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Original Article

Speciation of clinically significant Coagulase Negative Staphylococci and their antibiotic resistant patterns in a tertiary care hospital.

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ABSTRACT

Coagulase Negative Staphylococci (CoNS) are the indigenous flora of the human skin and mucous membrane. They are usually contaminants, when isolated from a clinical specimen. These organisms are becoming increasingly recognized as agents of clinically significant nosocomial blood stream infections. The study was undertaken to identify the prevalence of clinical isolates of CoNS, their speciation and to determine the antibiotic sensitivity/resistant patterns of CoNS. A total of 96 isolates were collected from different samples and subjected to biochemical characterization and antimicrobial screening using conventional microbiological methods. Ninety-seven isolates were conveniently identified. *Staphylococcus.epidermidis*(43,44.8%),*S.saprophyticus*(26,27.1%),*S.haemolyticus*(19,19.7%), *S.lugdunensis* (2,2.1%), *S.warneri* (2,2.1%), *S.cohnii* (1,1%), and others(3,3.1%). These 3 isolates were not identified to the species level. Antibiotic susceptibility testing showed maximum resistance to ampicillin and penicillin with 85%-95% and sensitivity to piperacillin / tazobactam, vancomycin and linezolid (85%-100%). The increased recognition of pathogen potential CoNS and emergence of drug resistance among them demonstrates the need to adopt simple laboratory procedure to identify and determine the prevalence and antibiotic resistant patterns of CoNS. It will help for clinicians in treating as the first line of treatment in the hospital.

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1. Introduction

Coagulase Negative Staphylococci (CoNS) are common colonizers of the skin, anterior nares, and ear canals of human beings. 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 become the major cause of nosocomial bloodstream infections [1] and they account for 9% of nosocomial infections [2]. Patients at risk include those with intravascular catheters, or other foreign bodies in place,

prosthetic devices, postoperative sternal wound infections and immunocompromised hosts [3, 4, 5]. These infections are difficult to treat because of the risk factors and the multiple drug resistant nature of the organisms [5]. Hence, this study was undertaken to identify the most prevalent clinical isolates, to discriminate between the species and to determine the antibiotic resistant patterns of CoNS.

2. Material and Methods

The study was carried out in the Department of Microbiology, VIMS Bellary for a period of 6 months from January 2011 to June 2011. A total of 96 clinically significant CoNS isolates were collected from different clinical samples, and processed using conventional microbiological methods. 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].

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2. Materials and Methods

The tests which were simple, inexpensive and easy to perform, were selected from the scheme of Kloos and Shleifer to identify CoNS species [8,9]. Speciation of CoNS was done by Novobiocin resistance test, urease activity, ornithine decarboxylase & aerobic acid production from mannose [8,9,10].

The antimicrobial susceptibility profiles of all isolates were done by Disc Diffusion Method using Mueller-Hinton agar plates (Hi-Media, Mumbai) according to the NCCLS guidelines. Disc contains following antibiotic at the specific absolute concentrations -vancomycin (30µg), penicillin (10µg), ampicillin, (10µg), erythromycin (15µg), oxacillin(1µg), linezolid(30µg), piperacillin/tazobactam(100/10µg) and cefoxitin(30 µg). Staphylococcus aureus ATCC 43300 was used as control [11, 12]

3. Results

Among 96 isolates of CoNS, 32 (33.3 %) were isolated from pus, 26(27.1%) from the urine samples, 26(27.1%) from sputum samples, 5(5.2 %) from blood samples, 3(3.1%) from ear swabs, 1(1%) from fluid and 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(43, 44.8%), followed by *S.saprophyticus* (26,27.1%), *S.haemolyticus* (19,19.7%), *S.lugdunensis* (2,2.1 %), *S.warneri* (2,2.1%), *S.cohnii* (1,1%), and unidentified(3,3.1%) because of aberrant reactions. Out of 43 *S.epidermidis* isolates, 14(32.5%) were isolated from sputum samples followed by 12 (27.9%) each from urine and pus samples. Out of 26 *S.saprophyticus* 9 (34.6%) isolated from sputum samples followed by 8 (30.7%) each from urine and pus samples. Out of 19 *S.haemolyticus* 6(31.5%) each from urine and pus samples (Table-1). Out of 96 CoNS, 38(39.5%) cases in >40 years of age group. The isolations were more in males (63, 65.6%) than females (33, 34.4%). In males majority of the CoNS 30 (47.6%) were found in the age group of >40 years & in females 9 (27.2%) were found in age group of 30-40 years.

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

Species	Sputum	Urine	Pus	Blood	Ear	Throat	Fluid
<i>S. epidermidis</i> (43)	14 (32.5%)	12 (27.9%)	12 (27.9%)	1 (2%)	3 (6%)	-	1 (2%)
<i>S.saprophyticus</i> (26)	9 34.6%)	8 30.7%)	8 30.7%)	1 (3%)	-	-	-
<i>S.haemolyticus</i> (19)	3 15.7%)	6 (31.5%)	6 (31.5%)	3 (15.7%)	-	1 (5%)	-
<i>S.lugdunensis</i> (2)	-	-	2 (100%)	-	-	-	-
<i>S.warneri</i> (2)	-	-	2 (100%)	-	-	-	-
<i>S.cohnii</i> (1)	1 (100%)	-	-	-	-	-	-
<i>Others</i> (3)	-	-	2 33.3%)	1 (33.3%)	-	-	-
<i>Total</i>	26 (27.1%)	26 (27.1%)	32 (33.3%)	5 (5.2%)	3 (3.1%)	1 (1%)	1 (1%)

