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Detection of Biofilm Producer Methicillin Resistant *Staphylococcus hominis* Isolated From Different Clinical Sources

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A R T I C L E I N F O

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ABSTRACT

Staphylococcus hominis is an opportunistic gram positive bacteria and is a member of coagulase-negative staphylococci (CoNS) . During the study, 213 clinical specimens were collected from patients admitted to different hospitals in Erbil city, Iraq. Only 18(8.45%) isolates of *S. hominis* were isolated including 7 isolates (17.07%) from blood, 4 isolates (8.70%) from urine, 3 isolates (7.14%) from ear, 2 isolates (6.67%) from wound, and one isolate from each nasal swab (4.55%) and oral cavity (3.13%), all *S. hominis* identified based on morphology, cultural, biochemical tests, and further confirmed by Vitek 2 compact system. To determine the most accurate assay for measuring methicillin resistance *S. hominis*, compared the detection of *mecA* by PCR with detection by National Committee for Clinical Laboratory Standards methods using oxacillin and Cefoxitin disk diffusion method. The results of oxacillin showed 13 (72.22%) isolates resistant to methicillin and 5 (27.77%) isolates were sensitive to it. While, the results of cefoxitin demonstrated that 16 (88.89%) isolates were resistant to methicillin and only 2 isolates (11.11%) were sensitive to it. However, the same results of the Cefoxitin disk diffusion method was obtained by PCR and by using *mecA* gene which 16 isolates (88.89%) were carried mecA gene with product size 499bp. The results of microtiter plate method revealed that 16 (88.89%) isolates of S. hominis were biofilm producer and only 2 isolates (11.11%) were non-biofilm producer. Moreover,of 16 biofilm producer isolates, 14 isolates (77.77%) were categorized as strong biofilm producers and 2 (11.11%) isolates were identified as moderate biofilm producers.

1. INTRODUCTION

Staphylococcus hominis is the third important species of coagulase-negative staphylococci (CoNS) and is a part of the commensal bacterial microflora of healthy people, in addition to that it recognized as a major cause of nosocomial infections and frequently isolated from specimens of hospitalized patients(Szczuka *et al*., 2014) . *S. hominis* found asymptomatically on the skin, arms, legs, and surfaces of the axilla and is an opportunistic pathogen found in blood and capable of causing a different diseases including bacteremia, septicemia, and endocarditis especially in immunocompromised patients (d'Azevedo *et al*., 2008; Al Wohoush *et al*., 2010; Bouchami *et al*., 2011). Infections caused by *S. hominis* are often highly resistant to antibiotics which make the treatment difficult. Furthermore, a growing concern is the emergence of methicillin resistant *S. hominis* , which is the important pathogen among methicillin resistant CoNS especially in clinical isolates and the methicillin resistance *S. hominis* is associated with the *mecA* gene (Zhang *et al*., 2013; Mendoza-Olazaran *et al*., 2013). Detection of *mecA* gene by Polymerase chain reaction is considered as the gold standard for methicillin resistance as this gene is highly conserved among Staphylococcal species (Khan *et al*., 2012). Indeed, many infections caused by *S.hominis* appeared to be associated with biofilms and the nosocomial infections by CoNS are primarily associated with the use of medical devices, likely because of biofilm formation (Fredheim *et al*. 2009; Mendoza-Olazaran *et al*., 2015). A biofilm can be defined as a sessile community, which are sets of microorganisms in which cells affix to each other on a surface that is a polymeric mixture generally composed of proteins, extracellular DNA and polysaccharides which facilitates the adherence of these microorganisms to the surfaces and protect them from host immune system and antimicrobial therapy (Jabra-Rizk *et al*., 2006; Novick and Geisinger, 2008). Nevertheless, little information is available regarding the ability of methicillin resistant *S. hominis* to form a biofilm . Because of that the main objective of the present study was isolation and identification of *S. hominis* from hospitalized patients in Erbil city- Iraq, also to characterize their susceptibility profile to methicillin resistant antibiotics and comparing with *mecA*, also to investigate the ability of methicillin resistant *S. hominis* to form biofilm.

