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# Virulence Determination in Invasive and Commensal Staphylococcus Epidermidis

**Hind Hamid Muneam[1] and May Talib Flayyih[1,*]**
[1]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

Corresponding author: May Talib Flayyih (e-mail: maytalib@yahoo.com).



**Abstract** **Aims** research primarily focuses on the virulence factors in Staphylococcus epidermidis isolates and their role in human disease. **Methods:** 15 Staphylococcus epidermidis Isolates were obtained from urine, wound, and blood samples from various hospitals in Baghdad. Additionally, 15 Staphylococcus epidermidis isolates were collected from the urine and fingerprints of healthy individuals in Baghdad from July 2021 to January 2022. The resistance of the isolates to antibiotics was evaluated by conducting the disk diffusion test, which involves placing antibiotic-containing disks on a culture plate to observe the extent of bacterial growth inhibition around each disk. The antibiotics tested included Vancomycin (VA- 30µg), cefoxitin (CFX- 30µg), gentamicin (CN-10µg), tetracycline (TE-10 µg), levofloxacin (LEV- 5µg), oxacillin (OX- 5µg), penicillin G (P-10 µg), Rifampin (RA- 5µg), and clindamycin (DA-10 µg). The frequency of virulence genes (fdh, mecA, clf, IgG, sesI) was determined using PCR assay. **Results:** A total of 30 S. epidermidis isolates were detected by traditional biochemical tests, and Viteck 2 had higher rates of resistance to cefoxitin (80%), oxacillin (90%), penicillin (86.6%), Clindamycin (60%), Gentamycin (76.6%) levofloxacin (80%) Tetracycline (26.6%). The most effective antibiotics are vancomycin (90%) and Rifampin (86%). Moreover, the detection 3/15(20%), 12/15(80%), of commensal marker fdh, and 3/15(20%),1/15(6.6%) of invasive marker sesI genes 11/15(73.3%), 14/15(93.3%) while other virulence genes mecA, clf, IgG detected and 6/15(40%), 7/15(46.6%), 9/15(60%), 14/15(93.3%) in clinical and healthy individual isolates, respectively.

**Key Words** staphylococcus epidermidis, fdh, mecA, clf, IgG, sesI

## 1. Introduction

The skin is a crucial barrier protecting the body from the outside world. When this barrier is compromised due to injury, it weakens the body's natural immune system, making it more vulnerable to bacterial infections [1], [2]. Staphylococcus epidermidis (S. epidermidis) is a significant component of the human skin microbiota and plays a crucial role in infections related to medical implants. Non-spore-forming staphylococci are commonly found in nature. Humans and animals naturally harbor several species of Staphylococcus on the skin, mucous membranes, digestive tract, and respiratory system. Staphylococci colonize the skin, particularly in moist areas such as the scalp, face, hands, navel, armpits, and perineum. Both intact skin (via sweat ducts or hair follicles) and damaged skin (such as through lesions) provide entry points for staphylococci. Coagulase-Negative Staphylococci (CoNS) have traditionally been considered less harmful than coagulase-positive ones [3], [4].

Staphylococcus epidermidis is generally considered a beneficial bacterium with properties such as regulating the immune system [5] and protecting against pathogen colonization [6]. However, it also can cause hospital-acquired infections associated with implanted medical devices [7], [8].

Antibiotic resistance poses a serious threat to public health [9]. Methicillin-resistant staphylococci-caused urinary tract infections (UTIs) are a rising issue for many healthcare facilities, mainly because they are linked to the development of biofilms by these isolates on both living and nonliving surfaces [10].

On the other hand, nothing is known about the processes the bacterium uses to adapt to its environment during carriage, which is a need for pathogenicity [11]. The term "virulence factors" is defined broadly to include both genes and proteins that make it easier for an organism to infect and stay alive in a person's body.