Table 2: Table-2: Identification of CoNS by simple scheme

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

Table-3: Showing resistance patterns of CoNS to different antibiotics

Species	P	AMP	E	Cx	Ox	LZ	PIT
<i>S. epidermidis</i> (43)	41 (95%)	39 (91%)	24 (56%)	28 (65%)	21 (49%)	4 (9%)	6 (14%)
<i>S.saprophyticus</i> (26)	25 (96%)	23 (88%)	23 (88%)	16 (62%)	18 (69.2%)	12 (46%)	4 (15%)
<i>S.haemolyticus</i> (19)	18 (95%)	17 (90%)	17 (90%)	17 (90%)	19 (100%)	0	13 (68%)
<i>S.lugdunensis</i> (2)	2 (100%)	2 (100%)	2 (100%)	1 (50%)	2 (100%)	0	0
<i>S.warneri</i> (2)	2 (100%)	2 (100%)	0	1 (50%)	1 (50%)	0	0
<i>S.cohnii</i> (1)	1 (100%)	0 (100%)	1 (100%)	0	0	0	0 1
<i>Others</i> (3)	2 (66.6%)	2 (66.6%)	2 (66.6%)	2 (66.6%)	1 (33.3%)	0	(33.3%)
<i>Total No of species</i> (96)	91 (94.79%)	85 (88.54%)	69 (71.87%)	65 (67.7%)	62 (64.58%)	16 (16.66%)	24 (25%)

No resistance to VA, VA= vancomycin, P=penicillin, AMP=ampicillin, E=erythromycin, Ox=oxacillin, LZ=linezolid, PIT=piperacillin/tazobactam, Cx=cefoxitin.

Antibiotic susceptibility testing of the isolates showed maximum resistance to penicillin (91,94.8%) and ampicillin (85,88.5%) followed by erythromycin (69,71.9%), cefoxitin(65,67.7%),oxacillin(62,64.6%),piperacillin/tazobactam(24,25%)and linezolid(16,16.7%). No resistance to vancomycin was seen.

S.epidermidis showed a significant percentage of isolates resistant to penicillin (95%) followed by ampicillin (91%), erythromycin (56%) and cefoxitin (65%). *S.saprophyticus* also showed resistance to penicillin (96%) followed by ampicillin (88%) erythromycin (88%), oxacillin (69.2%) and cefoxitin (62%) (Table-3).

4. Discussion

Coagulase Negative Staphylococci form a part of the normal flora. Therefore an effective management may not be aimed at curtailing these 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 [11].

In our study, *S. epidermidis* was the most frequent isolate (43, 44.8%) followed by *S. saprophyticus* (26, 27.1%), *S. haemolyticus* (19, 19.7%), *S. lugdunensis* (2, 2.1 %) etc. This study correlates with various studies like Shubhra Singh, Gopa Banerjee et al, 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 [11], 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 et al study showed 72 among 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 [11], whereas our study showed, among 96 isolates of CoNS, 32 (33.3 %) isolates from pus, 26 (27.1%) from the urine samples, 26 (27.1%) from sputum samples, 5 (5.2 %) from blood samples, 3 (3.1%) etc. Our study reported, out of 43 *S. epidermidis* isolates, 14 (32.5%) were isolated from sputum samples followed by 12 (27.9%) each from urine and pus samples. But in a study by Shubhra Singh, Gopa Banerjee et al, out of 60 *S. epidermidis* isolates, 30 (50%) were isolated from blood samples followed by 12 (20%) from pus samples [11].

Our study reported, out of 96 CoNS, 38 (39.5%) cases in >40 years of age group. The isolations were more in males (63, 65.6%) than females (33, 34.4%). In males, majority of the CoNS 30 (47.6%) found in the age group of >40 years & in females, 9 (27.2%) in found in age group 30-40 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].

In our study, antibiotic susceptibility testing showed maximum resistance to penicillin (91, 94.8%) and ampicillin (85, 88.5%), 62 strains showed resistance to oxacillin (64.6%) and no resistance to vancomycin was seen. This study correlates with a study by Shubhra Singh, Gopa Banerjee et al where the antibiotic susceptibility testing showed maximum resistance to penicillin and ampicillin with 80% and 38% strains showed resistance to oxacillin [11]. Other studies like Larry M. Baddour, David L. et al showed 18 of the 21 (86%) clinical strains were resistant to penicillin. G. methicillin resistance, which was present in five clinical strains, was not found in saprophytic strains [14] and Diekema, DJ, Pfaller, MA et al showed more than 80 percent of coagulase-negative staphylococcal isolates were resistant to methicillin and semi synthetic penicillin [15].

S. epidermidis showed a significant percentage of isolates resistant to penicillin (94.8%) followed by ampicillin (88.5%), whereas Shubhra Singh, Gopa Banerjee et al showed out of all species *S. haemolyticus* has the most antibiotic resistant profile. Approximately 76% of the isolates of *S. haemolyticus* showed resistance to oxacillin (76%), gentamycin (76%), and erythromycin (80%) although; *S. epidermidis* can also show significant multiple resistance patterns [11]. CoNS, primarily *S. epidermidis* and *S. haemolyticus* are often resistant to multiple antibiotics, and glycopeptides have been considered the drugs of choice for the management of infections caused by these organisms [1] and in a study by Del' Alamo L, Cereda RF, Tosin I et al showed that glycopeptide resistance is emerging among CoNS isolates [16].

5. Conclusion

CoNS have become the 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 (penicillin, ampicillin, oxacillin etc) and glycopeptides have been considered as the drugs of choice for the management of infections caused by these organisms. This simple, inexpensive methodology will prove useful in routine microbiology laboratory for the presumptive identification of CoNS and determination of antibiotic sensitivity/resistant patterns to avoid decreased susceptibility to glycopeptides.

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