2. MATERIALS AND METHODS

2.1 Collection, isolation and identification

During September 2015 to May 2016; 213clinical specimens were collected from different patients in Teaching, Rizgary, West Emergency hospitals, and Health Center Laboratory in Erbil city, Iraq. The included specimens were as follow: 41 specimens from blood, 42 from ear, 22 from nose infection, 32 from oral cavity, 46 from urinary tract infections, and 30 from wound infections . All specimens were taken by disposable cotton swabs or sterile containers. The specimens were plated on mannitol salt agar (MSA) media (Oxoid, England), and incubated overnight at 37^{o}C for 24 hours. All isolates were identified based on cultural, morphological, and different biochemical tests, Single, well isolated colonies with the typical appearance of *S. hominis* were sub cultured and confirmed by Vitek 2 compact system . On the other hand, the ability of isolated bacteria to produce some virulence enzymes were investigated depending on (Harley and Prescott. 2002; Cheesbrough, 2006; Atlas, 2010). The enzymes were catalase, oxidase, coagulase, gelatinase, lipase, DNase, protease, lecithinase, urease , beta lactamase and hemolysin .

2.2. 1. Phenotypic detection of methicillin resistant *S. hominis*

The disk diffusion method with antibiotic oxacillin 1µg and cefoxitin 30 µg (BBL, England) was performed in Mueller Hinton agar as described by (CLSI, 2015).

2.2.2. Detection of *mecA* gene by PCR

The extraction of genomic DNA from the isolated bacteria was performed by using Presto^{TM} Minig DNA bacterial kit following the manufacturer's recommendations. Lysostaphin was added for effective extraction of DNA from the *S. hominis*. The extracted DNA was stored at -20°C until PCR was performed. PCR was performed using the primer *mecA* primer were: 5'AGCTGATTCAGGTTACGGACAAGGT 3'and5'GCAACCATCGTTACGGATTGCTT CA 3'with expected size 499bp. The PCR reactions were prepared in 20µl volume, consisting primer 1.3µl of each forward and reverse, and 2.5µ of DNA template were added to AccuPower PCR tub. The amplifications were conducted using thermal Cycler (Eppendorf, Germany) programmed with the initial denaturation at 94°C for 5 min with 30 cycles, denaturation at 94°C for 60s, annealing at 63°C for 60s and extension at 72°C for 60s, and final extension at 72°C for 5 min. The PCR products were separated by gel electrophoresis

in 1.5% agarose gel (Igeltjorn, 2009 ;Mishra *et al*., 2010).

2.3.Detection of the bacterial ability to produce biofilm

All isolated bacteria were tested for biofilm formation by using the Microtitre plate method as described by (Mathur *et al*., 2006) .The isolated bacteria were inoculated in brain heart infusion broth and incubated for 24 hours at 37 °C. After incubation each culture was diluted 1:100 with sterile fresh medium. Then, 200 µL of the samples were added to each well of a 96-well microplate , in addition broth was used as blank. The microplate were incubated at 37 °C for 24 hours. Content of each well was gently removed. The wells were washed three times with phosphate buffer saline (pH 7.2) to take off unbound bacterial cells subsequently the plates were exposed to air-dry and 200 µL of 0.1% w/v crystal violet solution was added to each well and incubated at room temperature for 30 minutes . The plates were washed off with distilled water and kept for air-dry. The bound bacteria were quantified by addition of 200 µL of absolute ethanol to each well and the absorbance of dissolved dye was measured at a wavelength of 570 nm by using 96-flat wells of ELISA. The isolates were classified according to biofilm production (Christensen *et al.,* 1985; Mathur *et al*., 2006).

3. RESULTS AND DISCUSSION

3.1. Bacterial isolation and identification

Two hundred and thirteen specimens were collected from patients admitted to hospitals located in Erbil city, Iraq. Only 18(8.45%) isolates of *S. hominis* were isolated from clinical specimens including 7 isolates (17.07%) from blood, 4 isolates (8.70%) from urine, 3 isolates (7.14%) from ear, 2 isolates (6.67%) from wound, and one isolate from each nasal swab (4.55%) and oral cavity (3.13%) as illustrated in table (1), and the above results similar to results obtained by (Chaves *et al.,* 2005). All *S. hominis* identified based on morphology, cultural, and biochemical properties (Kloos and Schleifer, 1986; Bannerman and Peacock, 2007). The isolates were yellow Gram positive cocci colonies that appeared round, smooth, raised, glistening, non- motile, and grow in 5-10% NaCl. Additionally, all isolates gave positive results for (catalase, lecithinase, and protease). In addition to that, they gave negative result for (novobiocin (5µg/disc), oxidase, coagulase, Dnase, lipase, ß-lactamase, urease, gelatinase, rhamnose, and dextrose). Moreover, they had the ability to ferment (fructose, lactose, maltose, sucrose, and produces acid from trehalose). Whereas, they

differ in their ability to produce hemolysin which 6 isolates (33.33%) were β- hemolysin and 12 isolates (66.67%) were γ- hemolysin producers (Figure 1)and these results were similar to results reported by (Abdulla and Barzani, 2016; Barzani *et al.*, 2017). All obtained *S. hominis* isolates were further confirmed by Vitek 2 system.

