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Molecular detection of Fibronectin-binding Protein genes in *Staphylococcus hominis* Isolated from Blood Samples

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Abstract: Coagulase-negative staphylococci (CoNS) are now recognized as a major cause of nosocomial infections in blood samples especially the species *Staphylococcus hominis*. *S. hominis* is often detected as a one of the pathogenic bacteria implicated for catheter-related blood stream infections and infective endocarditis. The aim of the study to isolation and identification of *S. Hominis*. The blood samples (150) was collected that this species was identified. The results of antibiotic resistance pattern showed a complete resistance (100 %) to pencilline and High resistance to Oxacillin (97.1 %) and Erythromycin (88.5 %). Also, high sensitivity was recorded for Teicoplanin, Vancomycin, and Linezolid. Molecular screening of fibronectin binding protein genes using PCR technique. It was concluded that *fnb*A gene was detected in all isolates (100%), while *fnb*B gene was present in 17.14 % of the isolates.

Keywords: *Staphylococcus hominis*, PCR, *fnb*A, *fnb*B.

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Introduction

Staphylococcus hominis is a species of the Coagulase-negative staphylococci (CoNS) it capable of causing infections particularly in immunocompromised patients where it has been reported to cause bacteremia, endocarditis, and endophthalmitis (1).

Due to the rising use of medical devices like intravenous catheters, vascular grafts, prosthetic heart valves, and devices used to treat joint disease, *Staphylococcus hominis*, a coagulase-negative staphylococcal strain, is a typical organism that is involved in nosocomial bacteremia (2). Both *S. hominis* species are nonmotile, nonspore-forming cocci that are Gram-positive and range in size from 1.0 to 1.5 m. All Staphylococci species, where the organisms appear singly or form tetrads and lower numbers of pairs, share the same cell layout.These exhibit sluggish and delayed development even when they are facultatively anaerobic. *S. hominis* lacks a capsule encasing the cell wall, in contrast to coagulase-positive Staphylococci like *S. aureus* (3). However, the cell wall gives the cell form and protection by being made of distinctive peptidoglycan and teichoic acid. A lipid-protein bilayer consisting of peptidoglycan and other proteins makes up the cell membrane. *S. hominis* pathogenicity is typically discovered through hospital acquired bacteraemias brought on by medical treatments (4). Several surface proteins are expressed by bacteria, including fibronectin-binding Adhesins from the family of MSCRAMMs (microbial surface components recognizing

adhesive matrix molecules) include proteins A and B (FnBPA and FnBPB). domains" (5). The aim of this study was to investigate the prevalence of fibronectin binding protein genes among *S.hominis* isolated from blood samples.

Materials and methods

Collection of samples

The blood samples (150) were collected from patients at the period from October 2022 to April 2023. The Ages from newborn to adults. The isolates from the blood of hospitalized patients were regarded as the causative agents of blood stream-infections (blood specimens taken from separate vein punctures). As far as possible, blood was collected from two different sites at the same time, from adults, 10-20 ml of blood, and from pediatric patients, 5-10 ml of blood. The sample was collected and added to BacT/ALERT® FA Plus Blood Culture Bottles and incubated in BacT/ALERT® 3D system (BioMerieux).

Isolation and identification of bacteria

In the laboratory of hospital under aseptic conditions, Sub-cultures were performed from the culture bottles flagged as positive. The specimen streak directly on blood agar, MacConkey agar and chocolate agar These plates incubated at 37℃ for 24 hours under aerobic and anaerobic conditions. The colony characteristics of sub-cultured organisms were examined for morphological characteristics and biochemical tests. Also, Gram staining was done. Vitek 2 compact system and molecular detection were employed to confirm the Identification.

Molecular identification

The following genes are detected using conventional PCR in this work. *fnb*A (460 bp) and *fnb*B (495 bp) genes were used as a for Identification of *S. hominis*, as stated in Table (1). In order to extract DNA from both Gram positive and Gram negative bacteria, a commercial purification method according to the Genomic DNA Mini Kit (Geneaid, Taiwan) was used to extract DNA from pure cultures of the examined strains. This kit uses bacterial procedure to extract DNA from both Gram-positive and Gram-negative bacteria.

