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A Microbiological Study on Clinical Isolates of Coagulase-Negative Staphylococci (CoNS) from Sulaimaniyah Hospitals

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Abstract

The purpose of this study was to identify and isolate different coagulase-negative staphylococci (CoNS) species associated with clinical samples with their virulence factors. For this purpose, 355 clinical samples were taken from various Hospitals in Sulaimaniyah city. Different species of CoNS were identified by using (blood, MacConkey, Mannitol salt) agars, biochemical tests, and VITEK® 2 compact system. The phenotypic characterization of hemolysin was based on the hemolysis pattern of CoNS on sheep blood agar. Moreover, the biofilm detection in CoNS was performed by using a microtiter plate (MTP). The various biofilm and virulence genes were detected using specific primers to detect *mecA, hla, hlb, hld, hlg* and the *icaAD, fnbA*, and *bap* genes, respectively. The number of MR-CoNS and MS-CoNS were 31 (96.8%) and 1 (3.1%), respectively, out of 32 isolates. The availability of the *mecA* gene, responsible for the resistance of CoNS to methicillin, was found in (100%) of CoNS species related to clinical samples. Interestingly, the hemolysin genes were detected on the plasmids instead of the chromosomal DNA, and these results indicated that the CoNS could be the primary cause of nosocomial infection.

Introduction

Staphylococcus has mainly consisted of several species and subspecies extensively spread in nature, mainly in the skin and in birds and mammalian mucous membranes [1]. They are divided into two groups, following the production of the coagulase enzyme; if they have the capability of coagulating blood plasma, they would be classified as coagulase-positive staphylococci, however other staphylococci, that do not, are known as coagulase-negative staphylococci CoNS [2]. Even though staphylococci have developed resistance, they are still naturally susceptible to antimicrobial agents [3]. Both MR-SA and MR-CoNS are important in human and animal medicine, and they are referred to together as methicillin-resistant staphylococci (MRS).

The *mecA* genes, which encode the characteristic of resistance to methicillin and nearly all ß-lactam antibiotics, are conveyed by the staphylococcal cassette chromosome *mec* (SCC*mec*) large mobile genetic element [4]. CoNS can generate a variety of enzymes and toxins that are usually associated with S. aureus, like hemolysins - which are responsible for invading the host cell. The staphylococcal hemolysins are classified into four types as follows: alpha (α), beta (β), gamma (γ), and delta (δ) [5]. Alfa toxins, toxin's pathogenicity, are attributed to hemolytic, dermonecrotic, T cells, monocytes, and macrophages [6]. Beta toxins have a high phosphorylase activity and have a significant affinity for the cell membranes of many different cells. As a result,

the membrane becomes unstable [7]. Delta toxins: this toxin degrades erythrocytes by acting as a detergent and may cause a problem like intestinal diseases that vary from acute diarrhoea to severe enteritis [8], [9]. Gamma toxins impact neutrophils and macrophages, and gamma-hemolysin may lyse many different types of mammalian erythrocytes. Due to the inhibitory effect of agar on toxin activity, (γ) toxin is not visible on blood agar plates [10]. CoNS may contact and attach to medical devices and surfaces through a slime layer with a mucopolysaccharide structure. They can, therefore, swiftly penetrate and spread to the hospital surroundings. The slime factor plays a significant role in infections; it helps to protect pathogens against antibacterial and phagocytose agents [11].

Biofilm development is a complicated procedure that is further split into four stages: attachment, accumulation, maturation, and dispersal [12]. The bacteria then proliferate, further creating a multilayered biofilm linked to the formation of polysaccharide intercellular adhesion (PIA) that enables cell-to-cell attachment [13]. The intracellular adhesion (ica) operon's products are involved in PIA production. The icaABCD genes in this operon produce a biofilm matrix polysaccharide (PIA/PNAG), which is made up of linear ß-1-6-linked N-acetyl glucosamine residues [14]. The intracellular adhesion, initial attachment, and biofilm development are aided by the biofilm-associated protein (Bap) [15]. Bap activates a biofilm-inducing pathway that is also present in the S. epidermidis [16]. A major role is played by the fibronectin-binding proteins (FnbA) during the accumulation phase. FnbA promotes the formation of biofilms by binding proteins to surface-located receptors on neighbouring cells, a process known as homophilic interactions [17]. As a result, this research's goal was to better understanding the virulence factors of CoNS acquired from human clinical samples in Sulaimani city hospitals and looked into antibiotic resistance in CoNS.

Materials and methods

A. Sample collection

Three hundred and fifty-five samples were randomly obtained from different clinical specimens, from June to December 2020: including 219 (61.69%) samples from Shar hospital, 73 (20.56%) from the Emergency hospital, and 63 (17.74%) from Dr. Jamal Ahmad Rashid's Pediatric Teaching hospitals in Sulaimaniyah, Iraq. The bacterial isolates were collected from urine, swab, blood, tip catheters, central venous line (CV Line), endotracheal aspirate (ETA), sputum, cerebrospinal fluid (CSF), and peritoneal fluid (Table 1).