Table 1. The number and percentage of *Staphylococcus hominis* isolates isolated from different clinical sources.

Specimens sources	Specimens number	Number of *S. hominis*	Percentage (%) of *S. hominis*
Blood	41	7	17.07
Ear	42	3	7.14
Nasal swab	22	1	4.55
Oral cavity	32	1	3.13
Urine	46	4	8.70
Wound	30	2	6.67
Total	213	18	8.45

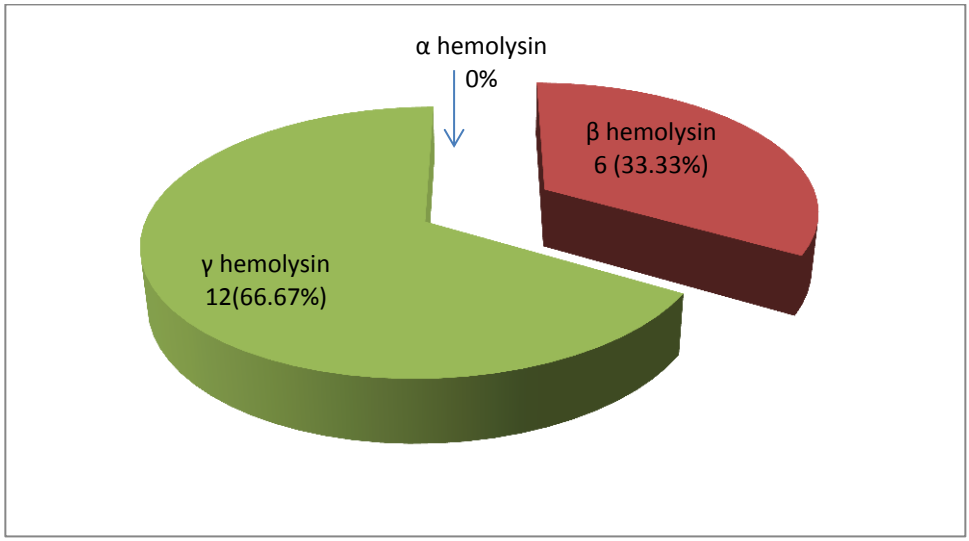

Figure 1. Hemolytic activity of isolated *S. hominis*

Detection of methicillin resistant *S. hominis*

To investigate the distribution of methicillin resistance *S. hominis* in the our community and among the patients in the Erbil city hospitals, the methicillin sensitivity test was applied to all 18 isolates, the test was performed by using disk diffusion method with antibiotic oxacillin and cefoxitin, additionally the *mecA* gene by using PCR was used to confirm the detection of methicillin resistant *S. hominis*. The results of oxacillin disk diffusion revealed that from all 18 isolates, 13 isolates (72.22%) showed resistant to the methicillin while 5 isolates (27.77%) were sensitive to the methicillin. On the other hand, the results of cefoxitin disk diffusion demonstrated that 16 isolates (88.89%) were resistant to methicillin and only 2 isolates (11.11%) were sensitive to methicillin (Table 2). However, the same results of the Cefoxitin disk diffusion method was obtained by PCR and by using *mecA* gene which 16 isolates (88.89%) were carried *mec*A gene with product size 499bp (Figure 3), and these results similar to results recorded by (Palazzo *et al*., 2008; Mendoza-Olazarán *et al*., 2015). Additionally, all above methicillin resistant isolates of *S. hominis* which detected phenotypically were harbor *mecA* gene, it means the Cefoxitin disk diffusion method was more accurate method for detection of methicillin resistant isolates in addition to PCR methods and *mecA* gene. Although, the rate of methicillin resistance *S. hominis* isolates were differ according to clinical specimens sources and also similar results obtained in both Cefoxitin disk diffusion method and by PCR (*mecA* gene). Generally methicillin resistant Staphylococci have become a serious problem in many country of the world. In spite of, the incidence of methicillin resistant strains varies from country to another, and from hospital to another, and may be also due to specimens size and source of isolation, it has been steadily increasing resistant to methicillin and oxacillin worldwide in the last decade (Calderon-Jaimes *et al*., 2002; Fung-Tomc *et al*., 2002). The most satisfactory explanation to this phenomenon is that even before methicillin resistance was reported for *S. aureus*, it was recognized in coagulase-negative Staphylococci (Chambers, 1988) and many data support the hypothesis that *mecA* originated in a coagulase-negative Staphylococcus species and resistance to methicillin is due to the acquisition of *mecA*, that encodes PBP2a -a transpeptidase with a low affinity for beta-lactam antibiotics

(Enright *et al*., 2002; Brased and Weigelt, 2007).

