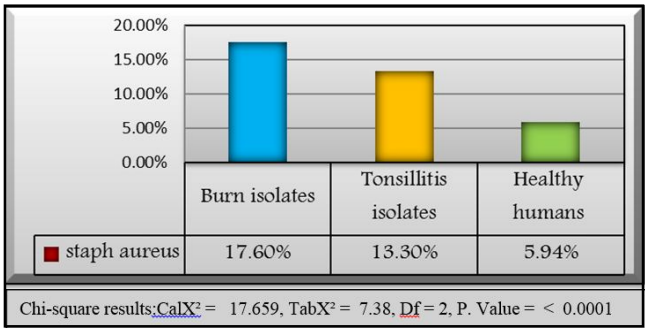

Fig. 2: Prevalence of staph aureus in patients and healthy humans.

Table 3: Samples of healthy people and number of positive *staph.aureus*.

S. aureus Sample Site	Negative		*S. aureus*		Overall total	
	No.	%	No.	%	No.	%
Skin Chest	79.0	98.75	1.00	1.25	80.0	25.0
Throat	68.0	97.1	2.00	2.90	70.0	21.9
Hands	55.0	91.7	5.00	8.30	60.0	18.8
Forearm	48.0	96.0	2.00	4.00	50.0	15.6
Anterior Nose	31.0	77.5	9.00	22.5	40.0	12.5
Anxilla	20.0	100	0.00	0.00	20.0	6.3
Overall total	301	94.06	19	5.94	320	100
$CalX^2 = 26.199$	$TabX^2 = 11.07$		DF = 5		P. Value = 0.0001	

respectively.

The variation in percentages may be due to the geographical characteristics of the Iraqi environment. Most Iraqi research had been published about *S. aureus* nasal carriage while the isolation of *staph. aureus* was very limited from other carriages. Estimating the percentage of pathogens in all possible parts of the human body was very necessary because it may be important in the stages of the development disease. Our study suggested a significantly higher prevalence of *S.aureus* from the anterior nostrils of healthy individuals. These outcomes was of critical significance as carriage of *S.aureus* in the nose might seem to play an essential role in the pathogenesis of infection as well as epidemiology (Fomda *et al*., 2014).

Antibiotic susceptibility test

All the 69 *Staphylococcus aureus* isolates were tested to determine their antibiotic susceptibility patterns by antibiotic disc diffusion method and Minimum inhibitory concentration (MIC) of vancomycin were performed using the E-test method (bioMerieux, France). This was according to CLSI (2019). All isolates showed multiple antibiotic resistances to the antibiotics tested, the results showed in Fig. 3.

Molecular analysis

Diagnosis of samples

Recently several molecular methods based on PCR have been introduced as an alternative way to accurately identify. The diagnostic gene, *16srRNA* gene was used in this study to denote isolates belonging only to *staphylococcus aureus* (Ali *et al*., 2014). PCR analysis was applied in all study *S.aureus* isolates, previously phenotypic diagnosed, table 4 and Fig. 4.

The appearance of negative results may be related to a genetic mutation in *16srRNA* gene which can be supported by Kabbashi *et al.,* (2019) and Mohammad (2018) whose confirmed the occurrence of a genetic

