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Prevalence of Clinically Significant Coagulase Negative Staphylococci and their Speciation in Tertiary Care Centre

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Coagulase-negative *Staphylococci* (CoNS) are common colonizers of the human skin and usually contaminants when isolated from a clinical specimen. These organisms have become increasingly recognized as agents of clinically significant nosocomial blood stream infections. The study was undertaken to identify the prevalence of clinical isolates of CoNS and their speciation. A total of 84 isolates were collected from different samples and subjected to biochemical characterization using conventional microbiological methods. 96% isolates were conveniently identified. *Staphylococcus .epidermidis* (36, 42.8%), *S.saprophyticus* (25,29.7 %), *S.haemolyticus* (15,17.8%), *S.lugdunensis* (2,2.3 %), *S.warneri* (2, 2.3%), *S.cohnii* (1,1.1%), and others(3,3.5%).These 3 isolates were not identified to the species level. The increasing recognition of pathogen potential CoNS and emergence of drug resistance among them, demonstrates the need to adopt simple laboratory procedure to identify CoNS and determine the prevalence and their speciation. The species identification and differentiation help in monitoring the reservoir and distribution of CoNS involved in nosocomial infections and determining the etiological agents in the hospitals.

Key Words: Coagulase Negative Staphylococci, Ornithine decarboxylase, Speciation.

Coagulase Negative *Staphylococci* (CoNS) are normal commensals of the skin, anterior nares, and ear canals of humans. They have long been considered as non-pathogenic and were rarely reported to cause severe infections. However, as a result of the combination of increased use of intravascular devices and an increase in the number of hospitalized immunocompromised patients, CoNS have emerged as a major cause of nosocomial bloodstream infections [1] and they account for 9% of nosocomial infections [2]. Patients at risk include those with prosthetic devices, intravascular catheters, any foreign bodies in place, postoperative sternal wound infections and immunocompromised hosts [3,4,5]. These infections are difficult to treat because of the risk factors and the often multiple drug resistant nature of the organisms [5]. Hence, this study was undertaken to identify the most prevalent clinical isolates and to discriminate between the species of CoNS.

MATERIALS AND METHODS

This study was undertaken in the Department of Microbiology, VIMS Bellary from January 2011 to May 2011. A total of 84 clinically

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significant CoNS isolates were collected from different clinical samples, and processed using conventional microbiological methods. The strains were isolated from sputum, blood, pus, urine samples etc. The isolates were considered clinically significant when isolated in pure culture from infected sites.

The isolates collected were initially identified by colony morphology, Gram staining, catalase, slide and tube coagulase test and anaerobic acid formation from mannitol [6,7,8]. The tests (minimum number), which were simple, inexpensive and easy to perform, were selected from the scheme of Kloos and Shleifer to identify CoNS species groups [9,10]. Speciation of CoNS was done by novobiocin resistance test, urease activity, ornithine decarboxylase & aerobic acid production from mannose [9,10,11].

RESULTS

Among 84 isolates of CoNS, 26 (31 %) were isolated from sputum, 25(29.8%) from the urine samples, 24(28.6%) from pus samples, 4(4.8 %) from blood samples, 3(3.5%) from ear swab, 1(1.1 %) from fluid and 1(1.1%) from throat swab Table 1. All isolates of CoNS were negative for blood clumping factor and tube coagulase**.** Identification of CoNS by simple scheme Table 2. showed *S. epidermidis* as the most frequent isolate(36, 42.8%),followed by *S.saprophyticus*(25,29.7 %), *S.haemolyticus* (15,17.8%), *S.lugdunensis* (2,2.3 %), *S.warneri* (2, 2.3%), *S.cohnii* (1,1.1%), and unidentified(3,3.5%) because of aberrant reactions. Out of 36 *S.epidermidis* isolates, 12(33.3%) were isolated from sputum samples followed by 11 (30.5%) from urine samples. Out of 25

Table 1. Frequency of Clinically significant CoNS in different clinical samples.