Table1: Studied samples sources from different three hospitals.

	Hospital's name			
Sample source	**Shar**	**Dr. Jamal Ahmad Rashid Pediatric Teaching**	**Emergency**	**Total (%)**
Urine	64	40	25	129 (36.33)*
Blood culture	55	-	5	60 (16.9)
Tip catheter	27	-	-	27 (7.6)
ETA	25	-	-	25 (7)
Swabs	16	15	33	64 (18)
CSF	13	-	-	13 (3.6)
Sputum	10	8	10	28 (7.88)
Peritoneal fluid	6	-	-	6 (1.69)
CV line	3			3 (0.8)
Total	**219**	**63**	**73**	**355**

*Number inside brackets represents percentages.

B. *Isolation and identification of CoNS*

Conventional methods investigated coNS. Primary isolates were subcultured on blood, MacConkey, and Mannitol Salt Agar (MSA) at 37°C for 24 h. Colonies were recognized by gram stain, coagulase, catalase, and

the oxidase test [18]. VITEK® 2 compact system was used to identify staphylococcal species and antibiotic susceptibility. According to the manufacturer's instructions, the system cards were automatically filled, sealed, and loaded into the VITEK® 2 compact system instrument for incubation and reading. The *mecA* gene was used to identify MR-CoNS by polymerase chain reaction (PCR).

C. *Hemolysins characterization*

The characterization of the different types of hemolysins is based on the lysis zone of each staphylococcal isolate on the blood agar base plates supplemented with 5% sheep blood [19]. After the incubation for 24 h at 37°C, the plates were observed for growth; they noted colony colour, morphology, and hemolysis [20].

D. Biofilm assay by microtiter plate

This quantitative test is the best approach for detecting the development of bacterial biofilms, as reported by Mathur et al. [21] and Pragyan et al. [22]. About 200 µL of bacterial cultures were placed in an MTP and then incubated at 37°C for 24 h, by removing the medium and the wells washed thrice with 200 µL phosphate buffer saline, pH 7.2. After 10 minutes, the wells became dry, and the connected cells were stained with crystal violate (CV) 0.1% for 15- 30 min. The liquid was removed after staining, and the wells were washed three times with distilled water (DW). After allowing the plate to dry at room temperature, 200 µL of ethanol (95%) was poured into the wells to solubilize the stain [23]. A microplate Spectrophotometer (Biotech Quant, USA) was used to detect absorbance at 570 nm. The non, weak, moderate, and vigorous biofilms categorized are the isolates based on the average optical density (OD) values shown below: non-biofilm-producing= OD isolates ≤ OD control; weak-producing= OD control ≤OD isolate ≤ 2OD control; moderate-producing= 2OD control ≤ OD isolate ≤ 4OD control; strong-producing= 4OD control ≤ OD isolate [24].

E. Chromosomal and Plasmid DNA extraction of CoNS

The growth of CoNS took place overnight at 37 °C on blood agar plates. Later on, one colony was suspended in a nutrient broth (LAB/UK) at 37 °C for 24 h. Chromosomal DNA (Geneaid, Taiwan) extraction kit was used for studying biofilm genes and *mecA* genes. According to the manufacturer's protocols, the plasmid extraction kit (Add a bio, Korea) was used for studying hemolysin genes. The bacterial culture was harvested by centrifugation at 8000 rpm for 3 min then the supernatant was discarded. Two hundred and fifty µl of resuspension solution (RNase solution was added to resuspension solution) was added to the collected cells and completely resuspended by vortexing. In the lysis step, 250 µl of lysis solution was added and mixed by inverting the tube 3-5 times gently. Three hundred and fifty µl of neutralization solution was added and immediately mixed by inverting the tube 3-5 times gently. Genomic DNA, cell debris, and the insoluble complex were formed. The tube was centrifuged at 13,000 rpm for 10 min in a microcentrifuge. A compact white pellet appeared at the bottom of the tube. The cleared lysate was transferred to the spin column with a collection tube and centrifuged at 13,000 rpm for 1 min. The flow-through was poured off and assembled the spin column with the 2.0 ml collection. Seven hundred µl of washing solution (Ethanol was added to the washing solution) was added to the spin column with the collection and centrifuged at 13,000 rpm for 1 minute. The flow-through was poured, and the spin column was assembled with the 2.0 ml collection tube. In this step, the spin column was dried by additional centrifugation at 13,000 rpm for 1 minute to remove the residual ethanol in the spin column. The spin column was transferred to the new 1.5 ml micro-centrifuge tube. In the last step, 50 µl of elution solution was added to the spin column with the micro-centrifuge tube and was left standing for 1 minute, and then the plasmid DNA was eluted by centrifugation at 13,000 rpm for 1 min. After this process, 1 µL was extracted from the chosen DNA sample and loaded on a Nanodrop 2000 UV spectrophotometer (Thermo Fisher Scientific, UK).