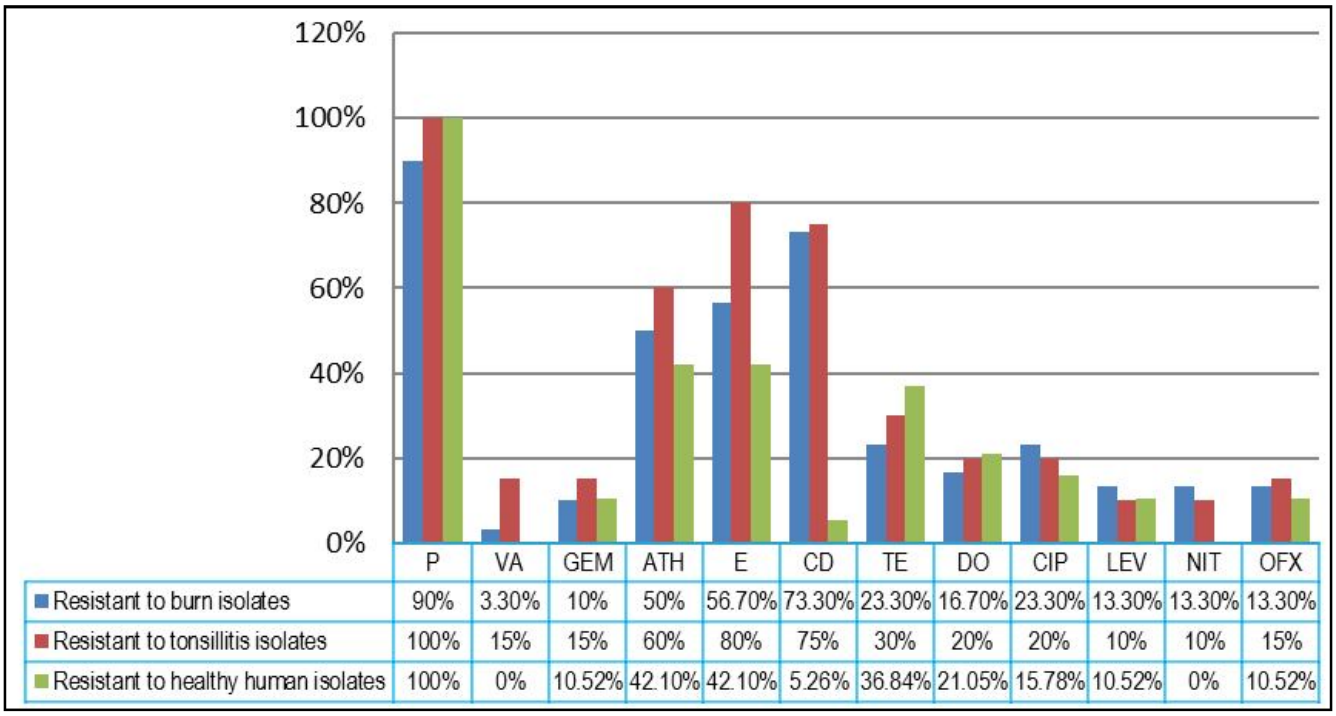

Fig. 3: Antibiotic resistance of *S aureus* isolates.

mutation in *16srRNA* genes in *E. coli* and *staphylococcus.* spp, respectively.

Plasmidic Virulence genes

***Edin-c* gene**

Staphylococcal invasion of the skin or mucosa is a significant possibility of bacterial transfer to internal tissues and bloodstreams. *Edin-c* as well as other alleles of *Edin* are produced from specific strains and carry out similar biological pathways towards Rho proteins but are encoded in different genetic backgrounds. These toxins attack host Rho GTPases proteins, in particular RhoA proteins. RhoA proteins are the professionals responsible for the regulation of host cell actin cytoskeleton and important in controlling cell stickiness, mobility and phagocytosis. Inhibition of these proteins lead to blocks of the major mechanism that are responsible for both actin filament elongation and its assembly in contractile actomyosin cables, RhoA activation in endothelial cells helps to control the creation of intercellular gaps by activating contractile actomyosin fibers, that are pulled along intercellular borders. Scientific discoveries demonstrate that inhibition of RhoA affects the function of the endothelial barrier by generating transcellular tunnels known to as macroapertures (Popoff, 2014; Munro *et al.*, 2011; Munro *et al.*, 2010). The PCR technique has been used to confirm the presence of *edin-c* in all *S. aureus* isolates in: burn and tonsillitis patients as well as healthy humans. The results were 48.27% (14/29) to burn and 42.10% (8/19) to tonsillitis, but *edin-c* did not give any bands in healthy humans, Fig. 5 and 6.

Penicillin G resistance were 100% in tonsillitis patients but 90% in burn patients; therefore there were consistent of our results with Maleki *et al.,* (2019) who had mentioned that most of the sensitive isolates to Methicillin carried the gene (*edin-c*) in rate 42.85% for MSSA but MRSA were 25.92%. Munro *et al*., (2011) had indicated that 90 percent of all *edin* gene carrying *S. aureus* isolates may have the type-C genes. Other studies by Madzgalla *et al*., (2016) in Pakistan showed 31.11 percent of *edin-c* in patients with skin and soft tissue infections, as well as other studies by Yu *et al*., (2015) in China, recorded 5.5 percent of *edin* genes to same sample previously mentioned. A study by Yang *et al*., (2020) on cases of subclinical bovine mastitis was unable to identify

Table 4: Comparison between phenotypic and genotype diagnosis of *Staphylococcus aureus* was isolated from patients and healthy humans.