It is evident that most of these elements also play significant roles in S. epidermidis commensal life as a benign resident of human skin. Therefore, they may not be classified

as "virulence factors" in the strictest sense. The two most significant virulence factors of S. epidermidis were biofilm formation and multidrug resistance [12]. The molecular pathways underlying virulence, S. epidermidis, is by far the most thoroughly researched member of the CNS.

virulence of Staphylococcus strains attributed to various factors like the clumping factor clfA, IgG-binding region genes, host specificity, and diverse immunological reactions. which found to possess IgG-binding regions, these are crucial in understanding the pathogenicity of Staphylococcus strains [13].

Genetic markers (fdh) that correctly differentiate between infection and contaminant or commensal sources may assist in the diagnosis of S. epidermidis infections [14], [15].

The struggle of S. epidermidis isolates methicillin is due to the mecA gene, which is carried by a mobile genetic element called the cluster chromosomal cassette (SCCmec). The mecA gene encodes a modified penicillin-bound protein (PBP2a) with low affinity for beta-lactam antibiotics [16]. Based on the categories of the MEC gene pool and the types of the CCR gene pool, eleven types (from the first to the eleventh) of SCCmec were assigned to Staphylococcus aureus [17]. The mecA gene is responsible for both virulence and methicillin resistance traits observed in S. epidermidis strains isolated from human clinical infections [18], [19].

S. epidermidis ability to adhere to various surfaces is due to its extensive repertoire of surface proteins, each of which has unique adhesive properties that contribute to enhancing its cling ability [20] The surface proteins of Staphylococcus epidermis (S. epidermidis), especially SesI, have attracted great attention due to their immunomodulatory properties [13] and their association with invasive isolates [21].

Microorganism antibiotic resistance is a severe public health issue. Numerous antimicrobial resistance strategies have been developed due to the rising infection rates of bacteria resistant to almost all antibiotics [22]. Antimicrobial resistance has also brought attention to how linked people, animals, and the environment are and how important these factors are in the spread of resistance genes [18], [23]. The rise of methicillin-resistant S. epidermidis strains and the clinical significance of such strains have presented several therapeutic hurdles in recent decades [24]. Hospitals now have a severe challenge with resistant S. epidermidis [25], [26]. In the United States, annually, approximately 100,000 cases of infections are caused by staphylococcal strains that are resistant to treatment. These infections have a mortality rate of around 10% [26]. The main focus of the study has been on virulence factors in invasive and commensal S.epidermidis isolates and opportunistic human disease.

## 2. Methods

### A. *Ethical Statement*

The College of Science Research Ethics Committee has accepted this work (ref. CSEC/1220/0081). Each participant consented to the researcher obtaining the specimens. According to the Declaration of Helsinki, each participant obtained informed permission.

### B. *Samples collection*

The commensal isolates of S. epidermidis were collected from the skin and urine of healthy individuals. In contrast, invasive isolates were obtained from wounds, urine, and blood of hospital patients in different hospitals in Baghdad. All isolates were identified using biochemical tests and the Vitek 2 system.

### C. *Assessment of Antibiotic Susceptibility*

The antibiotic's susceptibility was evaluated by employing a disc diffusion technique that followed the established protocols provided by the Clinical and Laboratory Standards Institute (CLSI) [27] (Table 1).