Table 2. Detection of methicillin resistant *S. hominis* isolates by different methods.

Specimens sources	Number of *S. hominis*	Oxacillin disk diffusion (No. & %)	Cefoxitin disk diffusion (No. & %)	PCR (*mec*A gene) (No. & %)
Blood	7	5 (71.43%)	7 (100%)	7 (100%)
Ear	3	2 (66.67%)	2 (66.67%)	2 (66.67%)
Nasal swab	1	1 (100%)	1 (100%)	1 (100%)
Oral cavity	1	1 (100%)	1 (100%)	1 (100%)
Urine	4	3 (75%)	3 (75%)	3 (75%)
Wound	2	1 (50%)	2 (100%)	2 (100%)
Total	18	13 (72.22%)	16 (88.89%)	16 (88.89%)

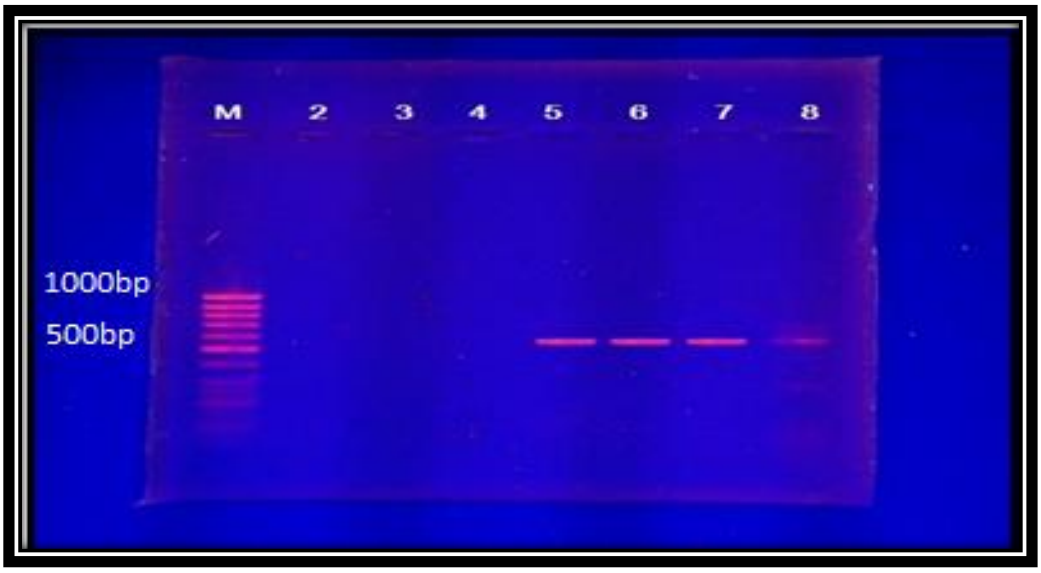

Figure 3. Polymerase chain reaction products on gel electrophoresis (1.5%) for *mecA* gene. M: DNA ladder (1000bp). Lanes 5,6,7, 8 : Amplified PCR product of *mecA* gene (499 bp) for *S. hominis* isolates. Lanes 3, 4: *S. hominis* isolates negative for *mecA* gene .Lane 2: negative control.

