

## Simplified scheme for species identification and antibiotic susceptibilities of coagulase negative staphylococci isolated from clinical specimens

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### Abstract

Coagulase negative staphylococci (CONS) species identification is difficult for most clinical laboratories. The schemes proposed by Kloos and Schleifer is the reference method used for the identification of staphylococcal species and subspecies. It requires the utilization of a large number of biochemical tests. Antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method. Out of the total 51 CONS isolates subjected for species identification the most common species identified was *S. epidermidis* (58.82%). *S. cohnii* and *S. haemolyticus* being the second and third most frequently encountered species. Common source of most species was pus followed by blood and urine. *S. epidermidis* and *S. cohnii* showed resistant to multiple antibiotics. The most effective antibiotic for all the species of CONS were linezolid, teicoplanin and vancomycin. So CONS isolated from clinical samples should always be considered as potentially significant pathogen. The species identification with antibiogram will be of benefit for both epidemiological and patient care purpose.

**Keywords:** Coagulase negative staphylococci

### Introduction

Forty species of the genus *Staphylococcus* have been identified thus far.<sup>(1)</sup> *Staphylococcus aureus*, a coagulase positive species which produces a series of enzymes and toxins, is the best known and has been implicated frequently in the etiology of a series of infections and intoxications in animals and humans, whereas coagulase negative staphylococci (CONS) species naturally colonize humans and are considered as commensals or rarely pathogenic.<sup>(2)</sup> Over the last decade, CONS have been recognised as the etiological agents of a variety of pyogenic infections,<sup>(3)</sup> blood stream infections and UTIs<sup>(4)</sup> etc. It can colonize artificial prostheses in association with a bacteremia.<sup>(5)</sup> The emergence of CONS as potential pathogen in different infections may be due to increasing use of invasive procedures such as intravascular catheters and prostheses in patients undergoing intensive treatment.<sup>(6)</sup> In view of the known pathogenic potential of CONS, these microorganisms need to be identified properly up to the species level.

### Aims and Objectives

- To identify potentially significant CONS obtained from different clinical specimens received in the Department of Microbiology, SCB Medical College and Hospital. The study was done from May 2013 to July 2013. Identification of CONS was done up to species level.
- To do antibiotic susceptibility testing of CONS isolates.
- To evaluate the most common CONS species causing infection in humans.

### Materials and Method

All slide and tube coagulase negative staphylococci obtained from blood, sterile body fluids, tissues, high colony count urine samples, endotracheal tube, wounds and abscesses were submitted for species identification. Species identification was done using the criteria of Kloos and Schleifer.<sup>(2)</sup> This method includes: Production of hemolysin, utilization of sugars like arabinose, sucrose, mannose, maltose, fructose, trehalose and lactose, nitrate reduction, presence of urease and ornithine decarboxylation and resistance to novobiocin. Reading of the tests were obtained after 24, 48 and 72 hours of aerobic incubation.<sup>(2)</sup> Sugar fermentation test was done using commercially available disks (Procured from HIMEDIA) specific for each sugar on phenol red agar plate.<sup>(7)</sup> Susceptibilities of the isolates was done by Kirby-Bauer disc diffusion method on Mueller-Hinton Agar according to the CLSI Guideline along with screening for methicillin resistance using cefoxitin disc.<sup>(8)</sup>

### Results

The observation obtained from this study was given in the tables. Majority of CONS isolates were identified from pus samples (68.62%) followed by blood and urine (Table 1). *S. epidermidis* (58.82%) was the commonest CONS species identified followed by *S. cohnii* and *S. haemolyticus*. Majority of the *S. epidermidis* species were isolated from pus sample (3.3%) (Table 2)

Antibiotics susceptibility showed highest resistance to cefoxitin (72.54%) followed by amoxycylav (70.58%) and cefadroxyl (64.70%). Most effective antibiotics against CONS species were Linezolid, Teicoplanin & Vancomycin each showing 100% sensitivity. (Table 3)

**Table 1: CONS isolated from various clinical samples**

Specimen	CONS isolated
Pus	35(68.62%)
Blood	09(17.64%)
Urine	04(7.84%)
Body of fluids	02(3.92%)
Others	01(1.96%)
Total	51(100%)