### D. *Detection of some virulence genes by conventional polymerase chain reaction (PCR)*

All S. epidermidis isolates were inspected for the presence of the commensal marker fdh gene, methicillin resistance mecA gene, clumping factor clf gene, IgG binding protein gene, and invasive marker S. epidermidis surface protein sesI gene. Total DNA extraction was obtained by culturing each isolate overnight at 35°C with shaking in BHI broth (BD et al., USA). The PrestoTM Mini gDNA Bacteria Kit (Geneaid, Taiwan) was used to extract the genomic DNA from the S. epidermidis isolates, and the AccuPower® PCR PreMix and Gradient master cycler (Eppendorf, Germany) was used for all amplifications. For PCR, a 50 µl final volume containing master mix 25µl (Roche et al.), upstream primer 10µm 0.5-5.0µl, downstream primer 0.5-5.0µl, DNA template 1-5µl, and nuclease-free water to 50µl was used (Promega kit). The conditions were as follows: one cycle of initial denaturation at 95°C for 2 minutes, then 30 cycles of denaturation at 95 °C for one minute, annealing at a temperature dependent on the gene annealing temperature for 20 sec, elongation at 72 °C for 1 minute, and a final extension cycle at 72 °C for 5 minutes. Electrophoresis in 2% agarose gel was used to analyze amplicons. The positive and negative controls were S. epidermidis ATCC 35984 and S. aureus ATCC 29213, respectively. The National Center for Biotechnology Information's GenBank sequence database obtained the fdh, mecA, clf, IgG, and sesI gene sequences. The primers were created by the Microgene company [28] using specific primers, respectively (Table 2).

### E. *Statistical Analysis*

The System—SAS (2018) [29] program was used to detect the effect of different factors on study parameters. This study used the chi-square test to compare percentages (0.05 and 0.01 probability) significantly.

## 3. Results

| Name of Antibiotic(µg/disk) | code | Classification | Diameter of zone inhibition (mm) | | |
|---|---|---|---|---|---|
| Cefoxitin (30µg) | CFX | Penicillinase stable penicillin | S | I | R |
| | | | ≥25 | – | ≤24 |
| Oxacillin (5µg) | OX | Penicillinase stable penicillin | ≥18 | – | ≤17 |
| Penicillin G (10 µg) | P | Penicillinase label penicillin | ≤ 28 | - | ≥ 29 |
| Clindamycin (10 µg) | DA | Lincosamides | ≥21 | 15-20 | ≤14 |
| Levofloxacin (5µg) | LEV | Fluoroquinolones | ≥19 | 16-18 | ≤15 |
| Gentamicin (10µg) | CN | Aminoglycosides | ≥15 | 13-14 | ≤12 |
| Tetracycline (10 µg) | TE | Tetracyclines | ≥19 | 15-18 | ≤14 |
| Rifampin (5µg) | RA | Ansamycins | ≥20 | 17-19 | ≤16 |
| Vancomycin (30µg) | VA | glycopeptides | – | – | – |

Table 1: The antibiotics used in this study (CLSI, 2021)

| Name | Abbreviation | Annealing temperature | Primer sequence (5-3) | Amplicon size (pb) | References |
|---|---|---|---|---|---|
| format dehydrogenase | fdh | 46 | F -ATA ATG GTG ATA TTC ATT CG<br>R -CCG TAT TAG TAA AAG TTC CA | 204 | 47 |
| Methicillin resistance | mecA | 55°C | F:AAAATCGATGGTAAAGGTTGGC<br>R: AGTTCTGCAGTACCGGATTTGC | 532 | 42 |
| Clumping factor | clf | 47°C | F: GGCTTCAGTGCTTGTAGG<br>R: TTTTCAGGGTCAATATAAGC | 980 | 42 |
| IgG-binding region | IgG | 53°C | F: CACCTGCTGCAAATGCTGCG<br>R: GGCTTGTTGTTGTCTTCCTC | 920 | 42 |
| Staphylococcus epidermidis surface protein | sesI | 48 | F: GCTGATTATGTAAATGACTCAAAT R: AGCTTTTGTTGTTTGAGCTTC | 200 | 44 |

Table 2: Primer sequences

Figure 1: Antibiotic Resistance Test

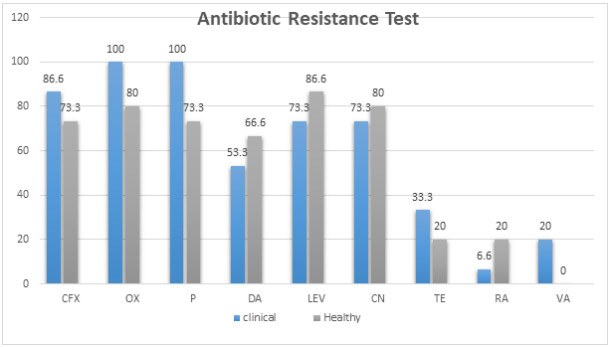


Figure 2: The percentage of antibiotic resistance of healthy and clinical S. epidermidis isolates

### *A. Staphylococcus epidermidis isolates diagnostic*

One hundred and fifty samples were collected in our study to characterize the human skin microbiome in healthy individuals (HIs) [30], and 150 samples were collected from different body parts of clinical patients. The results showed 30(10%) detection as S. epidermidis was detected by traditional biochemical tests and Vitek 2.

