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Molecular Detection of Some Virulence Genes among *Staphylococcus aureus* Isolated from Skin Infections

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Abstract : The study included the collection of (100) samples were collected from different skin sites(Acne, impetigo, cellulitis, folliculitis). Clinical samples were collected from patients who were admitted and visit in Al- Imam Al-Sadiq Hospital and Maternity and Children's Hospital in Al-Hilla city, at the period from October (2023) to January (2024). Twenty isolates were showed positive and identified as *Staphylococcus aureus* by using selective media, biochemical tests and Vitek 2 system. and it was found that, out of the total 100 samples, 89(89%) samples showed positive bacterial culture. No growth was seen in other 11(11%) samples, which indicate the presence of microorganisms that may be cultured with difficulty such as virus, fungi and other anaerobic agents or may be due to difference in the size and nature of the samples. Among (89) positive culturing selective media, 89/100 positive cultures ,were divided into 48/89 (53.93%) Gram-negative bacteria, and 41/89 (46.07%) Gram-positive bacteria based on Gram stain and culture medium. Among Gram positive bacteria 20/41 (48.78%), isolates of *Staphylococcus aureus* were obtained. *icaA*, and *fnbA* were detected by PCR amplification, *fnbA* was found in 70% of the isolates while *icaA* 60%. and 191 bp 689 were considered positive for the presence of *fnbA* ,and *icaA* respectively.

Key words: Acne, impetigo *Staphylococcus aureus*, Vitek 2 system

Introduction : The fundamental goal of the skin is to keep microbial populations on its surface under control and prevent diseases from colonizing the underlying tissue (1).

Staphylococci are Gram positive bacteria that are seen as cocci under the microscope. according to its ability to produce coagulase enzyme , these bacteria are classified into coagulase -positive bacteria and coagulase -negative bacteria . The more bacteria related to this genus that is positive for coagulase is *S.aueuse* which is considered one of the most important pathogen in some clinical cases in the human. However coagulase negative Staphylococci should also be considered where there are two important species should be taken into account in some clinical cases such as *S. epidermidis* and *S.saprophyticus* (2)

Although most species of staphylococci are considered as normal flora of the skin , but under certain circumstances , they will transform into opportunistic pathogens and their ability to produce different virulence factors (3)

It can cause various disorders, including skin and soft tissue infections (SSTIs), invasive infections, and toxin-mediated disorders (4). Since it produces several virulence factors and acquires multidrug resistance (MDR) to various antibacterial agents, it is a major infectious agent in communities and hospitals (5).

S. aureus on the other hand possesses many virulence gene that share in its ability to cause diseases. These virulence genes may be present as distractive loci or as genetic elements (6)

Methicillin-resistant *S. aureus* (MRSA) is a common inhabitant of a large part of the healthy population and can cause a wide range of illnesses, from minor skin infections to life-threatening diseases (7).

The MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories(8). The MDR of *S. aureus* strains has been linked to longer hospital stays, higher mortality rates, and concomitant costs. The presence of many virulence factors, such as surface proteins, biofilms, exoenzymes, exotoxins, and exfoliative toxins, is linked to the ability of *S. aureus* to cause different infections. All these factors allow bacteria to attach to tissues, causing pathogenesis, and to penetrate the immune system, causing toxicity(9). One of the virulence factors of *S. aureus* is a cytolytic, pore-forming toxin, such as α-hemolysin, which is involved in the pathogenesis of *S. aureus*(10).

Many *S. aureus* strains, particularly MRSA, release one or more distinct staphylococcal exotoxins, including staphylococcal enterotoxins, the most important pathogenic components belonging to the superantigen family. The ability of the microorganism to successfully persist within the hospital and community and several cell wall-associated adhesive molecules, such as *fnb* (encoding fbronectin-binding protein) is responsible for the possibility of severe animal and human diseases(11).