F. Studying PCR amplification assay

Four sets of hemolysins genes, *hla, hlb, hld and hlg*, were used to identify the hemolysin encoding genes of CoNS. Three sets of primers were used for amplification of biofilm genes *icaAD, fnbA*, and *bap*. One set was used for the *mecA* gene. These primers were prepared to conform to manufacturer protocol (Sina Clon, Iran) and were ready for PCR amplification assay. The PCR mix reaction constituent concentration is done with a final volume of 25 µL. The template DNA (3 µL), Forward (F) and Reverse primer (R) 10 pmol / µL (1 µL), one PCRtm 2X (10 µL) and dH_2O (DNase, RNase free) 10 µL were conformed. The reaction tubes were placed in a thermal cycler (AB applied biosystem, Singapore) and run under an optimized amplification condition (Table 2). Then, the process of extension (72 ° C for 30 sec), the process of denaturation (94 °C for 5 min), and the final process of extension (72 °C for 5 min) were used for all genes. PCR products (5 µL) were examined on one and a half percentage agarose gel stained with 1x Ethidium Bromide (EtBr) and visualized under UV transillumination.

Table 2: Used primers for amplification of genes and annealing temperatures for detection genes.

Genes	*Name of the primers*	*Primer sequence*	*Length (bp)*	*Annealing temperature (Ta)*	*References*
Hemolysin	*hlaF*	*CTGATTACTATCCAAGAAATTCGATTG*	*209*	*58 ºC 40 sec*	[5]
	hlaR	*CTTTCCAGCCTACTTTTTTATCAGT*			
	hlbF	*GTGCACTTACTGACAATAGTGC*	*309*		
	hlbR	*GTTGATGAGTAGCTACCTTCAGT*			
	hldF	*AAGAATTTTTATCTTAATTAAGGAAGGAGTG*	*111*		
	hldR	*TTAGTGAATTTGTTCACTGTGTCGA*			
	hlgF	*GTCAYAGAGTCCATAATGCATTTAA*	*535*		
	hlgR	*CACCAAATGTATAGCCTAAAGTG*			
Methicillin-Resistant	*mecAF*	*TGGCTATCGTGTCACAATCG*	*310*	*57 ºC 40 sec*	[25]
	mecAR	*CTGGAACTTGTTGAGCAGAG*			
Biofilm	*icaADF*	*GACAGTCGCTACGAAAAG*	*211*	*52 ºC 40 sec*	
	icaADR	*AATAAGCTCTCCCTAACTA*			
	fnbAF	*CCCTCTTCGTTATTCAGCC*	*422*		
	fnbAR	*CAGGAGGCAAGTCACCTTG*			
	bapF	*GGCGCAAGCAGCAGAATTA*	*901*		
	bapR	*CATAGTTCTTTGTGGTGTTGC*			

Result and discussion

A. Isolation and prevalence of CoNS

A total of 355 clinical samples from different sources were obtained from three hospitals. Based on the results from bacterial cultures, Gram stain, and biochemical assays, it was confirmed that the isolates are (32) CoNS; the isolates were common in urine culture [11 (34.37%)] followed by swab cultures [9 (28.12%)], blood cultures [6 (18.75%)], Tip catheter cultures [4 (12.5%)], CV line [1 (3.12%)], and ETA cultures [1 (3.12%)] (Figure 1).

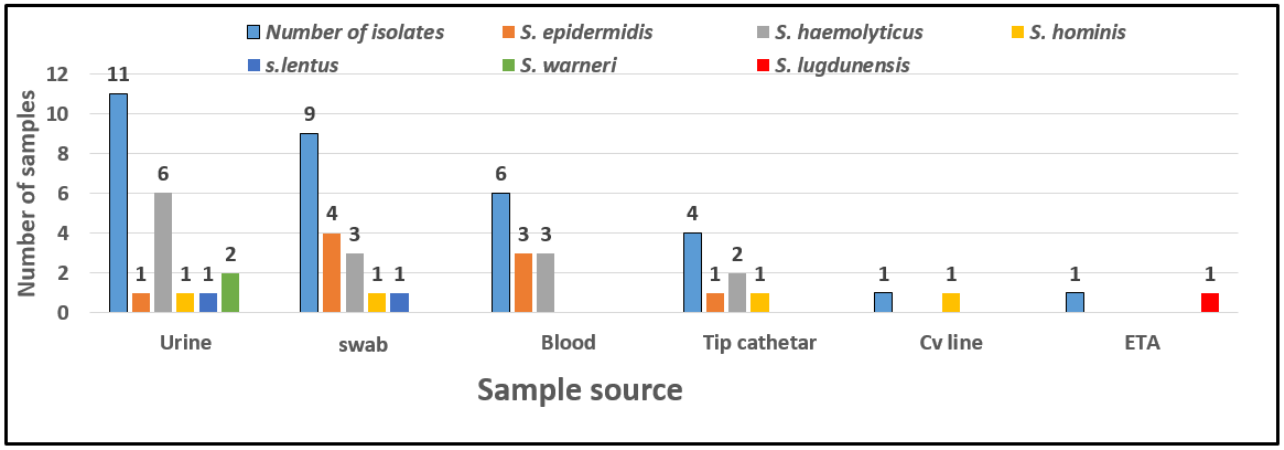

Figure 1: Distribution of CoNS isolates among their source (%).