### *B. Assessment of Antibiotic Susceptibility*

In clinical samples, S. epidermidis was the most frequently isolated CONS. Additionally, among the discovered CONS, we noted variations in antibiotic reluctance. S. epidermidis had higher rates of resistance to cefoxitin 13/15 (86.6%), 11/15 (73.3%) in, Oxacillin 15/15(100), 12/15(80%) penicillin15/15 (100%), 11/15(73.3%) in clinical and healthy isolates respectively, while the most effective antibiotic the vancomycin 12/15(80%) 15/15(100%) in clinical and healthy isolates respectively. Figure 1, 2 and Table 3.

### *C. Detection of the Staphylococcus epidermidis virulence gene by Polymerase chain reactions*

S. epidermidis isolates were obtained from clinical and healthy individuals subject to investigation about the virulence genes by using the PCR technique. The results show commensal marker gene (fdh) found in 3/15(20%), 6/15(40%), and the invasive marker gene (sesI) present in 3/15(20%), 1/15(6.6%) from clinical and healthy isolates respectively Figure 3 and Figure 4 Figure 5.

While the virulence factors genes as mecA present in 12/15(80%), 7/15 (46.6%) clf gene were found in 11/15(73.3%), 9/15(60%) in clinical and healthy isolates, respectively Figure 6 and Figure 7, the IgG gene was detected in 14/15(93.3%) Figure 8 in both clinical and healthy individual isolates. Figure 9 Table 4.

## 4. Discussion

Coagulase-negative Staphylococcus (CoNS) isolates are believed to be opportunistic pathogens that are a widespread component of the human microbiota and can lead to several serious infections, particularly in patients who have medical indwelling devices [31], [32]. Many S. epidermidis-related infections involve intravascular devices (such as artificial heart valves and shunts) and frequently affect prosthetic joints, catheters, and big wounds [33]–[35]. Most routinely given antibiotics no longer effectively treat CoNS [35]–