Source	No. isolates in phenotypic diagnosis	Perce-ntage	No. isolates in genotypic diagnosis	perce-ntage
Burn isolates	30\170	17.60	29\170	17.05
Tonsillitis isolates	20\150	13.30	19\150	12.66
Healthy humans	19\320	5.94%	19\320	5.94%
Total	69\640	10.78%	67\640	10.46%
CalX^2 = 26.102	TabX^2 = 12.59	DF = 6	P. value = < 0.0001	

edin-c in the samples. A research conducted in Gabon by Schaumburg *et al*., (2011) isolated from infection and carriage samples and recorded 0.61 percent to carriage but 0 percent to infection. All of these results may reflect a broad concept of determining these genes and their distributed nature. The distribution of *edin-c* in burn and tonsillitis patients supported the theory that *edin-c* could did with the arsenal of *Staph.aureus* virulence factors that encourage bacteria a greater capacity to cause infection. This may allow us to predict the possible risks for serious infection and spread (Courjon *et al.*, 2015; Messad *et al.*, 2013).

Sej gene

In recent years, Iraqi researchers have become very important to screen about classical Staphylococcal enterotoxin genes in clinical samples (Hamim, 2017;

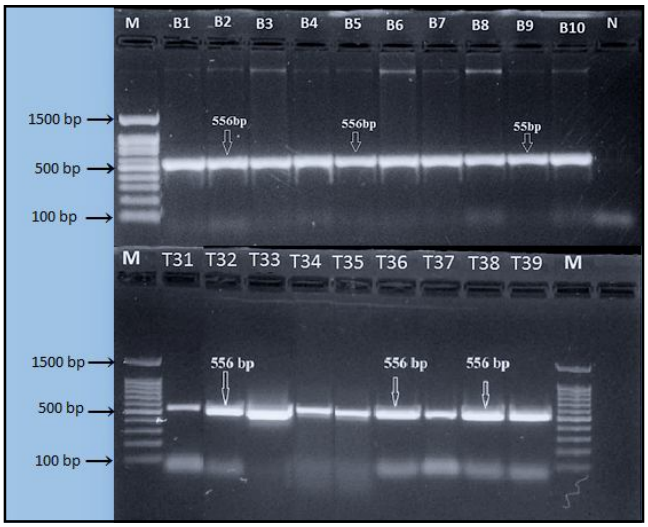

Fig. 4: Agarose gel electrophoresis that shown the PCR product determination of *16srRNA* gene, Where M: marker 1500bp, Agarose 1%, TBE buffer (1x), 100 V with 30min. then 50 V with 45min. , stained with ethidium bromide, visualized on a UV transilluminat or documentation system, Positive lane [Burn : B1-B10; tonsillitis: T31-T39].

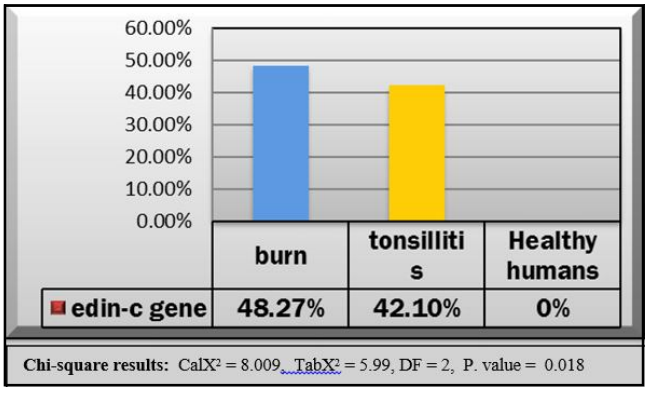

Fig. 5: The prevalence of *edin-c* gene in *S. aureus* clinical isolates.