The ability of *S. aureus* to build biofilms is linked to the antimicrobial resistance mechanism. Invasion isolates are more likely to form biofilm than healthy individual carriage isolates. The polysaccharide intercellular adhesin (PIA) is the most important component of biofilm. The N-acetylglucosamyl transferase enzyme responsible for PIA synthesis is known to be encoded by *icaA* (12).

Moreover , *S.aureus* can produce different types of toxins that participitate in their its pathogenicity such as Delta toxin which has heamolysin activity and is the only toxin that cause mast cell degradation and is linked to atopic dermatitis and some chronic skin disease. (13)

Identification of *S. aureus* virulence genes is important for evaluation of disease development. This study focused on *S. aureus* virulence genes and to detect their correlation to the skin infections.

Aim of the Study: This study aimed to investigate some virulence genes among *Staphylococus aureus* isolated from skin infections.

Objectives

1. Isolation of *Staphylococcus aureus* from different skin infections

2. Study some virulence genes(*fnbA* and *icaA*) among *Staphylococcus aureus* isolated from skin infections

Materials And Methods

Study Design: This study were included 100 patients, the specimens were collected from different skin infections (Acne, impetigo, cellulitis, folliculitis) from patients who were admitted and visited Al- Imam Al-Sadiq Hospital and Maternity and Children's Hospital in Al-Hilla city, during a period of three months from at the period from October (2023) to January (2024).

Ethical Approval

All subjects involved in this work are informed and the agreement was obtained verbally from each one before the collection of samples. This study was approved by the committee on publication ethics at college of medicine, University of Babylon, Iraq, under the reference No. BMS/0231/016.

Identification of bacteria

Colonial morphology and microscopic examination:

Depending on its morphological properties (colony form, size , color, borders, and texture), a single colony from each primary positive culture on blood, MacConkey and nutrient agar and classify it and examine it by light microscope after being stained with Gram's stain.

Biochemical tests

were performed on each isolate after inspection to complete the final identification according to (14) and it used the vitek2 method for *S.aurus* identification.

Identification of bacterial isolates with Vitek2 System

Vitek 2 clinical microbiology used as an automatic identification (ID) instrument device.

DNA extraction

This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company Geneaid, (Korea).

Detection of Some of Virulence Genes

DNA (extract from bacterial cells) was used as a template in specific PCRs for the detection of some of *S.aureus* virulence genes. DNA was purified from bacterial cells by using the Geneaid DNA extraction Kit. The primers used for the amplification of a fragment gene were listed in Table (1).

Table 1: The primers, sequences, and PCR conditions

Genes	Primer sequence (5′-3′)	Size of product bp	PCR condition	Reference
fnbA	F:GATACAAACCCA GGTGGTGG R:TGTGCTTGACCA TGCTCTTC	191	Step1:denaturation 95c for 1 min Step2 :denaturation 94c for 20 sec Annealing 65c for 4 min Extension 72c for 2 min 30 cycles Step3 :72c for 10 min	Tangchaisuriya.,et al.,2014
icaA	F:TCTGGAACCAAC ATCCAACA R;TCTGGAACCAAC ATCCAACA	181	Step1:denaturation 95c for 1 min Step2 :denaturation 94c for 20 sec Annealing 65c for 4 min Extension 72c for 2 min 30 cycles Step3 :72c for 10 min	Rohde.,*et* al.,2001

Results and Discussion

Isolation of pathogenic bacteria

The study included the collection of (100) samples were collected from different skin sites(Acne, impetigo, cellulitis, folliculitis). Clinical samples were collected from patients who were admitted and visit in Al- Imam Al-Sadiq Hospital and Maternity and Children's Hospital in Al-Hilla city, at the period from October (2023) to January (2024). All samples were subjected to aerobic cultivation on different culture media, and it was found that, out of the total 100 samples, 89(89%) samples showed positive bacterial culture. No growth was seen in other 11(11%) samples, which indicate the presence of microorganisms that may be cultured with difficulty such as virus, fungi and other anaerobic agents or may be due to difference in the size and nature of the samples. Among (89) positive culturing selective media, 89/100 positive cultures ,were divided into 48/89 (53.93%) Gram-negative bacteria, and 41/89 (46.07%) Gram-positive bacteria based on Gram stain and culture medium.