| Antibiotics | Clinical isolates | | | Healthy isolates | | | Total No(%) | | | Chi-square value |
|---|---|---|---|---|---|---|---|---|---|---|
| | R (%) | I (%) | S (%) | R (%) | I (%) | S (%) | R | I | S | |
| Cefoxitin (CFX- 30µg) | 13/15 (86.6) | 0 (0.00) | 2/15 (13.3) | 11/15 (73.3) | 0 (0.00) | 4/15 (26.6) | 24/30 (80) | 0 (0.00) | 6/30 (20) | 12.63 ** |
| Oxacillin (OX- 5µg) | 15/15 (100) | 0 (0.00) | 0 (0.00) | 12/15 (80) | 0 (0.00) | 3/15 (20) | 27/30 (90) | 0 (0.00) | 3/30 (10) | 16.78 ** |
| Penicillin G (P-10 µg) | 15/15 (100) | 0 (0.00) | 0 (0.00) | 11/15 (73.3) | 1/15 (6.6) | 3/15 (20) | 26/30 (86.6) | 1/30 (3.3) | 3/30 (10) | 16.03 ** |
| Clindamycin (DA-10 µg) | 8/15 (53.3) | 1/15 (6.6) | 6/15 (40) | 10/15 (66.6) | 2/15 (13.3) | 3/15 (20) | 18/30 (60) | 3/30 (10) | 9/30 (30) | 13127 * |
| Levofloxacin (LEV- 5µg) | 11/15 (73.3) | 2/15 (13.3) | 2/15 (13.3) | 13/15 (86.6) | 1/15 (6.6) | 1/15 (6.6) | 24 (80) | 3/30 (10) | 3/30 (10) | 8.61 ** |
| Gentamicin (CN-10µg) | 11/15 (73.3) | 2/15 (13.3) | 2/15 (13.3) | 12/15 (80) | 0 (0.00) | 3/15 (20) | 23 (76.6) | 2/30 (6.6) | 5/30 (16.6) | 8.52 ** |
| Tetracycline (TE-10 µg) | 5/15 (33.3) | 0 (0.00) | 10/15 (66.6) | 3/15 (20) | 3/15 (20) | 9/15 (60) | 8/30 (26.6) | 3/30 (10) | 19/30 (63.3) | 9.02 ** |
| Rifampin (RA- 5µg) | 1/15 (6.6) | 0 (0.00) | 14/15 (93.3) | 3/15 (20) | 0 (0.00) | 12/15 (80) | 4/30 (13.3) | 0 (0.00) | 26/30 (86.6) | 11.69 ** |
| Vancomycin (VA- 30µg) | 3/15 (20) | 0 (0.00) | 12/15 (80) | 0 (0.00) | 0 (0.00) | 15/15 (100) | 3/30 (10) | 0 (0.00) | 27 (90) | 11.84 ** |
| Chi-square value | 8.51 ** | 2.036 NS | 8.44 ** | 7.95 ** | 2.167 NS | 8.02 ** | 9.64 ** | 1.98 NS | 11.42 ** | — |
| ** (P≤0.), NS: Non-Significant. | | | | | | | | | | |

Table 3: Antibiotics susceptibility pattern of healthy and clinical S. epidermidis isolates

| Genes | Clinical isolates No(%) | Healthy isolates No(%) | Total |
|---|---|---|---|
| fdh | 3/15 (20) | 6/15 (40) | 9/30 (30) |
| mecA | 12/15 (80) | 7/15 (46.6) | 19/30 (63.3) |
| clf | 11/15 (73.3) | 9/15 (60) | 20/30 (66.6) |
| IgG | 14/15 (93.3) | 14/15 (93.3) | 28/30 (93.3) |
| sesI | 3/15 (20) | 1/15 (6.6) | 4/30 (13.3) |

Table 4: Polymerase Chain Reaction

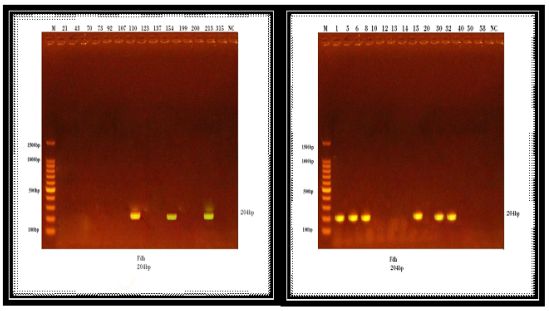

Figure 3: PCR Amplification of S. epidermidis fdh product size (204pb)

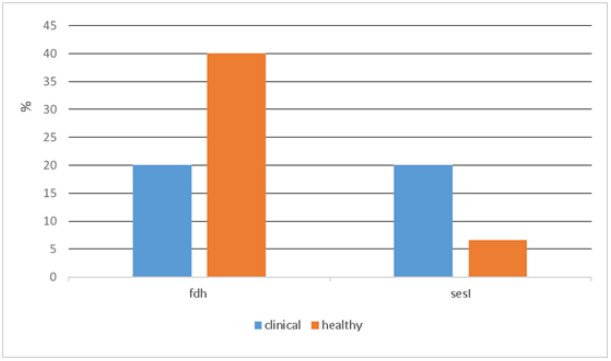


Figure 5: The Prevalence of fdh and sesI genes in healthy and clinical S. epidermidis isolates