It was reported, by previous studies, that the majority of CoNS species was isolated from blood in comparison to urine [26], [27]. Therefore, for further identification of *Staphylococcus* species, VITEK® 2 compact system was applied. The results showed that different species of *Staphylococcus* were identified in the tested samples, in which the most dominant species were 14 (43.75%) *S. haemolyticus* followed by 9 (28.12%) *S. epidermidis*, 4 (12.5%) *S. hominis*. The least commonly isolated CoNS species were 2 (6.25%) *S. lentus*, 2 (6.25%) *S. warneri*, 1 (3.12%) *S. lugdunensis.*

Results of the current study are in agreement with another study in Northern Thailand which reported that out of (55 MR-CoNS) isolates, *S. haemolyticus* (34.5%) was observed to be the most common species and the *S. epidermidis* (32.7%) was the second-most common isolate that was isolated from clinical samples [28]. Many reports demonstrated that the *S. haemolyticus* is the most frequent species and is highly prevalent in hospital environments, further causing hospital-acquired infections [25], [29], [30]. Another study by Secchi and his colleagues revealed that a total of 238 isolates were identified as CoNS. *S. haemolyticus* was the most frequent isolate (100/238), followed by *S. hominis* (70/238) and *S. warneri* (18/238) [31]. *Staphylococcus* spp. is different depending on the source of isolates [32].

B. Antibiotic susceptibility testing

The antibiotic susceptibility results were taken from the VITEK® 2 compact system depending on (MICs) of each antimicrobial agent [33]. Thirty-one (96.8%) had Cefoxitin resistance, which is considered MR-CoNS, and 1(3.1%) of isolates were Cefoxitin susceptible as MS-CoNS. CoNS were resistant to Benzylpenicillin and Oxacillin 32 (100%), Erythromycin 27 (84.3%), Tetracycline 21(65.6%), Tobramycin 20 (62.5 %), Gentamicin 18 (56.2%), Clindamycin 14 (43.7%), and more sensitive to linezolid 32 (100%), Nitrofurantoin 29 (90.6%), Vancomycin was 28 (87.5%), Tepicoplanin 26 (81.2%) Fusidic acid 7 (21.8%), levofloxacin 17 (53.1%). It was also shown by the present study that the CoNS are comparatively more resistant to numerous antimicrobial agents (Figure 2).

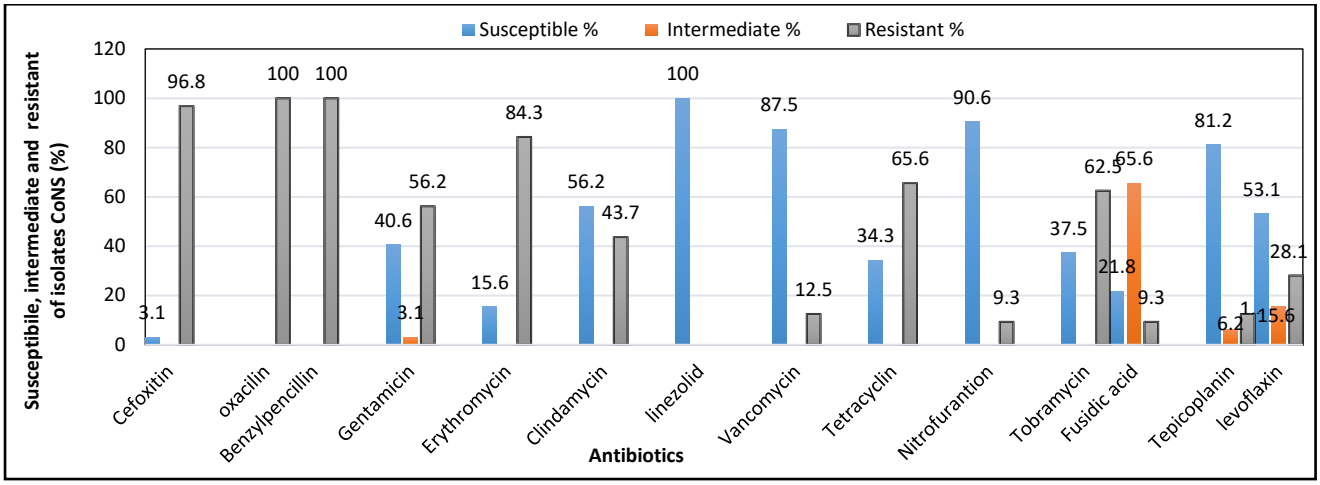

Figure 2: Antibiotic pattern of susceptibility for different CoNS isolates (%).

