

Original Research Article

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## Speciation and Detection of Virulence Factors of Coagulase Negative *Staphylococci* Isolated from Various Clinical Samples

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### ABSTRACT

Coagulase negative *Staphylococci* (CoNS), previously dismissed as contaminants are now emerging as important potential pathogens with the increase in number of severely debilitated patients and increased use of implants in hospitals. The objectives of this study are to isolate, speciate and to detect various virulence factors of CoNS from various clinical samples. A total of 500 various clinical samples from blood, urine, pus, central line tip and UVC tip were studied for a period of one year. Out of which, 62 isolates of clinically significant, non repetitive coagulase negative *Staphylococci* were identified by standard biochemical test. Following identification and speciation, the organism was studied for various virulence factors like Slime production, DNase and Haemagglutination. A total of 62 CoNS, isolated from 500 different clinical samples. Among various species of CoNS, *S. epidermidis* was most frequently isolated, corresponding to 20 (32.3%) isolates. Most of the isolates were positive for slime production (72.6%). Production of DNase and Haemagglutination activity were shown in 43.5% and 33.9% respectively. Because of the increasing clinical significance of CoNS, accurate species identification of CoNS is highly desirable to permit a more precise determination of host-pathogen relationship of CoNS and to report them as human pathogen.

#### Keywords

CoNS,  
Slime  
production,  
DNase,  
Haem-  
agglutination.

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### Introduction

Coagulase negative *Staphylococci* (CoNS), more commonly accounted as contaminants are now considered as a important potential pathogens with increase in use of implants and with the increase in frequency and number of severely debilitated patients. More than 30 species of CoNS are recognised but only a few are commonly incriminated in human infections.

Although CoNS were long considered non-pathogenic and having few virulence factors, new factors have lately been determined in the period of gradual clarification of pathogenesis. The majority of known factors are incorporated into the biofilm formation (Longuaerova, 2006). Production of biofilm by coagulase negative *Staphylococci* especially in higher incidence by *S.*

*epidermidis* is noted in indwelling medical devices and implants.

Although coagulase negative species other than *S.epidermidis* and *S.saprophyticus* are frequently found as contaminants in clinical specimens, several other species have now been reported as cause of human infection, principally in wounds, urinary tract infections, bacteremia, osteomyelites, catheter related sepsis, ventriculo-peritoneal shunt infection and both native-valve and prosthetic-valve endocardities. The more commonly implicated other species include *S.hemolyticus*, *S.lugdunensis*, *S.schleiferi*, *S.warneri*, *S.hominis*, *S.simulans* and *S.sacchrolyticus* etc.

Evidence indicates that pathogenicity might be related to the slime production and several other extra cellular enzymes to combat the host defense mechanism. The objectives of this study are to isolate, speciation and to detect various virulence factors of CONS isolated from clinical samples.

### **Materials and Methods**

A total of 500 clinical samples such as blood, urine, pus, central line tip and UVC tip were collected for a period of one year. Out of which, 62 isolates of clinically significant, non repetitive coagulase negative *Staphylococci* were isolated and identified by standard biochemical test.

### **Characterization of Coagulase Negative *Staphylococci***

The following test such as phosphatase production, acetoin production, ornithine decarboxylation, sugars (mannitol, trehalose, xylose, maltose, mannose, sucrose, lactose) fermentation test, novobiocin and polymyxin B (50 unit)

sensitivity test were done to speciate coagulase negative *Staphylococci*. (Koneman *et al.*, 1997) The organism were further subjected for detection of various virulence factors like Slime production, DNase and Haemagglutination.

### **Detection of Virulence Factor**

#### **Slime Production**

Slime producing ability of the CoNS were determined by using the congo red agar (CRA) method (Freeman *et al.*, 1989). Congo red agar is prepared by making aqueous solution of congo red 0.8g/l and autoclaved separately (121°C for 15 minutes) and cooled. It was added to the cooled agar base containing brain heart infusion broth 37g/l, sucrose 50g/l, and agar 10g/l.

Several colonies of single isolates were inoculated on medium and incubated aerobically at 37°C for 24hrs. Isolates which produced black colonies with dry crystalline consistency were regarded as slime positive. Non slime producer usually remains pink.

#### **DNase Test**

This test was performed by spot inoculating the isolates on the DNase agar and incubated at 37°C for 4 days. After incubation 1N HCl was poured on the agar. Clear halo around the bacterial growth was considered as positive (Deighton *et al.*, 1988).

#### **Haemagglutination Test**

The test was performed in U shaped micro titre well. Individual CONS isolates were added in to the phosphate buffer saline (PBS) and adjust the turbidity to McFarland standard 1. Two serial dilutions of the

bacterial suspension were made in the microtiter plates to give a total volume of 50 µl per well. Then, 50 µl of the 1% human erythrocyte suspension in PBS was added to each well. After shaking, the plates were incubated at room temperature for 2 hours and the hemagglutination was recorded as positive or negative. (Rupp and Archer, 1992).

**Results and Discussion**

A total of 62 CoNS, were isolated from 500 different clinical samples. CoNS were most commonly isolated from Pus (40.3%), followed by urine (29%), blood(24.2%) and least isolated from indwelling devices like central line tip(4.8%) and UVC tip (1.6%) (Table 1)

Among the CoNs species, *S. epidermidis* was most frequently isolated, corresponding to 20 (32.3%) isolates. The remaining species isolated were *S. haemolyticus* (15 isolates, 24.2%), *S.saprophyticus* (9 isolates, 14.5%), *S. lugdunensis* (7 isolates, 11.3%), *S. hominis* (7 isolates, 11.3%), *S. schlieferi* (2 isolates, 3.2%), *S. warneri* (1 isolates, 1.6%), and *S. warneri* (1 isolate, 1.6%).(Table 2)

Detection of virulence factors of CoNS species are shown in Table 3. Most of the isolates were positive for slime production

(72.6%). Production of DNase and Haemagglutination activity were shown in 43.5% and 33.9% respectively.