Table (1): Primers sequences used in this study

Target gene	Oligonucleotide primer sequence 5' to 3'	Amplicon size (bp)
***fnb*A**	F : CCCTCTTCGTTATTCAGCC	460 bp
	R : CAGGAGGCAAGTCACCTTG	
***fnb*B**	F : TAAACACCGACGATAATAACCAAA	495 bp
	R : GGTCTAGCCTTATTTTCATATTCA	

F= forward primer, R= reverse primer.

PCR amplification

Using 25µL of PCR reaction mixtures as follows: (12.5 µl GoTaq Green Master Mix, 5.5 µl Nuclease free water, 1 µl Forward Primer, 1 µl Reverse Primer and 5 µl Extracted DNA. The program is first 5 min at 95°C , followed by thirty cycles at 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec and followed with final extension at 72°C for 7 min and hold at 4°C for 10 min. The presence of a PCR bands were confirmed by 1.5% agarose gel electrophoresis and visualization with ethidium bromide.

Results and discussion

Isolation and identification

A total of 150 clinical samples (blood) were collected from medical city hospitals in Baghdad. The results

included 35 (28.45%) positive samples of blood were given the typical biochemical tests and characteristics of morphology that is specific to *Staphylococcus hominis*, while the other 88 (71.54%) isolates belong to other species of staphylococci or other pathogenic bacteria and fungi (Figure 1), while 27 clinical sample was as no growth . In this study, a total of patients were, 67(54.47%) females, whereas 56 (45.52%) were from men. Patients' ages for the study were between newborn and adults for both genders.

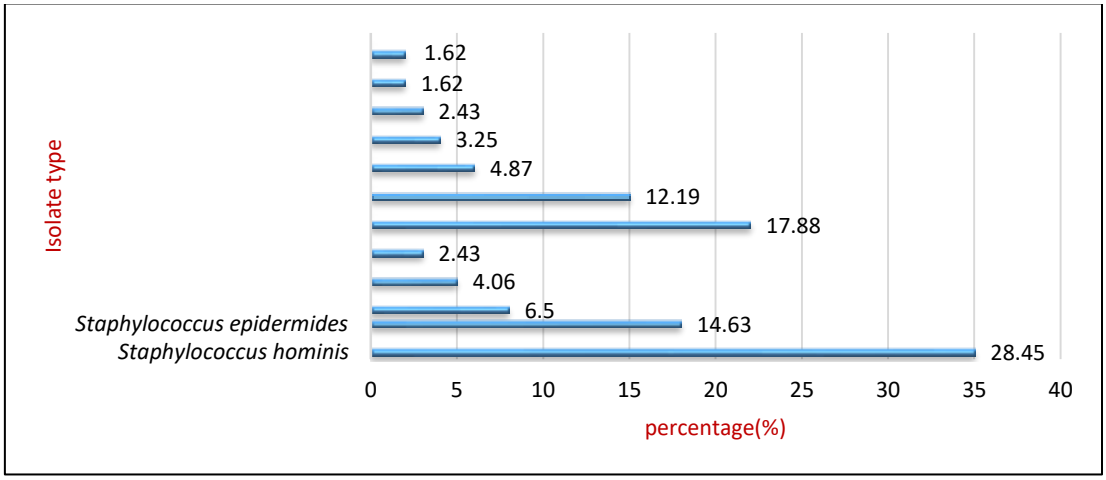

Figure (1): The percentages of Microbial species isolated from blood specimens.

The clinical specimens were cultured on blood agar media and chocolate agar media. The results found that isolates of *S. hominis* have smooth, slightly and spherical shape (Figure 2).

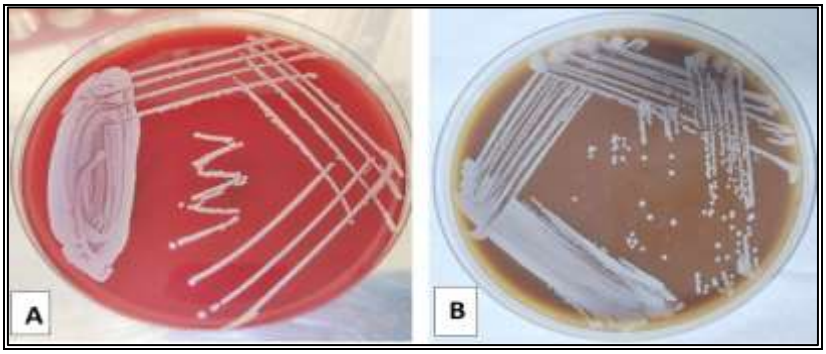

Figure (2): Colonies of *S. hominis* on A) Blood agar B) Choclate agar.