Another study from Turkey reported that 200 CoNS isolates were obtained from the patients' blood samples; (67.5%) of CoNS isolates showed their resistance to methicillin. Resistance rates are shown as follows, (25%) Fusidic acid, (60%) Tetracycline, (72%) Clindamycin, (80%) Erythromycin, (90%) Gentamicin. Resistance to Vancomycin and Teicoplanin was not shown [34]. Furthermore, another study by Zaid and Alaa in Babylon/Iraq reported that out of 15 CoNS isolates (86.7%), coagulase-negative Staphylococcus was resistant to Oxacillin and Ampicillin, 53.3% of the samples were resistant to Gentamicin. About 40% of Tetracycline and Ciprofloxacin (86.6%) were resistant to Erythromycin, (13.3%) were Rifampin resistant, (20%) Clindamycin resistant. On the other hand, all CoNS isolates (100%) were susceptible to linezolid, Teicoplanin, and vancomycin [35]. Another study reported that all 52 coagulase-negative staphylococcus isolates showed susceptibility to vancomycin and linezolid [11].

According to the findings of this study, linezolid, nitrofurantoin, and vancomycin (100%), (90.6%), (87.5%), respectively, showed the greatest effect against methicillin-resistant isolates. Our research findings were in line with previous works in which 5.4% of the 87 CoNS isolates showed reduced susceptibility to vancomycin [36]. The availability of the mecA gene, which is responsible for CoNS resistance to methicillin, was found in (100%) of CoNS species (Figure 3). This situation is following other studies [28], [35], [37].

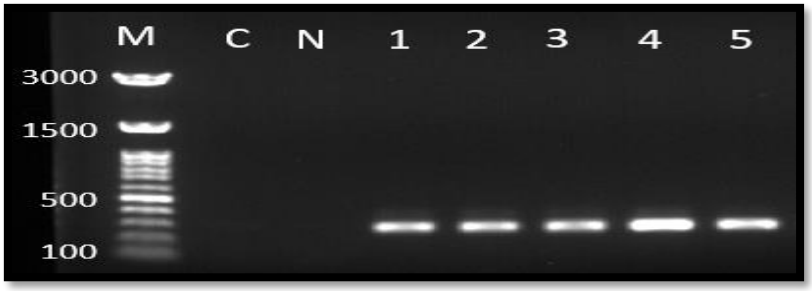

Figure 3: Agarose gel electrophoresis of (PCR) amplification of the *mecA* gene. Lane (M) ladder; lane C- blank, - Lane (N) – represent negative control, lanes (*1*, *2*, *3*, *4*, *5*) CoNS isolates have *mecA* gene length 310 bp.

C. Phenotypic and molecular detection of hemolysin:

Phenotypic detection of hemolysin

Phenotypic hemolysin activity on sheep blood agar in CoNS was found in 28 (87.5%) isolates, and 4 (12.5%) isolates were non- hemolysin producer isolates. Of the 28 isolates, 26 (81.2%) had alfa toxin activity; partial lysis of the membrane of the red blood cell (RBC) that creates medium green discolouration or a brown. Moreover, 2 (6.25%) had beta toxin activity, known as (β) hemolysis, which causes complete lysis of red blood

cells (RBC) and a near-transparent halo around the colony. Four (12.5%) isolates lacked hemolytic activity, known as gamma (γ). A similar study demonstrated that most of the human isolates produced alpha hemolysis and all isolates of CoNS lacked gamma hemolysin expression [5]. The hemolysin activity was more frequent in S. haemolyticus 13/14 (92.8%) than S. epidermidis 8/9 (88.8%). Other studies demonstrated that hemolytic activity was 100% for S. haemolyticus and 55% for S. epidermidis [38].

Molecular detection of hemolysin

The plasmid DNA extraction was performed to ensure the presence of the hemolysin genes. The PCR amplification of the plasmid gene encoding hemolysin of CoNS species with specific primers observed that the high frequency of hemolytic gene among CoNS isolated from urine followed by swap, blood, a tip of the catheter, and others as shown in table 3. Out of 32 isolates, all had the *hla* gene (100%), followed by *hld* gene, which was in 19 (59.3%) of the isolates, which means that 19 (59.3%) of isolates have both *hla* and *hld* genes from total isolates. None of the samples had *hlb* and *hlg* genes (Figure 4). According to this study, the *hla* and *hld* genes are found in abundance in CoNS. On the other hand, the other study showed that the existence of the *hlb* gene was not observed in any isolate of the CoNS species, while the *hla* gene was found in 20% of *S. epidermidis* [39]. In this study, out of 32 isolates were the largest number of the strains carrying the *hld* gene belonged to *S. haemolyticus* 10 (31%), then *S. epidermidis* 6 (18.7%), compared to another study, the most significant number of the strains carrying the *hld* gene belonged to *S. epidermidis* then *S. haemolyticus* [40**]**. Another research discovered that the *hld* gene was present in 95.3% of *S. epidermidis* but not in *S. haemolyticus* [7].

Table 3: Identification of encoding hemolysin genes among 32 of the CoNS isolates.