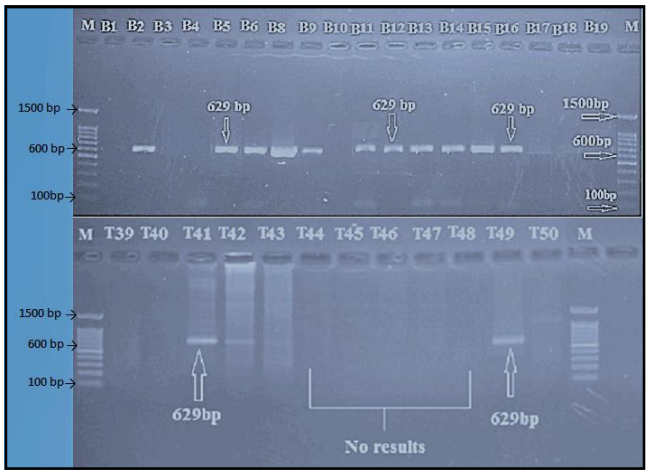

Fig. 6: Agarose gel electrophoresis that shown the PCR product determination of *edin-c* gene, Where M: marker 1500bp, agarose 1 %, TBE buffer (1x), 125 V with 25min Then 75 V with 1 hr and stained with ethidium bromide, visualized on a UV transilluminator documentation system, .Positive lane (Burn : B2, B5, B6, B8, B9, B11-B16 ; Tonsils : T 41, T42, T49).

Abed *et al.*, 2016). Staphylococcal enterotoxin J (SEJ) belongs to new types of SEs. Generally, it is classified as members of the pyrogenic toxin super-antigen family. They are marked by simultaneous associates to the major class two histocompatibility complex in the Antigen-Presenting-Cell and receptors of T cell, with the absence of specific antigens, leading in systemic toxicity like high fever and hepatic and renal disorders. These toxins are highly resistant to denaturation statuses such as low pH, heat treatment, and proteolytic enzymes. SEJ presents sequences similar to SED (classical SEs), corresponding to 51% and located on the same genetic origin that is related to plasmid pIB485 and the detection of one usually indicates the presence of the other (Hasan and Hoshyar, 2019; Alfouzan *et al.*, 2019; Jain *et al.*, 2019; Avila-Novoa *et al.*, 2018; Monistero *et al.*, 2018; Madahi *et al.*, 2014; Indrawattana *et al.*, 2013; Monecke *et al.*, 2011). The PCR results explained where it was 73.68% (14/19) and 62.01% (18/29) belong to tonsillitis and burn isolates, but we could not determine this gene in healthy humans, Fig. 7 and 8.

The prevalence of this gene in patients with tonsillitis may be appeared very high compared to patients with burns. This difference can be illustrated in many ways, for example: contamination or it may be genetic changes in the isolates of burns that prevented the expression of this gene. There were other studies about the detection of this gene, Rall *et al.,* (2010) found that *Sej* was 29.2% of Samples that were collected from the hands and anterior nares while Liu *et al.,* (2018) determined that *Sej* was 29.98% of samples that were gathered

from healthy animals and patients from Henan Province, China, but Ren *et al.,* (2020) prescribed that *Sej* was 7.7% of isolates that were subclinical bovine mastitis in southern Xinjiang, China; therefore the difference in the prevalence of this gene prospect due to the geographical location and other aspects mentioned previously.

etb gene

Desmosomes are the intercellular junctions necessary for strong intercellular solidarity mediation. This is made up of three families of proteins that include desmosomal cadherins, desmogleins (DSGs) as well as desmocollins (DSCs) (Hatzfeld *et al*., 2017). Desmoglein (Dsg1) is one type of protein belonging to desmogleins which recognized and hydrolyzed by exfoliative toxins (ETs). These toxins are serine proteases synthesized via *S. aureus* that cause the loss of cohesion between adjacent keratinocytes in the superficial epidermis and to a less extent in the mucous membranes (Imanishi *et al.*, 2019; Mohseni *et al*., 2018; Leke *et al.*, 2017; Arias *et al.*, 2016). There are many types of these toxins. *ETB* gene was the target in this current study, the results showed 62.06% (18\29) in burn isolates and 47.36% (9\19) in tonsil isolates as well as 21.05% (4\19) in healthy humans, Fig. 9, 10 and 11.

