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Molecular and Phenotypic Characteristics of *Staphylococcus Hominis* Isolates From Pediatric Infections

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Abstract

Background: *Staphylococcus hominis* has been recently emerged as an opportunistic hospital-associated pathogen. The aim of this study was to characterize the antimicrobial susceptibility pattern, biofilm formation, and staphylococcal cassette chromosome *mec* (SCC*mec*) types among clinical isolates of *S. hominis* obtained from pediatric infections. Methods: Totally, twenty- two clinically significant *S. hominis* isolates, mostly from blood samples, were examined for antibiotic resistance, SCC*mec* typing, and biofilm formation. Results: Multiresistance was observed in 86 % of isolates. Among *mec*A positive isolates (77%), SCC*mec* type I (28%), III (22%), IV (22%), and non-typeable ones (28%) were detected. More than half of the isolates (54.5%) did not produce biofilm. No significant association was found between biofilm formation and the presence of *ica* operon. Conclusion: As *S. hominis* isolates were not strong biofilm producers, biofilm formation cannot be considered as much as important multidrug resistance.in the pathogenesis of isolates.

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Introduction

Coagulase-negative staphylococci (CoNS) have got much attention as nosocomial opportunistic infectious agents especially in neonates and immunocompromised patients (1,2). While *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* account for the most frequent etiological factors of CoNS infections, recently *Staphylococcus hominis* has been found to be implicated in serious infections such as sepsis and endocarditis (3). Similar to other CoNS, the high prevalence of antibiotic resistance, especially methicillin resistance in *S. hominis* has limited therapeutic options. Notably, the development of resistance to vancomycin and even newer antibiotics such as linezolid and quinupristin- dalfopristin has been reported (4-6).

Resistance to methicillin is encoded by the *mec*A gene, which is located on a mobile genetic element known as staphylococcal cassette chromosome *mec* (SCC*mec*). These elements are more diverse in CoNS in comparison with *Staphylococcus aureus* (3,7). Additionally, there are some pieces of evidence for the horizontal transfer of SCC*mec*

elements between CoNS and *S. aureus* (3), implying that *S. hominis* might be the reservoir of resistance genes for the other staphylococci (7,8).

The other important factor involved in CoNS infections is biofilm formation. One of the important elements in this complex process is *ica* operon, a gene cluster encoding polysaccharide intracellular adhesion (PIA) (9). Although *S. hominis* is not regarded as a typical biofilm producer, some studies have linked the potential of this species for biofilm formation to its pathogenesis. However, there are some controversies over this subject (8,10,11).

Since in most investigations, the causative agent of CoNS infections is not identified at the species level, there is limited information about the characteristics of clinical *S. hominis* isolates worldwide. To the best of our knowledge, this is the first report of characteristics of clinically significant *S. hominis* isolates from our country. In this study, we seek to characterize biofilm formation, antibiotic resistance, and detection of SCC*mec* types of *S. hominis* isolated from pediatric infections.

Materials and Methods

Clinical isolates

In this analytical cross-sectional study, of 177 clinical CoNS isolates collected from February 2012 to December 2013, a total of 43 non-repetitive *S. hominis* isolates were identified. This collection contained isolates from different pediatric wards of three hospitals in Iran; two pediatric training hospitals and a tertiary care hospital. Simultaneously, clinical signs and some laboratory data related to each patient were recorded. Clinical relevance of the isolates was determined based on the criteria proposed by the Center for Disease Control and Prevention (CDC) for CoNS infections (12). Briefly, the isolates obtained from patients who met the following criteria were enrolled in the study: (1) risk factors for infections such as invasive procedures (vascular catheter, urologic manipulation, etc.), (2) clinical classical manifestations (fever, local or generalized inflammation, and fluctuant wounds), (3) related laboratory findings (elevated total leukocyte count with granulocyte predominance and the presence of leukocytes in cerebrospinal fluid or urine).

In addition to the above-mentioned criteria, for bloodstream infections in neonates, monobacterial positive blood cultures combined with C-reactive protein >10 mg/L within two days of blood culture were included. For the other patients, the isolates from at least two positive blood cultures on separate occasions were considered as clinically significant (12,13). CoNS isolates from internal fluids or foreign bodies in pure primary cultures were also included in this study. All CoNS recovered from non-pure primary cultures were excluded. According to this classification, 22 isolates were considered as clinically relevant and included in this study. More than half of these isolates (n= 14.64%) were from patients less than one year old. They were mainly originated from the blood (n= 19, 86%) followed by the other sources including wounds (n= 2) and the tracheal tube (n= 1).

