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ORIGINAL ARTICLE

Molecular investigate of slim and adhesion genes of *Staphylococcus saprophyticus* isolated from pregnant women with asymptomatic bacteriuria in Thi-Qar governorates

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ABSTRACT

Aims: to investigate and compared the frequency of biofilm formation *ica A* and adhesion genes *clf A*, *cnaA*, *fnbA* and *fnbB* in chromosome and plasmid of *S. saprophyticus*.

Materials and methods: 48 isolates about *S. saprophyticus* were selective randomly from total 1019 isolated from pregnant women with asymptomatic bacteriuria where the samples were collected in AL Habboby Hospital and Al-Hussein teaching, and bent Al-Huda hospitals from the first of April 2022 until the first of September 2023 and bacteria of study were identified depend on previous method.

Results: PCR appeared, higher existence percentage of study virulence factors genes was in chromosome that reached 399.9% of study bacteria, more than in its in plasmid 66.5%, however the frequency rate of study genes in chromosome was as following: ica A and fnb A genes were complete, 100% (48/48), while cna A and clf A genes percentage were identical and came in second grade that arrived significant rate 83.3% (40/48), the lowest results were showed 33.3% (16/48) isolates harbored fnb B gene, while they were decreased in its plasmid as following: ica A and fnb A genes were 12.5% (6/48) and 22.9% (11/48) respectively, the cna A and clf A genes percentage 6.2 % (3/48) and 4.1% (2/48) respectively, fnb B gene 20.8% (10/48).

Conclusions: Depend on above data may be ica A, fnb A, clf A, cna A genes more occurrence in comparison to fnb B genes among *S. saprophyticus* and plasmid may double the rate of same virulence study genes.

KEY WORDS: molecular, slim and adhesion genes, chromosome, plasmid, *S. Saproph*yticus, asymptomatic bacteriuria

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INTRODUCTION

Staphylococcus genus is present in different clinical site as normal flor causes opportunistic infections in human. *S. saprophiticus* causes urinary tract infections, especially in girls. It is present in over 40% of all young sexually active women as part of their normal genitourinary flor [1-4]. It represents serious to public health by its capability to give rise to infections in human and fixed in the ambience, obtain and freight plasmids, which can accentual antibiotic resistance and virulence [5, 6]. The capacity of different *Staphylococcus* species and strains to causes disease related to release a large number of virulence factors that are product of virulence gene in chromosomes. Some of these factors are importance in distinct sickness or at various phases of a pathogenesis of limited infections which may lead to morbidity and mortality [7]. Generally, many pathogenic bacteria carry genes for virulence in plasmids or other mobile genetic elements [8]. The bacteria can infected humans by virulence factors carried on virulence plasmids through a different mechanisms, which are either toxins that caused damage and kill cells of animal, or others which introduce help to bacteria for connect and invasive cells when others act to protect bacteria from the action of immunity system [9]. Most plasmids can be transferred between different bacteria by conjugation [8]. *S. saprophyticus* can develop and carry plasmids contain antibiotic resistance and virulence genes [5, 6, 10]. Recently, attentions focused on the adhesions in staphylococcal infections. When declared, protein receptors in the cells suppose the remarkable functions in adhesion of microbe [7]. The presence of adhesions genes such as cna, clf A and biofilm formation (ica loci) genes in clinical *Staphylococcus* were considered important factors of virulence [11]. The ica loci of biofilm formation is involved polysaccharide intercellular adhesion, that called PIA and also biofilm formation that is most interest one of virulence identifiers that making easy of cohesion and settlement of bacteria [12]. Synthesis of PIA is neatly back to the manifestation of ica A and ica D genes. The substantial function of ica A, synthesis