Among Gram positive bacteria 20/41 (48.78%), isolates of *Staphylococcus aureus* were obtained as shown in Table(2).

Table (2) Screening for the growth of bacterial isolates from skin infection :

Isolates	NO.	%
Gram Negative Bacteria	48	53.93
Gram Positive Bacteria	41	46.07
Negative Growth	15	0
Total	100	100

The distribution of *S.aureus* isolates among clinical samples were shown in the Table (3). *S.aureus* were isolated mainly from Acne samples at rate 11(55%), followed by Folliculitis at rate 4(20%), 3(15%) from Impetigo samples, while these bacteria were isolated from Cellulitis at rate 2(10%). These results are in agreement with study done by (15) who showed that *S.aureus* were isolated from skin infections at rate (52%). (16) found that, most of *S.auerus* isolates (34.14 %) were recovered from skin and wound infections.

Table (3): Distribution of *S.aureus* isolates among clinical samples

Sources Of Isolates	No. Of *S.Aureus*	Isolates %
Acne	11	55
Folliculitis	4	20
Impetigo	3	15
Cellulitis	2	10
Total	20	100

Detection of Some Virulence Genes Related to Skin Infections

To test the virulence genes of the isolates in this study, *icaA*, and *fnbA* were detected by PCR amplifcation. Table (4) shows that of the isolates. *fnbA* was found in 70% of the isolates, followed by *icaA* (60%) of the isolates). Amplicon sizes 689, and 181 bp were considered positive for the presence of *fnbA* , and *icaA* respectively(Figuer1and 2).

Table (4) distribution of virulence genes

Gene		%
Fnba		**70**
Icaa		**60**

The incidence of some major virulence indicators of *S. aureus* in skin specimens was examined in this study. This study concentrated on a small number of genes linked to *S. aureus* pathogenicity. These genes (*icaA, and fnbA*) were chosen because they were the most frequent in aggressive isolates. These targeted genes spread across the isolates after PCR amplifcation. Furthermore, the bulk of the isolates demonstrated a wide range of genes combinations, indicating that the study sample has a level of genetic diversity.