Type of hemolysin gene	***S. epidermidis (n=9)***	***S. haemolyticus (n=14)***	***S. hominis (n=4)***	***S. lentus (n=2)***	***S. warneri (n=2)***	***S. lugdunensis (n=1)***	***Total***
hla	*9*	*14*	*4*	*2*	*2*	*1*	*32*
hld	*6*	*10*	*3*	*0*	*0*	*0*	*19*
	Urine	***Swab***	***Blood***	***Tip catheter***	***ETA***	***CV line***	
hla	*11 (100)**	*9 (100)*	*6 (100)*	*4 (100)*	*1(100)*	*1(100)*	
hld	*5 (45.4)*	*6 (66.6)*	*4 (66.6)*	*3 (75)*	*0 (0)*	*1(100)*	

*Number inside brackets represents percentages.

The genotypes of CoNS hemolysin in this study did not appear to be associated with the expression of their phenotypes, suggesting that it may be impacted by a variety of variables at the genetic or phenotypic levels, similar to the findings of another study [5]. The detection of the genetic determinants of β- and α-toxins in *S. epidermidis* and *S. haemolyticus* is not an easy and convenient process [7]. In this study, the toxin's presence and the gene's absence was detected in 2 (6.25%) *S. haemolyticus* isolates. This might be due to changes in the gene sequences, such as insertion sequences that interfere with PCR amplification of the gene, a comparison related to this circumstance noticed in (5) *S. haemolyticus* isolates and (1) *S. epidermidis* was isolated from blood culture [7]. The presence of *hla* and *hld* genes in *Staphylococcus aureus* and CoNS are necessary for isolates related to staphylococcal infection that cause animal and human disease [41].

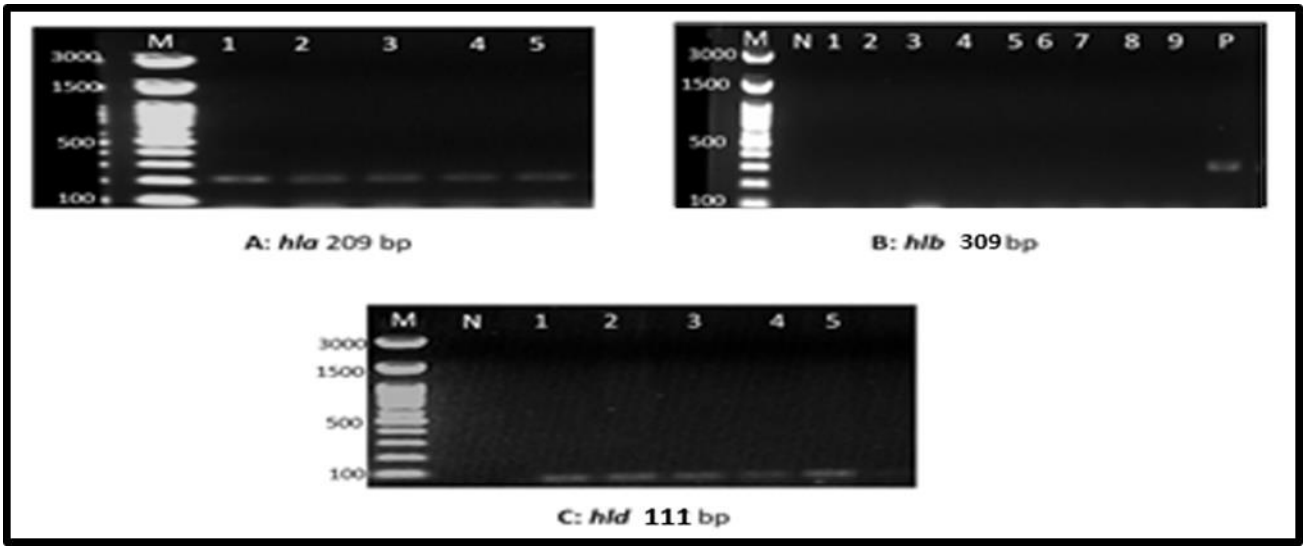

Figure 4: Agarose gel electrophoresis of (PCR) amplification of hemolysin genes. A- *hla* gene; lanes (1- 5) CoNS isolates have *hla* gene, B- *hlb* gene; lanes (1-9) CoNS isolates no *hlb* gene C- *hld* gene; lanes (1- 5) CoNS have *hld* gene, lane P: positive control has *hlb* gene length, lane (N): negative control, Lane (M) ladder 100 bp.

D. Phenotypic and Molecular detection of biofilm producer

Phenotypic of biofilm producers

In this study, 32 CoNS isolates were tested for biofilm production using MTP within 24 h, and results showed that 18 (56.2.1%) were biofilm producers and 14 (43.7%) isolates were no biofilm producers. Ten (31.2%) of the isolates had weak biofilm production, while 1 (3.12%) isolate showed strong biofilm production, and 7 (21.8%) isolates showed moderate biofilm production (Table 4) (Figures 5 and 6).

Table 4: Classification of bacterial biofilm formation by (MTP) method.