of N-acetyl-Dglucosamine polymer structure [13]. All tested strains of *Staphylococcus* sush as *S. saprophyticus* that can biofilms formed in vitro were contain specific locus called ica. Sequence analysis was found the omit of the locus of ica caused to loss of the capacity to biofilms formed, PIA production, or mediate activity of *N*-acetylglucosaminyl transferase during in vitro. The experiments of hybridization with cross-species clarified the the ica A found in some other species of *Staphylococcus*, proposed, cell-cell adhesion and the ability to biofilms formed is saved in the genus *Staphylococcus* [14]. The ingredients of microbe surface distinguishing, the molecules of adhesion matrix that called MSCRAMMs, are mediate adherence to the extracellular matrix ingredients of host, such as collagen, fibrinogen and fibronectin [15]. Important type of these genes is collagen adhesion gene (cna) encoding CNA is determinant of the ability to bind collagen. It is not present in all *Staphylococcus* types and strains. The compare between the strains of cna-positive *S. aureus* and *its cna*-negative strains appeared that CNA product is in *S. aureus* infection [16]. Clumping factor (Clf) is cell surface associated protein and main mediator factor of the adherence of *S. aureus* to fibrinogen and fibrin. The binding of *staphylococcal* to fibrinogen has been appeared to be important in the infection with endocarditis by invasiveness and perhaps in the attachment of bacteria to implanted biomaterials. ClF acts as a virulence agent in specific infections by repression phagocytosis. The connection of *S. aureus* with fibronectin is interposed by two neatly concerning proteins FnBPA and FnBPB, that encoded with fnb A and fnb B genes respectively. FnBPs interposed adhesion with epithelial cells of human, involving endothelial cells, airway epithelium, and fibroblasts, that are may subsequently act in these cells to the bacteria internalization [17]. Not all virulence factors that play an interest role in pathogenesis of *Staphylococcus* spp., permitting its transition from any virulence stage to other infections are produced by each type and strain, there are evidence that *Staphylococcus* strains express different virulence patterns according to isolation sources. Definition virulence factors about bacterial pathogenicity in each infection are important for treatment decision [18].

AIM

The aim of the study was to investigate the occurrence rate of virulence factors genes about slims (ica A) and adhesions (cna A, Clf A, fnb A and fnb B) genes in plasmid and chromosomes among same bacteria isolated from asymptomatic bacteriuria by molecular scanning with PCR.

MATERIALS AND METHODS

CLINICAL ISOLATES SOURCE

Forty-eight isolates about *S. saprophyticus* were selective randomly from total 1019 isolated from pregnant women with asymptomatic *bacteriuria* where the samples were collected in AL Habboby Hospital and Al-Hussein teaching, and bent Al-Huda hospitals from the first of April 2022 until the first of September 2023 and bacteria of study were identified depend on method of [19-21].

DETERMINE THE PRODUCTION OF SLIME

The production of a slime in *S. saprophyticus* isolates of the study was phenotypically estimated by cultured bacterial isolates on Congo red agar method as described by Ilham B [12]. Congo red agar contained: 37 gm/L brain heart infusion broth, 0.8 gm/L Congo red, 50 gm/L sucrose agar. The incubation of plates was at 37°C for 24 hrs. and incubation of whole plates accrue according on altered the color after period of incubation about 24 to 48 h. A position test result was regarded reliantly black discoloration of the colony. The colonies color of study bacteria did not altered Congo red agar, when the slim did not producing by bacteria grown in plate of culture.

MOLECULAR ANALYSIS

CYCLING PROFILES.

The assays of PCR were occurred in a 25 µl of reaction volume, and conditions of PCR amplification were in thermal cycler (Memmert, Germany) that were particular to each single primer set singly, rely on procedure of their reference which were initial denaturation temperature (94°C/30 s), elongation temperature (72°C/60 s) and extension (72°C/1min) for each gene where denaturation and annealing temperature as following:

1. fnbA: 94°C/30 s and 50°C/30 s respectively;
2. fnbB: 94°C/30 s and 50°C/30 s respectively;
3. Ica A: 95°C/30 s, 60°C/1 min respectively;
4. Cna: 90°C/30 s, and 55°C/1 min respectively;
5. Clf A: 90°C/30 s, and 57°C/1 min respectively.

EXTRACTION OF GENOMIC DNA.