### Discussion

From the total of 51 CONS isolates, eight different species were identified taking only seven biochemical

characters from Kloos and Scjhleifer scheme as the reference methods.<sup>(2)</sup> Most predominant species isolated was *S. epidermidis*(58.82%). *S.cohnii* and *S. haemolyticus* being the second and third most frequently encountered species respectively. Our findings are similar to those of Nicolle et al<sup>(9)</sup> who found 70% of *S. epidermidis*. *S. cohnii*, *S. haemolyticus* and *S. saprophyticus* were identified using two additional characters like nitrate reduction and urease tests. The disk method was also found to be highly efficient and practical since it does not require prior preparation of sugars thus preventing the loss of culture media.

**Table 2: Different CONS species identified in various clinical samples**

Cons	All total sources	PUS	Blood	Urine	Body fluids	Others
<i>S. epidermidis</i>	30(58.82%)	22	6	-	2	-
<i>S. cohnii</i>	10(19.6%)	8	1	1	-	-
<i>S. hemolyticus</i>	3(5.8%)	2	1	-	-	-
<i>S. warneri</i>	2(3.92%)	2	-	-	-	-
<i>S.capitus</i>	1(1.96%)	-	-	-	-	1
<i>S. simulans</i>	1(1.96%)	-	-	1	-	-
<i>S. xylosus</i>	1(1.96%)	-	-	1	-	-
<i>S. saprophyticus</i>	1(1.96%)	-	-	1	-	-
Unidentified	2(3.92%)	1	1	-	-	-
Total	51	35(68.62%)	9(17.64%)	4(7.84%)	2(3.92%)	1(1.96%)

Majority of CONS isolates were found from Pus (68.62%) followed by Blood (17.64%) and Urine (7.84%), whereas Gill et al showed that Blood (50%) is the major specimen from where CONS were isolated. Pus was found to be the most frequent source of *S.epidermidis* in our study.

Antibiotic susceptibility testing showed highest resistance to Cefoxitin (72.54%), Amoxyclav (70.58%) and Cefadroxyl (64.70%). Most effective antibiotics against CONS species were Linezolid (100%). Teicoplanin (100%) and Vancomycin (100%). *S. epidermidis* and *S. cohnii* showed highest resistant to multiple antibiotics (Table 3).

Our laboratory had been reporting the various species of staphylococcus as coagulase negative staphylococcus. But by using this method of Kloos and Schleifer proper species identification can be done. Antibiotic susceptibility testing should be done in each CONS isolate to guide proper therapy as resistant to multiple antibiotics is more common.

**Table 3: Antibiotic Susceptibility Testing**

Cons	Total	AMC		VA		LZ		TEI		CQ		Cn		CIP		AZM		GEN	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
<i>S.epidermidis</i>	30 (58.82%)	8	22	30	0	30	0	30	0	10	20	6	24	10	20	11	19	12	18
<i>S.cohnii</i>	10(19.5%)	5	5	10	0	10	0	10	0	5	5	3	7	3	7	4	6	5	5
<i>S.haemolyticus</i>	3(5.8%)	0	3	3	0	3	0	3	0	0	3	1	2	2	1	1	2	1	2
<i>S.warneri</i>	2(3.92%)	1	1	2	0	2	0	2	0	1	1	2	0	1	1	2	0	2	0
<i>S.capitus</i>	1(1.96%)	0	1	1	0	1	0	1	0	0	1	1	0	0	1	1	0	1	0
<i>S.simulans</i>	1(1.96%)	0	1	1	0	1	0	1	0	0	1	0	1	1	0	0	1	0	1
<i>S.xylosus</i>	1(1.96%)	0	1	1	0	1	0	1	0	0	1	0	1	0	1	0	1	0	1
<i>S.saprophyticus</i>	1(1.96%)	0	1	1	0	1	0	1	0	1	0	0	1	0	1	0	1	0	1
Unidentified	2(3.92%)	1	1	2	0	2	0	2	0	1	1	1	1	0	2	1	1	1	1

## **Conclusion**

Coagulase negative staphylococci isolated from different clinical samples should always be considered as potentially significant pathogens. Species identification of these organisms help us to learn more about the diversity, resistance pattern, epidemiology and virulence. Proper identification of CONS species with antibiogram will be of benefit for patient care and epidemiological purpose.

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