Identification and Susceptibility Testing

The initial identification was performed based on the conventional microbiological methods: Gram staining, catalase, and coagulase tests, DNase activity, and carbohydrates fermentation. Then, they were confirmed at the species level using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics) as described previously (14).

The disk diffusion method was used to determine the susceptibility to antibiotics including gentamicin, erythromycin, clindamycin, fusidic acid, ciprofloxacin, trimethoprim-sulfamethoxazole, and linezolid (Mast, UK).

Additionally, the minimum inhibitory concentration for vancomycin was detected using E-test (Liofilchem, Italy).The results were analyzed according to the Clinical and Laboratory Standard Institute (CLSI) guidelines (15). Herein, the intermediate level of susceptibility was considered nonsusceptible. Multiresistance was defined as showing resistance to at least three different classes of antibiotics.

Biofilm formation

Microtiter plate assay

Semi quantitative biofilm production was carried out as described previously (8,16). Briefly, overnight cultures of bacteria in trypticase soy broth (TSB) were diluted 1:100 in the TSB medium containing 0.25% glucose and were then inoculated (200µl) into 96 well flat-bottom polystyrene microtiter plates. Following 24 h incubation at 37 °C, the wells were washed three times with normal saline and stained with 0.1% safranin (Merck, Germany). After the addition of 95% ethanol solution to each well, the absorbance of biofilm-bound-safranin was measured at 490 nm using a microtiter plate reader (Biotek ELX 800). The tests were performed in triplicate. An optical density (OD) ≥ 0.25 was considered as strong biofilm formation and $0.15 \leq \text{OD} \leq 0.24$ was classified as weak biofilm production. *S. epidermidis* RP62A and *S. epidermidis* ATCC 12228 were used as positive and negative controls, respectively.

Detection of *ica* operon

Genomic DNA was extracted by the Cetyltrimethylammonium Bromide (CTAB) method (17) and all isolates were examined for the presence of *ica* operon as described previously (16) (Table1).

Table 1. Primers and PCR condition for amplification of genes

Target	Primer sequences (5′→3′)	Annealing temperature (°C)	Product size (bp)
***mec* A**	AAAATCGATGGTAAAGGTTGGC AGTTCTGCAGTACCGGATTTGC	56	532
***ica* ADB**	TTATCAATGCCGCAGTTGTC GTTTAACGCGAGTGCGCTAT	55	546
SCC*mec* II, IV	ATTGCCTTGATAATAGCCYTCT TAAAGGCATCAATGCACAAACACT	55	937
SCC*mec* III, V	CGTCTATTACAAGATGTTAAGGATAAT CCTTTATAGACTGGATTATTCAAAATAT	55	518
SCC*mec* I, IV	GCCACTCATAACATATGGAA CATCCGAGTGAAACCCAAA	55	415
SCC*mec* V	TATACCAAACCCGACAACTAC CGGCTACAGTGATAACATC	55	359

Detection of methicillin resistance and *SCCmec* typing

Resistance to methicillin was detected using the cefoxitin disk test (11).The presence of *mec*A gene was demonstrated by PCR. All *mec*A- positive isolates were investigated for SCC*mec* typing (types I-V) using multiplex PCR assay (18) (Table 1). The isolates showing patterns different from the defined types were classified as non-typeable.

Statistical methods

The statistical analysis of the data was performed using the chi-square test by IBM SPSS Statistics version 21 at the significance level of $P < 0.05$. The χ^2 test was used to examine the association of biofilm formation with the presence of *ica* operon and/or multiresistance phenotype.

Results

Methicillin resistance and antimicrobial susceptibility profile

Most of the isolates (n= 17, 77%) showed methicillin resistance. No resistance to linezolid and vancomycin was found. The rates of non-susceptibility to the other antibiotics tested were as follows: trimethoprim-sulfamethoxazole (100%), erythromycin (95.5%), clindamycin (82%), ciprofloxacin (54.5%), fusidic acid (54.5%), and gentamicin (36%). Notably, multiresistance was seen in 19 (86%) isolates (Table 2).