The chromosomal DNA Mini Kit and plasmid DNA Mini Kit were used to extracted and purified of chromosomal and plasmid DNA from *S. saprophyticus* that was occurred rely on manufacture company instructions which was Favorgen-China. The refrigerator was

Fig. 1. Gel electrophoresis product of PCR amplification of ica A gene, DNA molecular size marker 1500bp ladder, TBE buffer was (1x), agarose 0.01 and 100 V for 30 min then 50 V for 45 min, red stain was used. Lanes 1-15 in chromosome and lanes 16-19 in plasmid show positive results of S. saprophyticus.

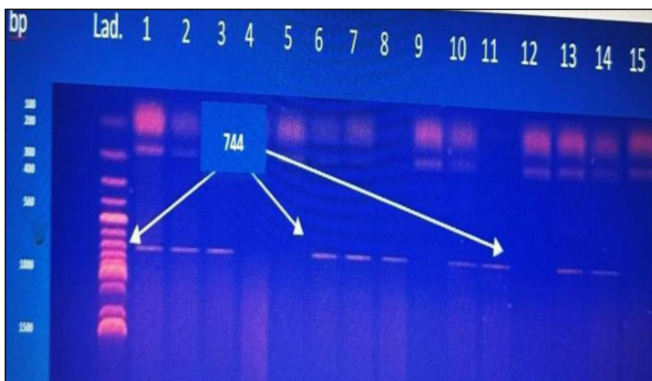

Fig. 2. Gel electrophoresis product of PCR amplification of cna A gene, DNA molecular size marker 1500bp ladder, TBE buffer was (1x), agarose 0.01 and 100 V for 30 min then 50 V for 45 min, red stain was used. Lanes 1,2,3,6,7,8,10 in chromosome and lanes 11,13,14 in plasmid show positive results of S. saprophyticus.

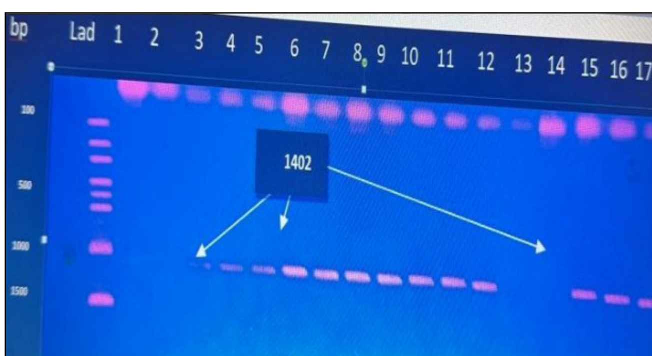

Fig. 3. Gel electrophoresis product of PCR amplification of clf A gene, DNA molecular size marker 1500bp ladder, TBE buffer was (1x), agarose 0.01 and 100 V for 30 min then 50 V for 45 min, red stain was used. Lanes 3-12,15 in chromosome and lanes 16-17 in plasmid show positive results of S. saprophyticus.

used to keep the elute production of DNA that was saved in for later until examined with PCR and gel electrophoreses.

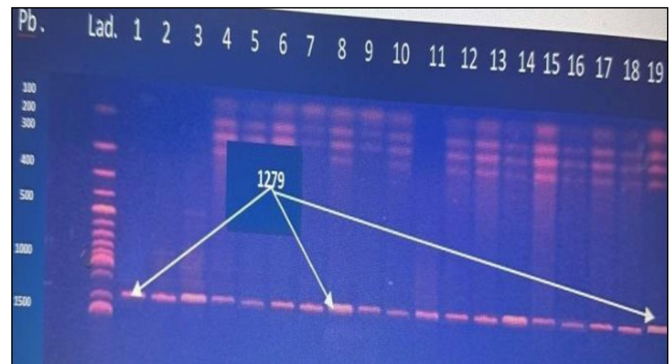

Fig. 4. Gel electrophoresis product of PCR amplification of fnb A gene, DNA molecular size marker 1500bp ladder, TBE buffer was (1x), agarose 0.01 and 100 V for 30 min then 50 V for 45 min, red stain was used. Lanes 1-9 in chromosome and lanes 10-19 in plasmid show positive results of S. saprophyticus.