Biofilm formation in *S. hominis* isolates

The results of microtitre plate method revealed that 16 (88.89%) isolates of *S. hominis* were biofilm producer and only 2 isolates (11.11%) were non-biofilm producers, and these results similar to those obtained by (Garza-González *et al.,* 2011; Soroush *et al*., 2017). Of the 16 biofilm producer isolates, 14 isolates (77.77%) were categorized as strong biofilm producers and 2 (11.11%) isolates were identified as moderate biofilm producers (Figure 4) as defined by (Christensen *et al.,* 1985; Mathur *et al*., 2006) , and these results accordance with those reported by (Mendoza-Olazarán *et al*., 2015, Abdulla and Barzani, 2017) . On the other hand, the results showed that all 16 biofilm producer isolates were methicillin resistant and carried *mecA* gene, whereas the two non-biofilm producer were methicillin sensitive and not contained *mecA* gene. A biofilm is a community of bacteria living together in an organised structure as cellular clusters or microcolonies and it is enclosed in amatrix composed of an extracellular polymeric substance . However, the biofilm allows bacteria to adhere to inert materials and to experience increased antibiotic resistance (Davies, 2003; Hoiby *et al*., 2010). Moreover, the biofilm producer CoNS species are more resistant to antibiotics than when they exist as free-swimming planktonic cells.The formation of a stable biofilm on medical devices or on host clinical specimens is thought to be the major pathogenicity factor of *S. hominis* (Götz 2006; Rodhe *et al*. 2006). Additionally, many infections caused by staphylococci species found to be associated with biofilms. In addition to that, the knowledge about the ability of these bacterial isolates to form biofilm is relatively limited especially in Iraq.

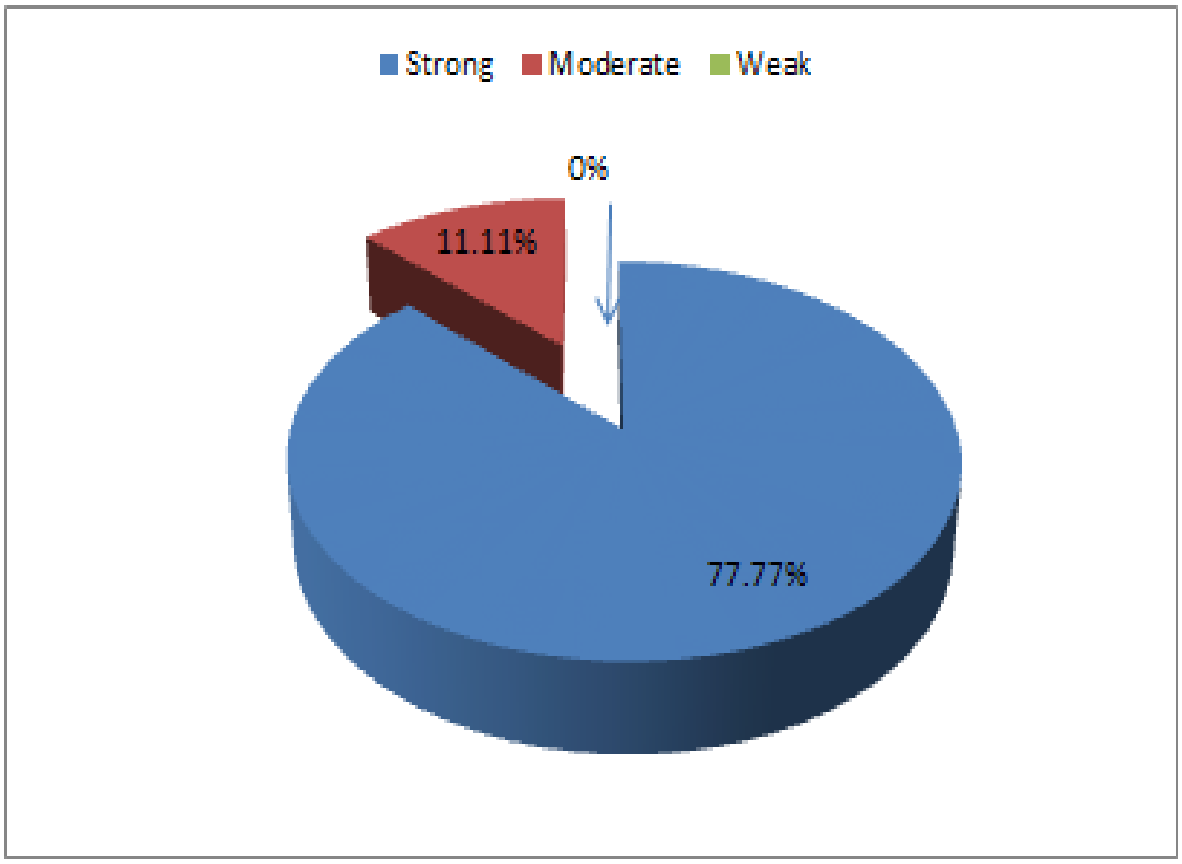

Figure 4. Percentage of strong, moderate and weak biofilm production of isolated *Staphylococcus hominis* by using microtiter plate method.

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