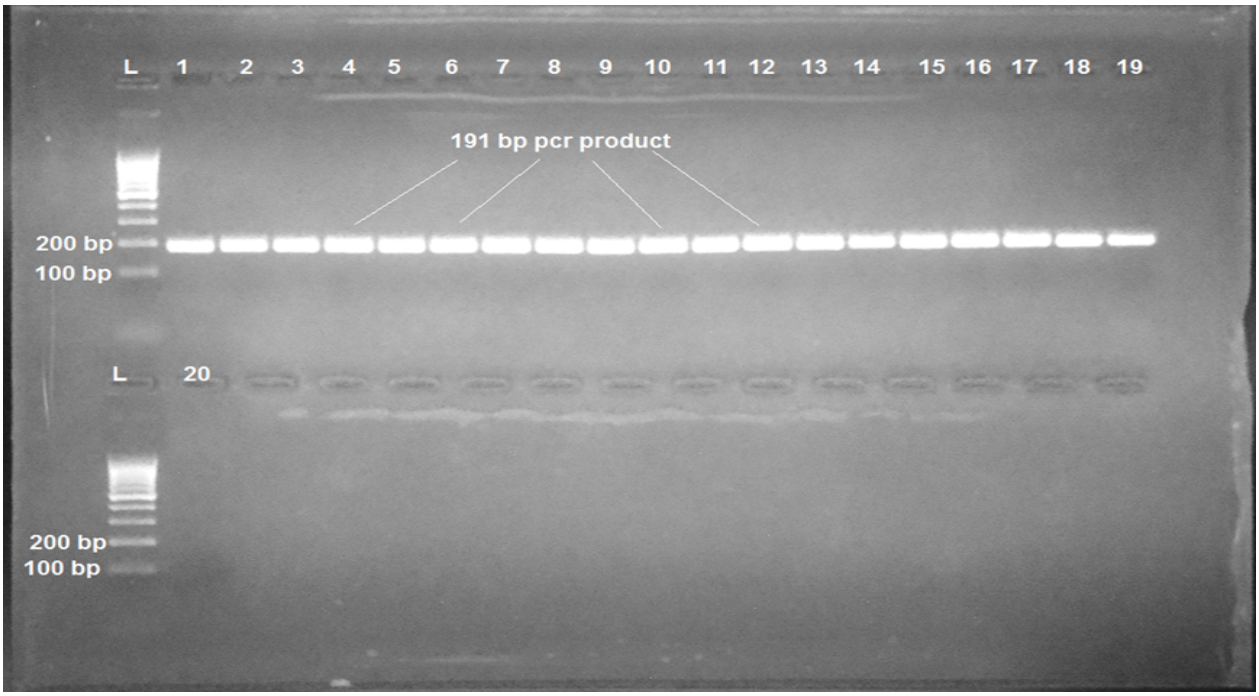

Figure (1): Ethidium bromide stained agarose gel showing PCR amplification products with *fnbA* gene 191bp) primers for *S.aureus* extracted DNA L: ladder, (1, 2, 3,……14) samples of *S.aureus* positive *fnbA* gene.

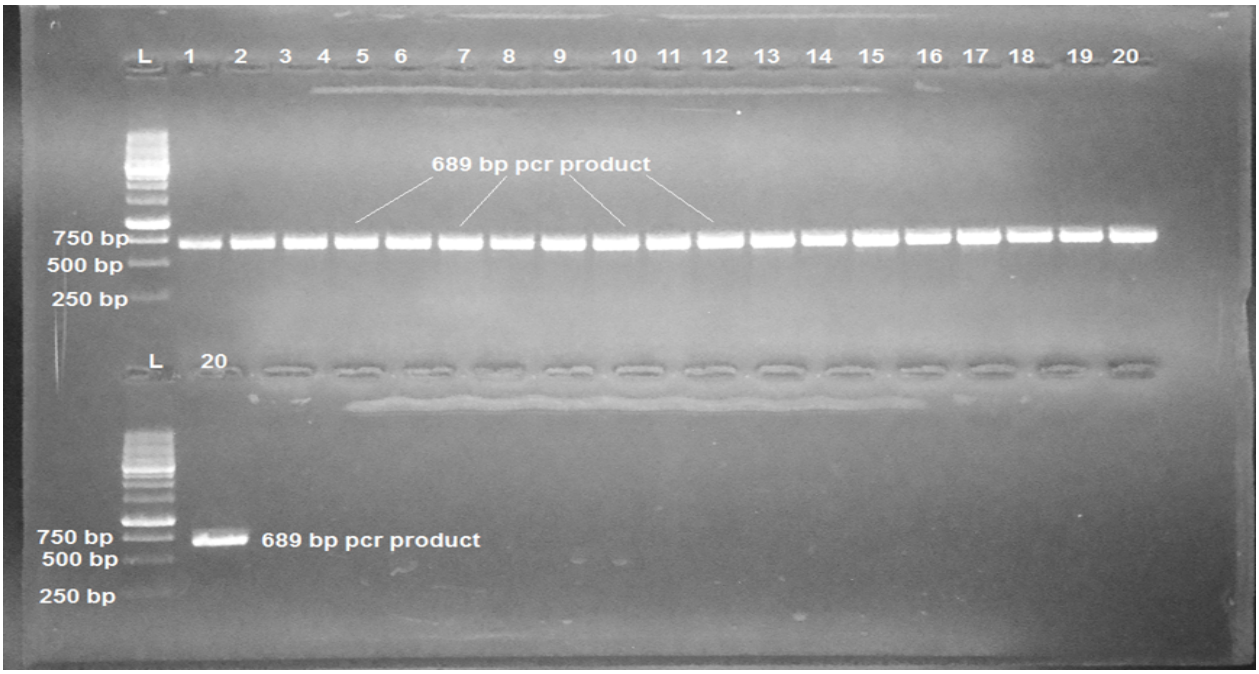

Figure (2): Ethidium bromide stained agarose gel showing PCR amplification products with *icaA* gene 689bp) primers for *S.aureus* extracted DNA L: ladder, (1, 2, 3,……12) samples of *S.aureus* positive *icaA* gene.