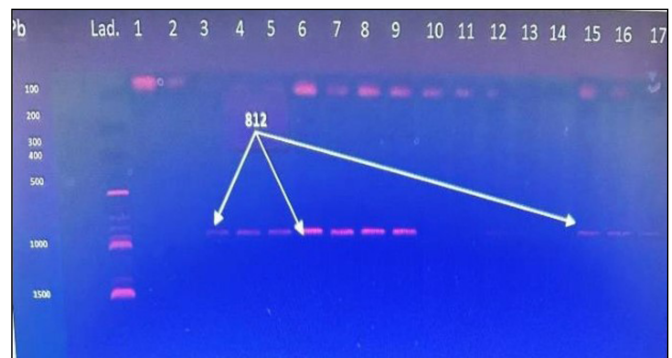

Fig. 5. Gel electrophoresis product of PCR amplification of fnb B gene, DNA molecular size marker 1500bp ladder, TBE buffer was (1x), agarose 0.01 and 100 V for 30 min then 50 V for 45 min, red stain was used. Lanes 3-9 in chromosome and lanes 15,16,17 in plasmid show positive results of S. saprophyticus.

PCR MATERIALS

Master mix used in this work was Go Tag DNA polymerase from Company Bioneer (Korea), was provided in reaction buffer that contain 2x Green Taq with pH 8.5, 400 µm of dATP, 400 µm of dGTP, 400 µm of dCTP, 400 µm of dTTP, and 3 mM of $MgCl_2$. The DNA marker of 100 bp, ladder from BioNeer (Korea) that compose 100-1500 base pairs.

PRIMER

According to manufacturer instructions, the BioNeer primers were prepared. The description of the primers presented in table 1.

ASSAY OF POLYMERASE CHAIN REACTION (ANALYSIS THE PRODUCT OF PCR)

AGAROSE GEL ELECTROPHORESIS

The amplified PCR products were analyzed with agarose gel electrophoresis (Lab net, U.S.A.) where

Table 1. The primers employed in the amplification of the study virulence genes with PCR assay

Gene	Orientation of primer	Primer Sequence (5-3-)	Product of amplification/bp	Reference
Fnb A	F F	5-TTT CCA ATA ACC ACC CGT TT-3	1279	Nashev et al., 2004
	R R	3-GCG GAG ATC AAA GAC AA-5		
Fnb B	F F	5-GGA GAA GGA ATT AAG GCG-3	812	Nashev et al.,2004 and Mohammadi et al.,2020
	R R	3-GCC GTC GCC TTG AGC GT-5		
ica A (intracellular adhesion)	F F	5TCAGACACTTGCTGGCGCAGTC -3	9936	Contreras et al., 2012
	R R	3TCACGATTCTCTCCCTCTCTGCCATT -5		
cna (collagen adhesion)	F F	5AGTGGTTACTAATACTG -3	7744	Kumar et al., 2009
	R R	3CAGGATAGATTGGTTTA -5		
clf (clumping factor)	F F	5GGCTTCAGTGCTTGTAGG-3	1402	Contreras et al., 2012
	R R	3- TTTTCAGGGTCAATA TAAGC-5		

Table 2. Phenotypic Slime production in S. saprophyticus isolated from pregnant women with asymptomatic bacteriuria

Isolate	isolates number	Positive phenotypic slime production isolates number	Percentage (%)
S.saprophyticus	48	48	100

loaded and visualized depend on method of Ghali JS [22].

STATISTICAL ANALYSIS

The study data were statistically analyzed using SPSS version 22 software, using the chi-square test, which was applied for two or more groups compared at a significance level of 0.05.

RESULTS

PHENOTYPIC SLIME PRODUCTION

Forty eight isolates of *S. saprophyticus* appeared phenotypic black color on Congo red agar (Table 2).