Coagulase-Negative *Staphylococci* are normal flora component of various part of skin and mucosa of respiratory and gastrointestinal tract were considered as contaminants bacteria formerly, but are now considered as important possible pathogens. As opposed to *Staphylococcus aureus*, though there are not equipped with various virulence factors they tend to establish infection when there is a breach in external barrier due trauma, wounds, inoculation and implantation of foreign bodies.

In this study, overall prevalence of clinically significant CoNS isolated from various clinical samples were 62/500 (12%) similar isolation rate of 14.4% and 17.5% were reported in various other studies (Sarathbabu *et al.*, 2013; Javadpour *et al.*, 2010).

In the current study, CoNS were predominantly isolated from pus sample (40%) followed by urine (29%). In another similar study, isolation rate of CoNS were 32 (33.3 %) from pus and 26(27.1%) from the urine samples (Asangi *et al.*, 2011). Few others also have reported 30% (Zilevica *et al.*, 2005) and 35.5% (Villari *et al.*, 2000) isolation rate from pus.

**Table.1** Number of Cons Isolated from Clinical Samples

Total no of Samples	Total no of CoNS Isolated (%)	Clinical Samples				
		Pus (%)	Urine (%)	Blood (%)	Central line tip (%)	UVC tip (%)
500	62(12.4%)	25(40.3%)	18(29%)	15(24.2%)	3(4.8%)	1(1.6%)

**Table.2** Species Distribution of CoNS Species from Various Clinical Samples(n=62)

Isolates	%	Blood	Urine	Wound	Central line tip	UVC tip
<i>S.epidermidis</i> (20)	32.3	4	3	10	2	1
<i>S.haemolyticus</i> (15)	24.2	4	6	4	1	—
<i>S.saprophyticus</i> (9)	14.5	1	4	4	—	—
<i>S.hominis</i> (7)	11.3	1	3	3	—	—
<i>S.lugdunensis</i> (7)	11.3	4	1	2	—	—
<i>S.schlieferi</i> (2)	3.2	1	—	1	—	—
<i>S.cohini</i> (1)	1.6	—	—	1	—	—
<i>S.warneri</i> (1)	1.6	—	1	—	—	—

**Table.3** Detection of Slime Production, DNase and Haemagglutination from CoNS

Isolates	No of slime production positive	No of DNase positive	No of haemagglutination Positive
<i>S.epidermidis</i> (20)	17	5	11
<i>S.haemolyticus</i> (15)	12	9	5
<i>S.saprophyticus</i> (9)	6	—	3
<i>S.hominis</i> (7)	3	5	—
<i>S.lugdunensis</i> (7)	4	2	2
<i>S.schlieferi</i> (2)	2	1	—
<i>S.cohini</i> (1)	—	—	—
<i>S.warneri</i> (1)	1	—	—
<b>Total(62)</b>	<b>45(72.6%)</b>	<b>27(43.5%)</b>	<b>21(33.9%)</b>

*S. epidermidis* was the CoNS species most frequently isolated, corresponding to 20 (32.3%) isolates. The remaining species were distributed among *S. haemolyticus* (15 isolates, 24.2%), *S.saprophyticus*(9 isolates, 14.5%),*S. lugdunensis* (7 isolates, 11.3%), *S. hominis* (7 isolates, 11.3%), *S. schlieferi* (2 isolates, 3.2%), *S. warneri* (1 isolates, 1.6%), and *S. warneri* (1 isolate, 1.6%).

*Staph. epidermidis* was the most common species identified, accounting for 32.3 % of isolates. *Staph. haemolyticus* was the second most common species, followed by *Staph. Saprophyticus*, *Staph. hominis* and, *Staph. lugdunensis* which was well in concordance

with various other studies (Goyal *et al.*, 2006; Sharma *et al.*, 2010; Surekha *et al.*, 2011).

The most important pathogenic mechanism of CoNS in establishing disease in human in foreign bodies is due to their biofilm forming capacity. Biofilm is an extracellular polysaccharide produced by these microorganisms which permits them to adhere to smooth plastic surfaces, colonizing catheters and prosthetic devices pacemakers. (Vogel *et al.*, 2000).

Detection of biofilm production showed 72.6% of the isolates were slime producers

well comparable to similar study.(Azuka Azih *et al.*, 2013) where they reported 75.95% of all CoNS species were positive for slime production.

Though DNase is not entirely reliable indicator of pathogenicity of CoNS, it was reported that CoNS also have DNase activity (Quinn *et al.*, 1994). In this study, the rate of DNase positiveness was 43.5%. This results was parallel with the various other studies on DNase activity (Rkyilmaz *et al.*, 2006; Daghistani *et al.*, 2000).

In our study, hemagglutination rates was 33.9%. Study by Rkyilma *et al.* 2006 and Rupp *et al.* 1992 reported 41.1% and 33% positivity for hemagglutination wellin concordance with our study.

In conclusion, in our study, *Staph. epidermidis* was the most common species identified among CoNS and >70% were biofim producers. Increasing clinical significance of CONS as an important cause of opportunistic and nosocomial pathogen, necessitates the need to isolate and characterize Coagulase negative Staphylococci so as to report them as pathogen in causing human disease.

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