Several investigations have prescribed different findings of instability in the prevalence of ETB toxin genes in those patients, including studies conducted by Islam *et*

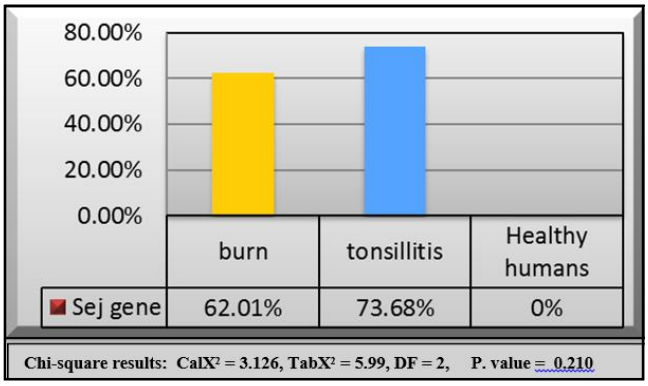

Fig. 7: The prevalence of *Sej* gene in *S. aureus* clinical isolates.

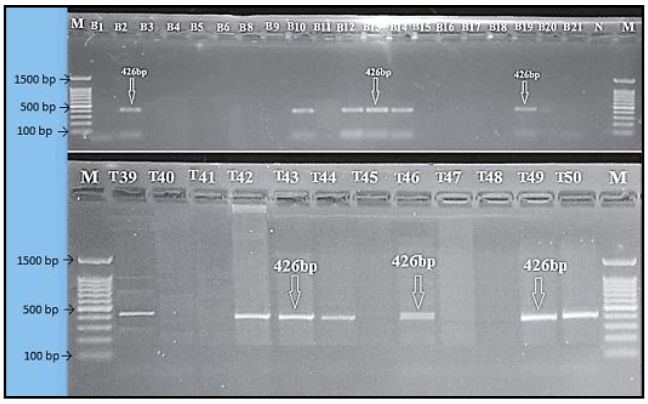

Fig. 8: Agarose gel electrophoresis that shown the PCR product determination of *sej* gene, Where M: marker 1500bp, agarose 1 %, TBE buffer (1x), 125 V with 25min Then 75 V with 1 hr and stained with ethidium bromide. Positive lane (Burn:B2.B6, B10-B12-B14, B19and Tonsils:T39, T42-T44, T46, T49, T50) N: negative control and visualized on a UV transilluminat or documentation system.

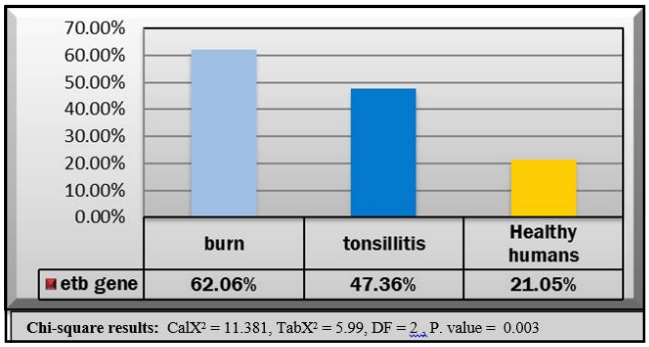

Fig. 9: The prevalence of etb gene in S. aureus isolates.

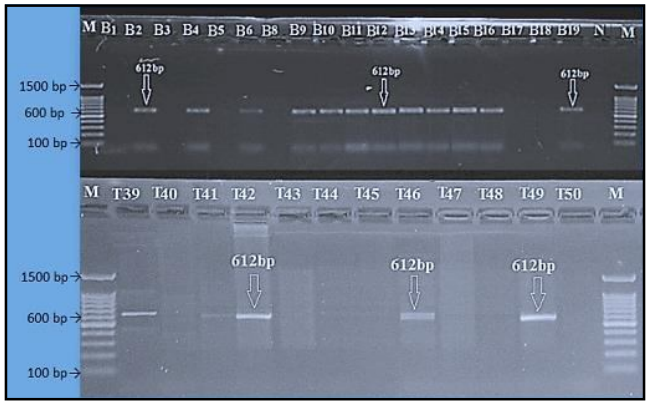

Fig. 10: Agarose gel electrophoresis that shown the PCR product determination of *Etb* gene, Where M: marker 1500bp, agarose 1 %, TBE buffer (1x), 125 V with 25min Then 75 V with 1 hr and stained with ethidium bromide. Positive lane (Burn:B2, B4, B6, B9-B16, B19; Tonsillitis:T31,T35-T37,T39,T41,T42,T46T,49), N:negative control and visualized on a UV transilluminator documentation system.