MOLECULAR ANALYSIS

Molecular analysis of ica A gene showed occurrence rate 100% (48/48) in the chromosome of S. saprophyticus, where its frequency in plasmid was 12.5% (6/48) and the total appearance was 112.5%, (Table 3, Fig. 1), while PCR study of cna A gene showed that the chromosomal rate was 83.3% (40/48) of isolates, but decreased in plasmid to 6.2 % (3/48), when total presence increased to 89.5%, (Table 3, Fig. 2.). The significant presence rate of Clf A gene in the whole isolates of the study was indicated as 83.33% (40/48) in chromosome and 4.16% (2/48) in plasmid, which cause the severity expression to 87.5% (42/48), (Table 3, Fig.3.), while fnb A gene in chromosome was 100% positive with a decrease in plasmid to 22.9% (11/48) and an elevated in the overall to 122.9% (59/48) (Table 3, Fig. 4.). Research data offered that only 33.33% (16/48) of the isolates had the fnb B gene in the chromosome, while 20.83% (10/48) of isolates harbored the same gene in the plasmid, which led to an increase in total gene presence, that reached 54.1% (26/48), (Table 3, Fig. 5.).

DISCUSSION

PHENOTYPIC SLIME PRODUCTION

The totally forty eight of *S. saprophyticus* isolates in research appeared phenotypic black color on Congo red agar therefore may be all isolates in study can slime production because study of Ilham B. [12] showed the black color isolates on Congo red agar were slime production isolates and Opeyemi LU et al. [23] explained the slime production was kept in the *Staphylococcus* genus.

Table 3. Frequency of some virulence slim and adhesions genes in the chromosome and plasmid of S. saprophyticus diagnosed with PCR assay.

Genetic site	Frequency of gene (%)					
	Ica A	***can A***	***clf A***	***fnb A***	***fnb B***	**Total**
Chromosome	100%(48/48)	83.3%(40/48)	83.33% (40/48)	100%(48/48)	33.3%(16/48)	399.9
Plasmid	12.5% (6/48)	6.2 % (3/48)	4.16 % (2/48)	22.9%(11/48)	20.8%(10/48)	66.5
Total	112.5	89.5	87.5	122.9	54.1	466.4
Value=0.05	CalX^2=105.10	CabX^2= 13.95	Df = 6			