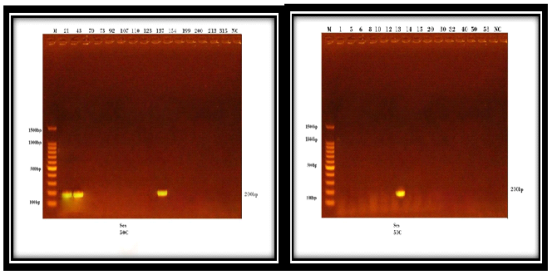

Figure 4: PCR Amplification of S. epidermidis sesI product size (200bp)

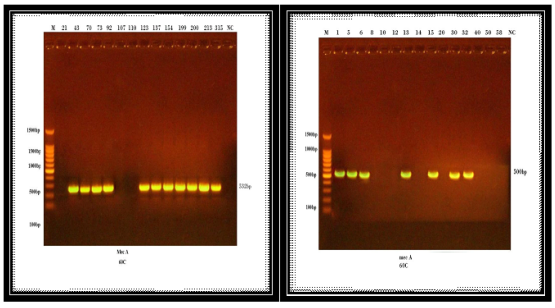

Figure 6: PCR Amplification of S. epidermidis mecA product size (532pb)

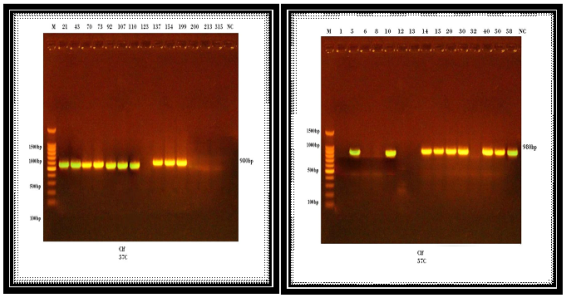

Figure 7: PCR Amplification of S. epidermidis clf product size (980pb)

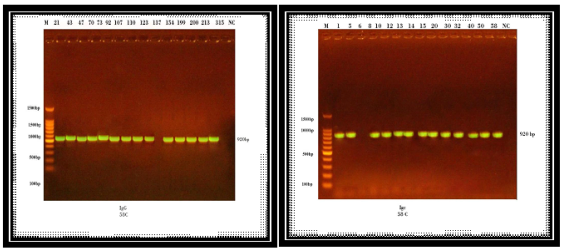

Figure 8: PCR Amplification of S. epidermidis IgG product size (920bp)

[37]. In hospital settings, it is believed that these infections have the potential to spread among both patients and staff members. The transmission of these opportunistic pathogens by healthcare workers (HCWs) may spread infections in different hospital wards. [36].

In this study, we conducted a survey to assess antibiotic resistance among S. epidermidis isolates. The findings from our research indicate a notable disparity in resistance rates to various antibiotics, such as oxacillin, penicillin, and cefoxitin, between clinical S. epidermidis isolates and healthy isolates. Specifically, the resistance rate was significantly higher among clinical S. epidermidis isolates than healthy ones. The result [37] of the cefoxitin diffusion disc test

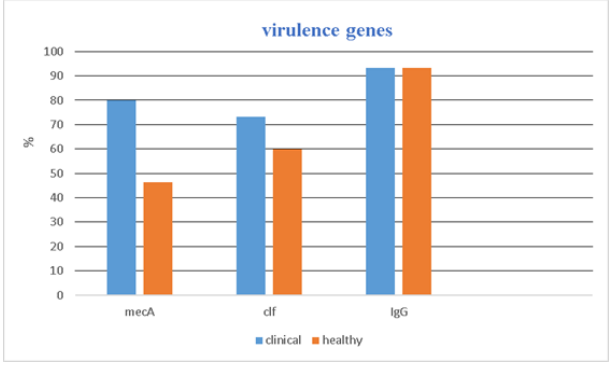


Figure 9: The Prevalence of clf, IgG, and mecA genes in healthy and clinical S. epidermidis isolates

findings was that 93% of S. epidermidis isolates had become resistant to methicillin. The newly released data [38]–[40] showed a significant incidence of S. epidermidis in human clinical infections. Our results agree with [41] and [42], which showed that the most common types of penicillin, tetracycline, and cefazolin resistance were found in S. epidermidis strains. Every strain of S. epidermidis resisted at least three distinct kinds of antibiotics. The primary cause of the increased frequency of antibiotic resistance is the unlicensed and illicit prescribing of medicines [42].