These results were agreement with results obtained by (17) who found that, molecular detection of (*fnbA,*and *icaA*) genes by used specific primers for these genes showed that (13.6% ,and 49.2%) of isolates were positive for these genes among *S.aureus*.

(18) found that, *icaA* genes regulate the production of biofilm that facilitate the skin infection, whereas results of showed positive to *icaA* gene in rate of (55%).

The *icaA* gene in *S. aureus*, which is involved in synthesis of poly Nacetylglucosamine for intercellular adhesion, may play a role in biofilm formation to initiate skin infections (19).

the fact that *ica A* expression is subjected to environmental conditions (20,21). Slime production and adhesion are considered to be a crucial virulence factor among the staphylococci presence of slime and adhesions genes shown to be exacerbation of keratitis (22), endocarditis in hemodialysis patients (23) and Skin infections(24). Biofilm formation by both *S. aureus* considered one of the most virulence factors of this bacteria.

S. aureus recognized as the most frequent causes of biofilm – associated infection to large variety of matrix components to initiate skin colonization, (25).

According to Lee *et al*. , *icaA* expression in *S. aureus* increased when the bacteria were exposed to NaCl, which has been widely used for food preservation, especially in processed meat products (26)

(27) and (28) found that fibronectin binding proteins A and B (*fnbA* and *fnbB*) increased biofilm accumulation.

Another study (29) suggested that the incidence of *fnbA* in skin swabs was (28.8%), which was lower than the present results.

The incidence of *fnbA* in *S.aureus* strains was (52.4%) and, However, the incidence of *icaA* in *S.aureus* was (84.5%). The percentages of *fnbA* were lower than the current study . However, the percentage of *icaA* was much higher than the present study (30).

PCR investigation revealed that *hla* was found in (62.5%) of 85 *S. aureus* isolated from Skin infection (31), close to the present findings.

The collagen – binding protein fibrinectin – binding proteins and fibrinogen – binding pr otein belong to this family (32). The specific surface proteins (*fnb A*) is expensed mainly by *S. aureus* strains (33).

These specific surface protein provide the specific interaction between bacteria and extracellular matrix proteins of the host cells. As a result they contribute to bacterial colorization (34).The bacterial adhesion thought to be an important step in the beginning of the infections.

The *fnb A* was the most important staphylococcal adhesion (35). It has been reported that *fnb A* gene was found to express in more than (88%)among *S. aureus* (36).

References

1. Ndip RN, Takang A, Echakachi CM, Malongue A, Akoachere J, Ndip LM, Luma HN.(2017). In-vitro antimicrobial activity of selected honeys on clinical isolates of Helicobacter pylori. *Afr Health Sci*.7(4):228–32.
2. Balachander, K., & Alexander, D. G. S. (2021). Antibiotic Resistance, Virulence Factors and Phylogenetic Analysis of Efflux Proteins of Coagulase Negative Staphylococcus Isolates from Sewage Samples. Pharmaceutical Science and Technology, 5(1), 14.
3. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr.(2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 28(3):603–61.
4. Morell EA, Balkin DM.(2010). Methicillin-resistant Staphylococcus aureus: a pervasive pathogen highlights the need for new antimicrobial development. Yale J Biol Med. 83(4):223–33.
5. Gauliard E, Ouellette SP, Rueden KJ, Ladant D.(2020) Characterization of interactions between inclusion membrane proteins from Chlamydia trachomatis. Front Cell Infect Microbiol. 5:13.