Mean of OD Values	***Biofilm formation***
<0.072	*No biofilm*
0.072<OD sample<0.144	*Weak*
0.144<OD sample<0.288	*Moderate*
0.288<OD sample	*Strong*

Figure 5: Biofilm assay. A: Microtiter plate technique of Biofilm essay: (1) non-biofilm, (2) Moderate, (3) Weak, (4) Strong, Crystal Violet staining was used in 96 wells of MTP. B: Percentages of biofilm formation ability by MTC method among CoNS isolates (%).

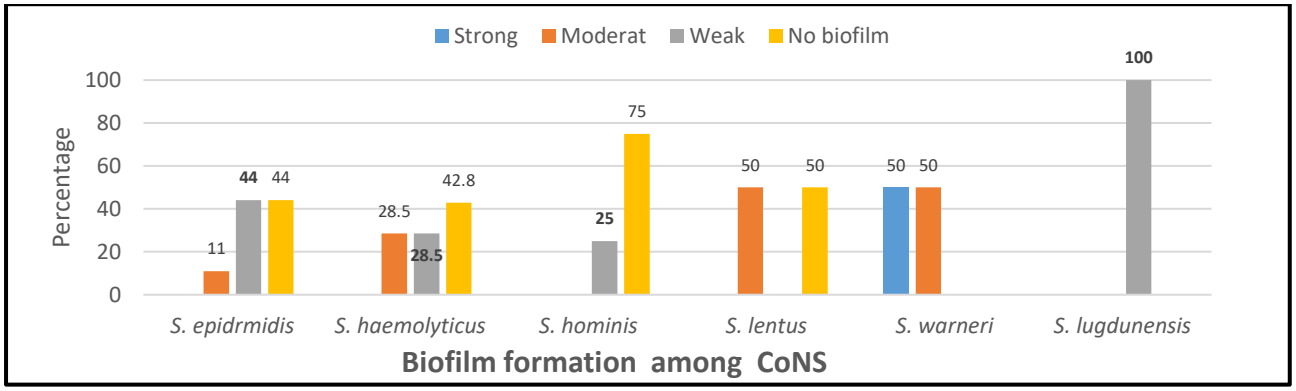

Figure 6: Distribution of biofilm formation among CoNS isolates (%).

Another study demonstrated that out of 6 CoNS isolates, 4 were no biofilm producers, 1 was a weak producer, and 1 showed moderate biofilm production. These isolates were recovered from intravascular catheters and blood samples [42]. On the other hand, 176 CoNS isolates were collected from newborns' blood cultures; 40.9% (72/176) of the isolates produced biofilms; 21% (37/176) were weakly adherent, and 19.9% (35/176) were firmly adherent [43]. In another study in India, it was found that not all clinical staphylococcal isolates expressed a biofilm-positive phenotype; only 82/152 (57.8%) were biofilm producers, 22/152 (14.47%) were strong producers, and 60 (39.4 %) were moderate. While in 70 (46%) isolates, weak or no biofilm production was detected. The isolates were recovered from blood, infected devices, and skin surfaces [21]. The ability to create biofilm is directly linked to bacterial virulence and persistence. Also, several chronic bacterial illnesses are now considered to be associated with biofilm formation [44].

Molecular detection of biofilm producers

Among 31 (96.8%) MR-CoNS isolates and 1 (3.1%) MS-CoNS, the *icaAD* and *fnbA* genes were present in all CoNS (100%) concerning chromosomal gene encoding biofilm formation. However, the *bap* gene was detected in 2 (6.25%) isolates. Figure 7 shows that PCR- a product of *icaAD*, and *fnbA* genes from CoNS isolates. In general, *icaAD* and *fnbA* genes were detected in 100% of the CoNS isolates from different clinical samples, and that 18 (56.2.1%) were biofilm producers, and 14 (43.7%) isolates were no biofilm producers; therefore, it was not possible to explore the relationship between the formation of biofilm, and the genes' presence in these isolates (Table 5).