Species	No.	Sputum	Urine	pus	Blood	Ear	Throat	Fluid
S. epidermidis	36 (42.8%)	12 (33.3%)	11 (30.5%)	9 (25%)	—	3 (8%)	—	1 (2%)
S.saprophyticus	25 29.7%)	10 (40%)	8 (32%)	7 (28%)	—	—	—	—
S.haemolyticus	15 (17.8%)	3 (20%)	6 (40%)	2 (13.3%)	3 (20%)	—	1 (6.6%)	—
S.lugdunensis	2 (2.3%)	—	—	2 (100%)	—	—	—	—
S.warneri	2 (2.3%)	—	—	2 (100%)	—	—	—	—
S.cohnii	1 (1.1%)	1 (100%)	—	—	—	—	—	—
Others	3 (3.5%)	—	—	2 (66.6%)	1 (33.3%)	—	—	—
Total	84 (100%)	26 (31%)	25 (29.8%)	24 28.6%)	4 (4.8%)	3 (3.5%)	1 (1.1%)	1 (1.1%)

Table 2. Identification of CoNS by simple scheme

Species	Clumping factor	Tube coagulase test	Ornitine decarboxylase test	Urease activity	Mannose fermentation	Novobiocin sensitivity (5µg)
S. epidermidis	-	-	+	+	+	S
S.saprophyticus	-	-	-	+	-	R
S.haemolyticus	-	-	-	-	-	S
S.lugdunensis	-	-	+	+	+	S
S.cohnii	-	-	-	+	+	R
S.warneri	-	-	-	+	-	S

S.saprophyticus 10 (40%) isolated from sputum samples followed by 8 (32%) in urine samples. Out of 15 *S.haemolyticus* 6(40%) from urine samples Table 1**.** Out of 84 CoNS, more cases 34 (28.5%) were found in the age group of >40 years. The isolations were more in males (59, 70.2%) than females (25, 29.7%). In males majority of the CoNS, 28 (47.4%) were found in the age group of >40 years & in females 7 (28%) found in age group of 21-30 years.

DISCUSSION

As CoNS form a part of the normal flora, effective management may not be aimed at curtailing Coagulase Negative *Staphylococci* (CoNS). Moreover, if CoNS is isolated along with another organism, its pathogenic potential may be totally neglected. Hence, it is necessary to speciate CoNS and understand the pathogenic potential of individual CoNS[12].

In our study, *S. epidermidis* was the most frequent isolate (36, 42.8%)followed by *S.saprophyticus*(25,29.7 %), *S.haemolyticus* (15,17.8%), *S.lugdunensis* (2,2.3 %) etc. This study correlates with various studies like Shubhra Singh, Gopa Banerjee etal, where they identified *S.epidermidis* (40%) as the most frequently encountered clinical isolates in their hospital followed by *S. saprophyticus* (14*%), S.haemolyticus* (12%), *S. hominis* (6%), *S.lugdunensis* (6%) and reported that various workers from India have shown *S.epidermidis* or *S.saprophyticus* to be the most common isolate similar to our study[12], P Manikandan, M Bhaskar, R Revathy *et al* showed *S. epidermidis* as the predominant CoNS (57.1 %) isolate followed by *S. hominis* (22.8 %) [13] and Larry M. Baddour, David L.*et al*, reported *S.epidermidis* as the most often identified species for both clinical and saprophytic strains[14] . Shubhra Singh, Gopa Banerjee etal study showed 72 out of 150 strains of CoNS (60%) were isolated from blood samples, 36 from pus samples, 15 from urinary catheter tip and 12 from the urine samples [12,] whereas our study showed, among 84 isolates of CoNS, 26 (31 %) were isolated from sputum, 25(29.8%) from the urine samples, 24(28.6%) from pus samples, 4(4.8 %) from blood samples etc. Our study reported, out of 36 *S.epidermidis* isolates, 12 (33.3%) from sputum samples followed by11 (30.5%) were isolated from urine samples. But in the study by Shubhra Singh, Gopa Banerjee etal, out of 60 *S.epidermidis* isolates, 30 (50%) were isolated from blood samples followed by 12 (20%) from pus samples [12].

Our study reported, out of 84 CoNS, 29 (40.37%) cases in >40 years of age group. The isolations were more in males (59, 70.2%) than females (25, 29.7%). In males, majority of the CoNS 28 (47.4%) found in the age group of >40 years & in females, 7 (28%) in found in age group 21-30 years, which is comparable with a study by Larry M. Baddour, David L.*et al*. who reported 19 (54.2%) of 35 patients were males and 30 (85.7%) patients were above the age of 40. Incidence of CoNS was significantly higher among those 60 and older [13].

CONCLUSION

CoNS have emerged as a major cause of nosocomial bloodstream infections as a result of the combination of increased use of intravascular devices and an increase in the number of hospitalized immunocompromised patients. *S.epidermidis* and *S.saprophyticus* are the more common isolates identified and CoNS are often resistant to multiple antibiotics and glycopeptides have been considered as the drugs of choice for the management of infections caused by these organisms. This simple and inexpensive methodology will prove useful in routine microbiology laboratory for the identification and differentiation of these isolates, which help in monitoring the reservoir and distribution of CoNS involved in nosocomial infections and determining the etiological agents in the hospitals.

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