6. Cheung, G. Y., Bae, J. S., and Otto, M. (2021). Pathogenicity and virulence of Staphylococcus aureus. Virulence, 12(1), 547-569.

7. Rasmi A. Helmi , Eman Farouk Ahmed, Abdou Mohammed Abdullah Darwish and Gamal Fadl Mahmoud Gad.(2022).Virulence genes distributed among Staphylococcus aureus causing wound infections and their correlation to antibiotic resistance. *Infectious Diseases* 22(1):652

8. Wolfensberger A, Kuster SP, Marchesi M, Zbinden R, Hombach M.(2019). The effect of varying multidrug-resistence (MDR) defnitions on rates of MDR gram-negative rods. Antimicrob Resist Infect Control.8:193 .

9. Rauber JM, Carneiro M, Arnhold GH, Zanotto MB, Wappler PR, Baggiotto B, Valim AR, d'Azevedo PA.(2016). Multidrug-resistant Staphylococcus spp and its impact on patient outcome. Am J Infect Control.44(11):261–3.

10. Costa AR, Batistão DW, Ribas RM, Sousa AM, Pereira MO, Botelho CM.(2013). Staphylococcus aureus virulence factors and disease. In: Mendez-Vilas A, editor. Microbial pathogens and strategies for combating them: science, technology and education, 1:702–10.

11. Rahimi F, Katouli M, Karimi S.(2019). Bioflm production among methicillin resistant Staphylococcus aureus strains isolated from catheterized patients with urinary tract infection. Microb Pathog. 98:69–76.

12. Motallebi M, Jabalameli F, Asadollahi K, Taherikalani M, Emaneini M.(2018). Spreading of genes encoding enterotoxins, haemolysins, adhesin and bioflm among methicillin resistant Staphylococcus aureus strains with staphylococcal cassette chromosome mec type IIIA isolated from burn patients. Microb Pathog. 97:34–7.

13. Chen, H.,Saisindhu Narala Kimerly,A. Chun, Paul,F. Kotol, Aminabou Slimani.(2017) . Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis.*SCIENCE TRANSLATIONAL* MEDICINEl 9 (378):1-11

14. McFadden, 2000. Biochemical tests for identification of medical bacteria . 3rd ed. Williams and Wilkins – Baltimor ; PP: 321-400.

15. Tangchaisuriya U, Yotpanya W, Kitti T, Sitthisak S (2014). Distribution among Thai children of methicillin-resistant Staphylococcus aureus lacking cna, fnbA and icaAD. Southeast Asian J Trop Med Public Health, 45 (1): 149-56.

16. Rohde,H., J. K. Knobloch, M. A. Horstkotte et al., "Correlation of Staphylococcus aureus icaADBC Genotype and Biofilm Expression Phenotype," Journal of Clinical Microbiology, vol. 39, no. 12, pp. 4595-4596, 2001.

17. Kothari, Bhavin.(2022). "Clinical study and surgical management of diabetic foot: An observational study. International Journal of Surgery 6(1): 130-133.

18. Abbas-Al-Khafaji, Z. K., & Aubais-aljelehawy, Q. H. (2021). Evaluation of antibiotic resistance and prevalence of multi-antibiotic resistant genes among Acinetobacter baumannii strains isolated from patients admitted to al-yarmouk hospital. Cellular, Molecular and Biomedical Reports, 1(2), 60-68.

19. Budzyńska, A., Skowron, K., Kaczmarek, A., Wietlicka-Piszcz, M., & Gospodarek-Komkowska, E. (2021). Virulence Factor Genes and Antimicrobial Susceptibility of Staphylococcus aureus Strains Isolated from Blood and Chronic Wounds. Toxins, 13(7), 491.

20. Lee, S., K.-H. Choi, and Y. Yoon. (2018). Effect of NaCl on biofilm formation of the isolate from Staphylococcus aureus outbreak linked to ham. Korean J. Food Sci. Anim. Resour. 34:257–261.