The bacterial cells that were diagnosed using a microscope. *Staphylococcus hominis* are visible after Gram staining as Gram-positive cocci and the characteristics include nonspore-forming, grouped in irregular clusters.

Biochemical tests of *S. hominis* isolates

The simplest biochemical test was employed to identify specimens that responded positively to catalase. However, the results of the coagulase and oxidase tests were negative.

The coagulase assays that demonstrate the capacity of the bacteria to produce the coagulase enzyme that distinguishes between *S. aureus* species (positive coagulase) and other *Staphylococcus* species (negative coagulase) (6), agreement with (7). Catalase was an enzyme that decomposes H_2O_2 (hydrogen peroxide)

to oxygen and water, it prevented the toxic metabolites accumulation (8). The *S. hominis* isolates were positive Catalase genus differentiates from others *Streptococcus* genus. *S. hominis* were negative for oxidase, which differentiates them from others *Micrococcus* genus (9).

Antibiotic susceptibility test

Antimicrobic susceptibilities of *S. hominis* were determined by the disk diffusion method on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute (2022). Resistance of *S. hominis* isolates were tested against several types of antibiotic (Table 2), including: pincillin (PEN); Ciprofloxacin(CIP);TrimethoprimSulfa methoxazole (SXT); Gentamycin (GEN); Moxifloxacin (MXF); Tetracycline (TET); Vancomycin (VAN); Clindamycin (CLI); Erythromycin (ER); Oxacilline; Rifampin (RIF); Linezolid (LZD); Teicoplanin (TEC); as shown in table (2). All *S. hominis* isolates have shown Highest resistance to Pincillin was recorded (100%), Oxacilline (97.1%) Erythromycin (88.5%), Clindamycin (74.2%): and intermediate resistance to gentamicin (65.7 %) the resistance rate was similar with other study reported by (10,11). Trimethoprim-sulfamethoxazole resistance has been shown low resistance (42.8 %), while it was shown to be 10–53% in Turkey and higher (47–76%) in European countries (12). with Moxifloxacin displaying the least resistance ratio (45.7%), Rifampin (48.5%), Linezolid (11.4%). In this study, the isolate was weak resistance to Vancomycin (22.8) and Teicoplanin (25.7%). The results presented here not agree with those of (13), who recorded None of CoNS strains were resistant to vancomycin and teicoplanin, This study agree with (14) shown Vancomycine were sensitive to all patients.

In the last years, the health benefits are under threat as many commonly used antibiotics have become less effective against certain illnesses not only because many of them produce toxic reactions but also due to the emergence of drug-resistant bacteria (15).

Table (2): Percentages of antibiotic susceptibility rate of 35 *S. hominis* isolates against 13 antibiotics.

Antibiotic	Resistance percentage
Pencillin	100 %
Oxacilline	97.1 %
Gentamycin	65.7 %
Erythromycin	88.5 %
Tetracycline	68.5 %
Ciprofloxacin	60 %
Moxifloxacin	45.7 %
Clindamycin	74.2 %
Trimethoprim-Sulfamethoxazole	42.8 %
Rifampin	48.5 %
Linezolid	11.4 %
Vancomycin	22.8 %
Teicoplanin	25.7 %
Chi-Square-χ^2 (P-value)	18.502 ** (0.0001)
** ($P \leq 0.01$).	

Molecular detection of fibronectin binding protein genes

The detection of *fnb*A and *fnb*B genes in *S. hominis* isolates was conducted used PCR technique according tothe specific primers of these genes, and the optimum conditions. The genes confirmed by agarose gel electrophoresis and photographed under ultraviolet (UV) transilluminator as shown in Figures 3 and 4. The amplification revealed the products of 460 bp and 495 bp for *fnb*A and *fnb*B genes, respectively. The rate of clinical *S. hominis* isolates that gave a positive result for the *fnb*A gene was 35 samples (100%); while the rate of a positive results for the *fn*bB gene was 6 samples (17.14%).