Table 2. Molecular and Phenotypic Characterization of S. hominis isolates

Isolate	Biofilm[a]	*ica* operon	SCC*mec* type	Resistance pattern[b]
3T	Strong	Positive	IV	E, CD, FC, Cip, TS
10T	Strong	Negative	III	E, CD, FC, Cip, TS
16T	None	Negative	N	TS
22T	Strong	Negative	IV	Gm, E, CD, FC, Cip, TS
26T	Weak	Negative	III	E, CD, TS
85T	None	Negative	III	Gm, E, CD, FC, Cip, TS
87T	None	Negative	III	Gm, E, CD, FC, Cip, TS
98T	None	Negative	NT	E, CD, FC, Cip, TS
102T	Strong	Negative	I	E,FC,TS
106T	Strong	Positive	IV	Gm, E, CD, Cip, TS
108T	None	Negative	None	E, CD,TS
110T	Strong	Negative	I	Gm, E, CD,TS
11MT	None	Negative	NT	Gm, E, CD, FC, Cip, TS
25MT	Strong	Negative	I	Gm, E, CD, Cip, TS
44MT	None	Positive	IV	E, CD,TS
47MT	Weak	Negative	I	E,TS
49MT	None	Negative	None	E, CD, FC, TS
59MT	None	Positive	None	E, CD, FC, Cip, TS
26M	Strong	Positive	NT	E, CD, Cip, TS
73M	None	Negative	III	Gm, E, CD, FC, Cip, TS
82M	None	Negative	NT	E, CD, FC,TS
84M	None	Negative	NT	E, TS

[a]Biofilm formation was detected by MTP assay.
[b]Gm: Gentamicin; E: Erythomycin; CD: Clindamycin; FC: Fusidic acid; Cip: Ciprofloxacin; TS: Trimethoprim/Sulfamethoxazole

Biofilm formation

MTP assay showed that more than half of the isolates (n= 12, 54.5%) did not produce any biofilm. The remaining ones (n= 8, 36%) and (n= 2, 9%) were classified as strong and weak biofilm producers, respectively. On the other side, in most of the isolates (n= 17, 77%), no *ica* operon was found (Table 2).

Among *ica* positive isolates (n= 5), two isolates did not produce any biofilm while three isolates were strong producers. By comparison, the majority of *ica* negative isolates (n= 10 out of 17) did not produce any biofilm. However, no significant correlation was found between biofilm formation and the presence of *ica* operon (p = 0.62). Similarly, the correlation between multiresistance phenotypes and biofilm formation was not statistically significant (p= 0.57).

SCCmec typing

Among 18 *mec*A-positive isolates, SCC*mec* type III was found in five isolates (28%) and each SCC*mec* types I and IV contained four isolates. The remaining ones were non-typeable (Table 2). According to our results, diversity was detected in SCC*mec* types dissemination.

Discussion

Among CoNS, *S. hominis* is known as the third most common species isolated from human infections especially from neonates and immunocompromised patients. Since this species (like the other CoNS) is a normal colonizer of skin and mucous membranes of humans, it is frequently recognized as a culture contaminant (19). Nevertheless, under specific circumstances (defined by the CDC) (12,13), it can be indicative of true infection. For this reason, its role as a true pathogen has been underestimated. Until now, no definite virulence factor has been identified in this species. Therefore, it is not clear why the number of *S. hominis* infections is increasing.

In this study, 22 clinically significant *S. hominis* isolates were examined. According to the data of this study, a high rate of methicillin resistance (77%) was found which has been reported similarly in previous studies (8,19,20). Most of the isolates showed multiresistance phenotypes. Moreover, the resistance profile of our isolates was the same as that found by Mendoza-Olazarán et al (8), but they reported a higher prevalence of resistance to trimethoprim-sulfamethoxazole in their study.

Based on the used SCC*mec* typing method, five *mec*A positive isolates (23%) were non-typeable, and no significant differences were found between the numbers of typeable and non-typeable ones. This finding is in contrast to most of the other studies which have reported the presence of new SCC*mec* types in their isolates (3,8,20). Since we used the typing method developed for *S. aureus* which might be not suitable for CoNS, our results should be interpreted cautiously.

Concerning biofilm formation, more than half of the isolates did not produce any biofilm and only eight isolates were strong producers. Five isolates carried *ica* operon, three of which were biofilm producers. This shows that biofilm formation might be independent of *ica* operon or might be affected by environmental conditions (8). Furthermore, the ability of seven *ica* negative isolates for biofilm formation can be justified by the fact that the composition of biofilm matrixes in *S. hominis* isolates is different. While PIA is encoded by *ica* operon, some studies have shown that biofilm matrixes in *S. hominis* are composed of other components such as proteins (3,4).

Conclusion: The most important factor in the increasing emergence of *S. hominis* infections might be the ability to acquire multiresistance against most of the antimicrobial agents. Preventive measures to control the dissemination of these isolates should be considered in hospital settings. Additionally, our results showed that biofilm formation may not be involved in the pathogenesis of this enigmatic pathogen. However, more detailed studies are needed to prove this subject.

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Conflict of interest

The authors declare that they have no conflict of interest.

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