21. Cue, D., M. G. Lei, and C. Y. Lee. (2012). Genetic regulation of the intercellular adhesion locus in staphylococci. Front. Cell. Infect. Microbiol. 2:38.

22. Cramton S.E., Ulrich M., Gotz, F., Doring, G., (2010). Anaerobic condition induces expression ofpolysaccharide intercellular adhesion in Staphylococcus aureus and Staphylococcus epidermidis. Infact.Immun 69:4079-4085

23. Zmantar, T, chaieb, K. Miladi , H. Mahdouani, K., Bakhrouf, A. (2006). Detection of the intracellularadhesion loci (ica) in clinical Staphylococcus aureus strains responsible for hospital acquired auricular infection

24. Jett, BD, Gilmone, MS. (2020). Internalization of S. aureus by human corneal epithelial cells: role ofbacterial fibronection – binding protein and host cell factors, Infect. Immun. 70: 4697 – 7400

25. Robinson, D.L, Fowler V. V, Sexton, D.J. (1997). Bacterial endocarditis in hemodialysis patients.

26. Otto,M. (2008). Basis of Virulence in Community-Associated Methicillin-Resistant *Staphylococcus aureus*. *Annual Review of Microbiology*.64:143-162.

27. Lee, J., J. Ha, S. Kim, S. Lee, H. Lee, Y. Yoon, and K.-H. Choi. (2016). The correlation between NaCl adaptation and heat sensitivity of Listeria monocytogenes, a foodborne pathogen through fresh and processed meat. Korean J. Food Sci. Anim. Resour. 36:469–475

28. O'Neill, E., C. Pozzi, P. Houston, H. Humphreys, D. A. Robinson, A. Loughman, T. J. Foster, and J. P. O'Gara. 2008. A novel Staphylococcus aureus biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. J. Bacteriol. 190:3835– 3850.

29. Vergara-Irigaray, M., J. Valle, N. Merino, C. Latasa, B. Garcia, I. Ruiz De Los Mozos, C. Solano, A. Toledo-Arana, J. R. Penades, and I. Lasa. 2009. Relevant role of fibronectin-binding proteins in Staphylococcus aureus biofilm associated foreign-body infections. Infect. Immun. 77:3978–3991.

30. Eftekhar F, Rezaee R, Azad M, Azimi H, Goudarzi H, Goudarzi M. Distribution of adhesion and toxin genes in staphylococcus aureus strains recoveredfrom hospitalized patients admitted to the ICU. Arch Pediatr Infect Dis. 2017;5(1): 39349

31. Tabandeh M, Kaboosi H, Armaki MT, Pournajaf A, Ghadikolaii FP. New update on molecular diversity of clinical Staphylococcus aureus isolates in Iran: Antimicrobial resistance, adhesion and virulence factors, biofilm formation and SCCmec typing. Mol Biol Rep. 2022;49(4):3099–111.

32. El-baz R, Rizk DE, Barwa R, Hassan R. Virulence factors profile of Staphylococcus aureus isolated from different clinical sources. J Microbiol.2016;43:126–44.

33. Bunyan,I.(2013).Detection of Adhesion Genes and Slim Production among Staphylococcus aureus and Staphylococcus epidermidis Isolatedfrom Hemodialysis Patients. Advances in Life Science and Technology.15:(27-32)

34. Zmantar, T., Chained, K., Makni, H. and Bakhrof, A. (2008). Detection by PCR of adhesions genes andSlime production in clinical S. aureus .J. Basic Microbial 48: 308 – 314

35. Arciola, CR, Campoccia and Montanaro, L. (2005). Occurrence of ica genes for Slime Synthesis in acollection of S. epidermidis strains from orthopedic prostheses infection. Acta Orthop. Scand. 74: 617 –621

36. Duran, n, Dogramaci, Y, Ozar, B, Demir, C, and Kalaci, A. (2010). Detection of adhesion genes andSlime production among Staphylococci in orthopedic surgical wound. African J Microbial Res. 4 (9):708-715.