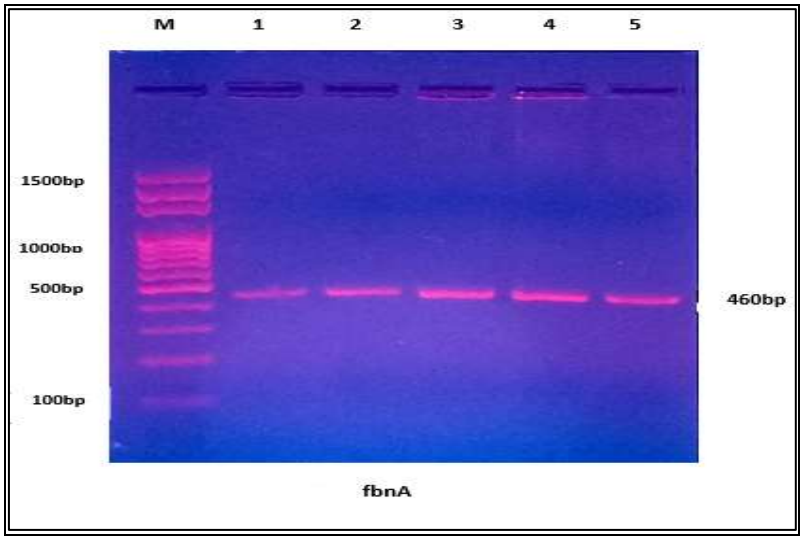

Figure (3): Gel electrophoresis of PCR products of *fbn*A gene in *S. hominis* isolates.

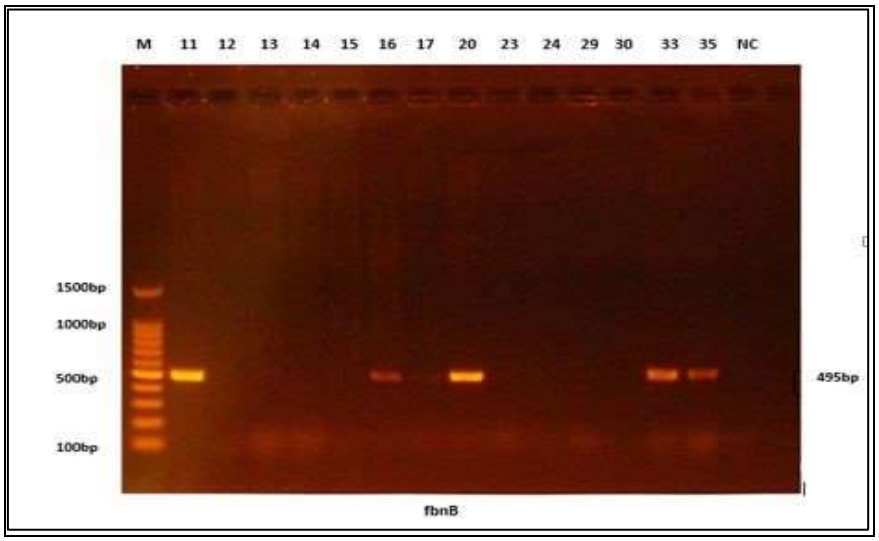

Figure (4): Gel electrophoresis of PCR products of *fbn*B gene in *S. hominis* isolates.

Among CoNS isolates, the prevalence rates of *ica*AD, *bap*, *fnb*A, and *cna* were 18.2%, 12.7%, 47.3%, and 27.3%, respectively. There were significant differences in the presence of these biofilm-associated genes among the MR-CoNS isolates (16). The gene *fnb*A presented in Methicillin-

resistant coagulase negative staphylococci (MR-CoNS) isolated from both community and hospital environments. The high prevalence of biofilm producing MR-CoNS strains indicates the persisting ability in environments (17).

Conclusion

The results of this study indicated to the importance of *S. hominis* as a main cause of blood infections with high resistance to most antibiotics used for treatment, also the predominant of *fnb*A gene among these isolates.

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