A notable level of antibiotic resistance was observed in hospital-acquired S. epidermidis isolates, likely stemming from the widespread use of antibiotics among healthy individuals and the resulting selective pressure. However, clinical isolates from healthy individuals displayed lower resistance to Clindamycin, Levofloxacin, and Gentamicin.

They were more susceptible to vancomycin and rifampin, suggesting their effectiveness in treating infections caused by S. epidermidis. The results of a study by [43] indicated that S. epidermidis isolates from VRSE had less self-degrading activity when exposed to antibiotics compared to no antibiotics. In addition, it was observed that the cell walls of VRSE isolates are thicker than those of VISE isolates.

Interestingly, no evidence of cell wall thickness was found in VSSE isolates. These explanations are consistent with several global studies that have looked at the prevalence of methicillin resistance among CoNS isolates. For example, a study in Iran revealed that 74% of S. epidermidis isolates were resistant to methicillin [23].

The present study aims to examine how genes related to virulence and antibiotic resistance are distributed among isolated S. epidermidis bacteria. It specifically examines the sesI gene found on the surface of S. epidermidis isolates from 15 clinical cases and 15 healthy individuals. Our findings indicate that the sesI gene was present in 20% of clinical isolates and 6.6% of commensal isolates.

These results align with those reported by Söderquist and colleagues [43]. The presence of the sesI gene in invasive isolates and its absence in commensal ones suggests that this gene could serve as a potential marker for virulence. The screening of S. epidermidis isolates revealed that only two carried the sesI gene, further supporting the notion that this gene is rarely found among commensal isolates. However, previous research by Bowden and colleagues found the sesI gene in 34% of skin isolates, 29% of contamination isolates, and 45% of S. epidermidis isolates from newborn bloodstream infections. Additionally, Söderquist et al. used the fdh gene as a marker to differentiate between nosocomial and commensal isolates, finding the fdh gene in 20% of clinical isolates and 40% of healthy isolates. These results agree with [44] that it was present in around 23% of commensal strains (16/71) but in roughly 4% of nosocomial strains (3/46). Intriguingly, the three nosocomial isolates that tested positive for fdh had minimal virulence markers. This would imply that these three isolates, identified as nosocomial after a blood sample, represent commensal contamination from the

venipuncture. Unlike conventional nosocomial markers, fdh is a venipuncture. Unlike conventional nosocomial markers, fdh is a commensal-associated marker with potential for discrimination [44].

In a study focused on infectious diseases caused by S. epidermidis, researchers identified the genes clfA and the IgG-binding region as the most significant markers among all virulence markers present in the S. epidermidis strains [44]. The findings, supported by previous research [45], [46], indicated that there was no significant variation in the clf gene (73.3% and 60%) and the IgG region gene (93.3%) between clinical and healthy isolates. However, a study by Eftekhar et al [47] reported that the frequency of the virulence genes clfB and clfA was 78.60% and 71.40%, respectively.

## 5. Conclusions

The investigation found that S. epidermidis isolates displayed high clonal diversity from clinical and healthy individuals. However, specific genes associated with commensal fdh and invasive marker ses I genes and virulence factors clf, IgG, and mecA genes were more prevalent in the clinical isolates. Despite all the S. epidermidis isolates being from individuals exposed to hospital settings, we observed significant variations in two genes that could indicate invasiveness in the future. S. epidermidis demonstrates high genetic plasticity, allowing it to acquire, lose, or alter genetic elements, contributing to enhanced host colonization and increased pathogenicity.

## Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

## Authors Contribution

All authors contributed equally in this paper.

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