MOLECULAR ANALYSIS

The ica A gene showed complete occurrence rate in chromosome of *S. saprophyticus* where it's rate on plasmid was lowered and total appearance was doubled to 113%. The complete prevalence of ica A genes in chromosome of *S. saprophyticus* isolates during the study may be because the ica A genes was saved in the genus *Staphylococcus* spp. as mentioned by Opeyemi LU et al. [23], who suggested that cell-to-cell adhesion and the potential to form biofilms is conserved within this genus by using cross-species hybridization technique with Staphylococcus species that revealed the presence of ica A in some other *Staphylococcus* species, lead to conclusion thet cell-to-cell adhesion and the possibility to compose biofilms was maintain in same genus. The frequency of presence of the same gene in plasmid may be because acquisition of these genes by the plasmid through re-joining with the chromosome of the same bacterium or the acquisition of the plasmid carrying this gene through conjugation with other bacteria such as *S. aureus* in the same environment which may lead to double the percentage of gene in study *S. saprophyticus* isolates [8, 22] which means there were in evaluation of bacteria may be related to the role of plasmid [6, 8]. Based on the above data, the isolates in the study may be clinically responsible for slime production, this was strongly supported by phenotypically identified in above step of Phenotypic Slime production, and may be indicates all isolates of *S. saprophyticus* can colonization to causes infection in those and another location of body when can transfer to these location. Santos SV et al. [6] showed phenotype, genotype and proteome varies in *S. saprophyticus* results reflected in the capability of bacteria to survive through interaction with cells of host, because the 9325 isolates appeared highest survival average after interaction of macrophage where, about 7108 strains were possessed minimal components about proteins that related to higher ability of virulence to biofilm form suggesting, this strains can be bester adjusted to preserved within the host and in nature. The possibility of protein of slime in strains of *S. saprophyticus*, can be invert in virulence and preservation, and may be more resistance to the antimicrobial action of antibiotics. *Staphylococci* are indicated as the most common causative agents of biofilm formation, which is associated with colonization of initiated infections, as well as mucus products that cause staphylococci to evade the host immune defense [12]. The total percentage of ica A gene exceeding the total percentage may be related to the role of plasmid in evaluation of bacteria [5, 24]. The results of study about ica A were identical to that in clinical source of several researches, the positively ica A were identified in whole *S. aureus* and *S. epidermidis* with 100% isolated from hemodialysis patients [12], while it differ with another research [25] shows, the slime genes average of ica A was about 69.6% (48/69) and 63.6% (56/88) within the isolates of *S. epidermidis* and *S. aureus* respectively. The data of study find that approximately 90% of isolates can bind to collagen of host cell and lead to infection, since CNA (product of cna gene) is the determinant bind ability to collagen. As mentioned by Allison GF et al. [26], when a comparison was made between strains that were positive and negative for cna in *S. aureus*, it significantly revealed the primary collagen binding ability by CNA. The height occurrence of this gene in *S. saprophyticus* may be resulted from the expected presence of *S. aureus* with *S. saprophyticus*, which were known to have virulence plasmid carriers of virulence genes when isolates from clinical samples in Thi-Qar- governorates that can transferred to other different bacteria by conjugation when present in same environment [22, 27]. The high occurrence of cna A gene in study isolates resembled to that in several clinical studies, as the cna A gene present 100% in all six *S. aureus* strains of hemodialysis patients [12]. Based on previous results, it is possible that a total of 87.5% of isolates can escape from host phagocytosis during infection` course, causing severity of disease, because phagocytosis of host can inhibited by clf A gene product during bacterial infection [14]. he significantly elevated rate of staphylococcal ClfA in clinical specimens is similar to other studies, which shows significantly higher ClfA in healthy persistent carriers than in healthy non-carriers [2], the clf A positive rate was confirmed in 45/88 (51.1%) of *S. aureus* isolates from hemodialysis patients [25], and in Al-Najaf city

of Iraq, the clfA gene was positive in all isolates of the *S. aureus* [28]. The rate of occurrence of each studied gene in the plasmid of the studied *S. saprophyticus* may be because each gene can transferred by recompilation or jumping from chromosome of same bacteria or by conjugation or transformation from chromosome or plasmid of *S. aureus*, which may be present with *S. saprophyticus* at the same clinical site of isolation, or other mobile genetic elements [8, 22]. The differences in the frequencies of ica A, clf A, cna A, fnb A and fnb B in this study and other studies may be related to difference in source of isolation, this suggests that staphylococcal types and strains express different virulence patterns according to isolation sources or may be back to the environmental differences between the regions of studies or may be revert to varies between species and strains in different studies [18]. The isolates harboring the genes of investigation may represent dangerous clinical isolates and participate in increasing the distribution of slim and adhesions virulence factors by transformed genes to other different isolates of pathogenic bacteria in society, that may causes severity invasive infection in carriers of pregnant women and further different infections in introduced human. The study indicated that *S. saprophyticus* isolates ware more developed in relation to the study of slim and adhesions virulence factors genes.

CONCLUSIONS

Based on the above data, there is a wider distribution of ica A, fnbA, clf A, cna A in comparison to fnbB genes among *S. saprophyticus* isolates of asymptomatic bacteriuria of pregnant women, therefore our study found the *S. saprophyticus* isolates harboring these genes may represent dangerous clinical isolates and participate in increase the distribution of slim and adhesions virulence factors by transformed genes to other various isolates of pathogenic bacteria in society, that may causes severity invasive infection and antibiotic resistance and fleeing host immune defenses in carriers of pregnant women and further different infections in introduced human. The study indicated *S. saprophyticus* isolates was more developed about study slim and adhesions virulence factors genes.

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CONFLICT OF INTEREST

The Author declare no conflict of interest

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