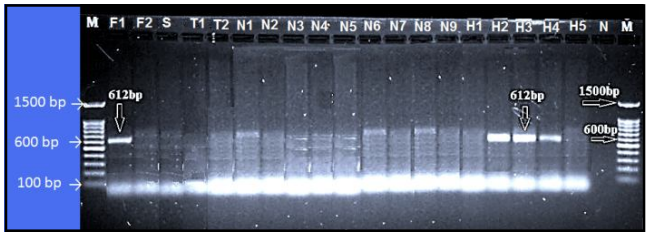

Fig. 11: Agarose gel electrophoresis that shown the PCR product determination of *Etb* gene, Where M: marker 1500bp, Agarose 1 %, TBE buffer (1x), 125 V with 25min Then 75 V with 1 hr and stained with ethidium bromide. Positive lane(F1: Forearm;H2-H4:Hands). Negative lane (F2: Forearm; S: Skin chest; T1-T2: Throat; N1-N9: Nasal swabs; H1: Hands), N:control.

al., (2019) and Katkowska *et al.,* (2019) whose had mentioned no positive results (0%) to the prevalence of *etb* genes in burn and tonsillitis patients but in other studies, they showed a very large variation of the recurrence of this gene, these studies were by Mahmoudi *et al.,* (2019) 92%; Tahbaz *et al.,* (2019) 30.5%; Yaslianifard, 2017 10%; Nezhad *et al*. (2017) 4.2% and Goudarzi *et al.,* (2019) 2.2%. The differences in the occurrence of *etb* genes in these researches suggest a geographic variation in the distribution of *etb* strains of *Staphylococcus aureus*. This variability may be reflect distinct ecological reservoirs present in different countries or may originate from differences in the sensitivity and specificity of techniques used to detect ETB toxins gene. Compared to healthy humans, Many studies researched *etb* gene identification in Healthy people, some of these studies had succeeded, and the other failed to find it. In Ukraine, Netsvyetayeva *et al*., (2014) documented 11.11 percent; in India, Abimanyu and Murugesan (2013) recorded 8.7 percent, while in Tunisia, Karim *et al*., (2014) recorded 3.63 percent, as well as in the current study in Iraq, recorded a higher percentage (21.05 percent) than the previous studies. In addition to the studies in Angola and china by Conceição *et al*., (2015) and Wang *et al*., (2019) recorded a lower percentage (0.2 percent) and (1.89 percent) of *etb* gene prevalence, respectively. On the other hand, no percentage was revealed to determine the prevalence of *etb* genes in Iran, Malaysia, Thailand, São Tomé and Príncipe and the United States (Mir *et al.*, 2019; Zarizal *et al.*, 2018; Blomqvist *et al.*, 2015; Conceição *et al.*, 2014; Jochmann, 2013).

Conclusion

In conclusion, The highest percentage of *Staphylococcus aureus* had been observed in burn patients, Most *Staph aureus* isolates can able to produce virulence factors such as hemolysins, urease, and protease. Anterior nose and Hands were more colonized by *staph.aureus* in healthy humans. *Staph aureus* isolates of study showed multiple resistance to several antibiotics used, especially β lactams, where these antibiotics now not active in the treatment of staphylococcal infection. Most antibiotic sensitivity in burn patients was to Vancomycin, but in tonsillitis patients was to Levofloxacin. Antibiotics including: Penicillin-G, Azithromycin, Erythromycin and clindamycin were most resistant by isolates of study *Staph aureus* in burn and tonsillitis. Plasmid virulence *sej* gene was most found in *S. aureus* isolates of tonsillitis patients whereas The highest prevalence of plasmid virulence *edin-c* and *sej* gene was in *S. aureus* isolates of burn patients.

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