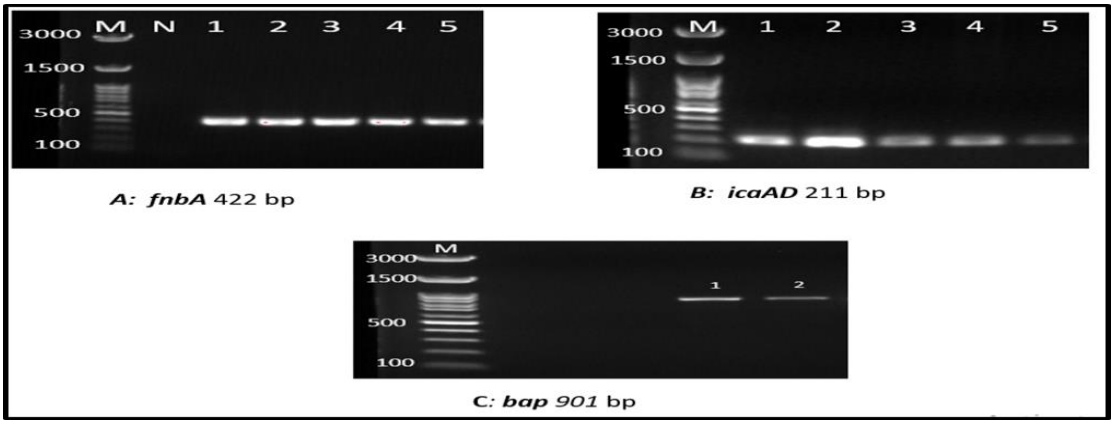

Figure 7**:** Agarose gel electrophoresis of polymerase chain reaction (PCR) for biofilm formation genes. **A-** *fnbA* 422bp, (1,2,3,4,5) samples have related gene, **B-** *icaAD* 211 bp (1,2,3,4,5) samples have related gen, and **C-** *Bap* (1,2) samples have related gene, L M: 100bp DNA ladder.

Table 5: Resistance antimicrobial patterns and phenotypic biofilm formation in 32 CoNS isolates.

Antibiotic's name	Susceptible		Intermediate		Resistance	
	Biofilm	No biofilm	Biofilm	No biofilm	Biofilm	No biofilm
Cefoxitin	1 (100)*	0	0	0	17 (54.8)	14 (45.1)
Benzylpenicillin	0	0	0	0	18 (56.2)	14 (43.7)
Gentamicin	7 (53.8)	6 (46.1)	1 (100)	0	11 (61.1)	7 (38.8)
Erythromycin	2 (40)	3 (60)	0	0	16 (59.20	11 (40.7)
Clindamycin	9 (50)	9 (50)	0	0	9 (64.20)	5 (35.7)
Vancomycin	16 (57.1)	12(42.8)	0	0	3 (75)	1 (25)
Tetracycline	7 (63.6)	4 (36.3)	0	0	11 (52.3)	10 (47.6)
Nitrofurantoin	14 (48.2)	15 (51.7)	0	0	3 (100)	0
Fusidic acid	4 (57.1)	3 (14.2)	12 (57.1)	9 (42.8)	1 (33.3)	2 (66.6)
Tobramycin	7 (58.3)	4 (41.6)	0	0	11 (55)	9 (45)
levofloxacin	8 (47)	9 (52.9)	2 (40)	3 (60)	8 (88.8)	1 (11.1)
Teicoplanin	15 (57.6)	11 (42.3)	1 (50)	1 (50)	3 (75)	1 (25)

*Number inside bracket represents percentages

Nasr *et al*. found that out of 50 clinical isolates, 16 (32%) staphylococcal isolates contained the *icaAD* gene, while 34 (68%) did not possess such gene and correlation between the phenotypic biofilm production methods with *icaAD* gene detection; 8 (50%) of the 16 *icaAD* positive staphylococcal isolates, showed MTP positivity; therefore the *icaAD* gene's presence was not completely related with the formation of biofilm [42]. It was shown, by several studies, that the presence of both the *icaD* and *icaA* genes is linked with biofilm development in staphylococci producing catheter-associated and nosocomial infections. To get the full phenotypic expression of biofilm in staphylococcal isolates, a co-expression of the genes would be necessary [45]. Despite the presence of *ica*, the lack of biofilm could result from several factors: an example is the inactivation of the *ica* operon by the action of the *icaR* repressor [46] or the post-transcriptional regulation [47].

Biofilm is a perfect medium for the exchange of resistance plasmids [44]. The difference between the phenotypic and genotypic characterization of biofilm formation may result in heterogeneity in the genetic origins and not because of the presence or absence of genes required for the biofilm [48]. Moreover, it was shown that the *bap* gene was present in 2 (6.25%) of CoNS isolates. Although *bap* genes have been involved in biofilm formation, previous studies showed that their presence is not required during the biofilm process [25]. This correlated with our finding that the presence of the *bap* gene did not correlate with biofilm production in CoNS isolates. Kitti and his colleagues found that (7-12.7%) out of the 55 MR-CoNS clinical isolates had *bap* genes and (21-38%) of isolates were biofilm producers [28]. The *fnbA* gene was present in all CoNS isolates and (56%) were produced biofilm. These findings correlated with other studies which detected the *fnbA* gene in (41.4%) of biofilm-producing isolates from patients with bloodstream infections [49], as well as another research that found the *fnbA* gene in (47.3%) of MR-CoNS isolates[28]. The *icaAD* gene has been linked to biofilm formation. In contrast, the *fnbA and bap* genes have been linked to adhesion to biotic or abiotic surfaces, which is the first step in biofilm formation [50].

Conclusion

The *mecA* gene was found in all MR-CoNS isolates. All observed isolates were shown to have multidrug resistance. Because of its capacity to build biofilm on many surfaces and include the *hla* and *hld* genes, *S. haemolyticus* was the most common bacterium recovered from patients. We also discovered that the *icaAD* and *fnb*A genes were linked to biofilm development as measured by MTP but that the *bap* gene was not. These findings suggested that the CoNS might be a significant source of nosocomial infection.

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