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Species distribution and antimicrobial susceptibility pattern of coagulase-negative staphylococci isolated from clinical specimens at a tertiary care hospital

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Abstract

Background and objectives: Coagulase-negative staphylococci (CoNS) are considered important causative agents of hospital acquired infection. These organisms are found in various clinical specimens from hospitalized patients. Present study was carried out to determine the species distribution and antimicrobial susceptibility pattern of CoNS isolated from clinical specimens at a tertiary care hospital.

Materials and methods: CoNS isolated from various clinical samples were included in this study. The isolates were identified by colony morphology, Gram's staining, catalase and coagulase tests. Further differentiation of species was performed by susceptibility to novobiocin, urease activity and ornithine decarboxylase test. Antibiotic susceptibility testing was performed according to the Clinical and Laboratory Standard Institute (CLSI) guidelines.

Results: Total 108 isolates of CoNS were included and analysed. Out of 108 CoNS, *S. epidermidis* was the most common species (36.1%) followed by *S. saprophyticus* (23.1%), *S. hemolyticus* (17.6%), *S. hominis* (13%) and *S. lugdunensis* (10.2%). Most of the isolates showed resistance to penicillin, oxacillin, amoxycillin, erythromycin, ciprofloxacin and ofloxacin. All the isolates were sensitive to vancomycin.

Conclusions: CoNS emerged as an important nosocomial pathogen and should not be neglected as contaminant. High rate of antimicrobial resistance warrants susceptibility testing prior to the treatment of CoNS.

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Introduction

Coagulase-negative staphylococci (CoNS) are part of normal flora of skin [1]. Previously, they were considered non-pathogenic with low virulence. But since 1950, cases of CoNS associated infections have been reported with increased frequencies. The predisposing factors for CoNS infections are patients with catheter, prosthetic implants, dialysis, oncological diseases, compromised immunity and neonatal state [2]. CoNS survive on synthetic medical devices and equipment such as intravenous catheters, prosthetic heart valves and various implants [3]. Currently more than fifty different CoNS species have been described. Out of this, *S. epidermidis*, *S. saprophyticus*, *S. hemolyticus*, *S. hominis* and *S. lugdunensis* have higher clinical significance [4].

The main challenge in the diagnosis is to correctly identify the cases in which CoNS are causative agents for infection rather than contaminants. This

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leads to under treatment (i.e., delayed or withheld antibiotics) and thereby contributing to increased morbidity and mortality [5]. Due to increasing clinical significance of CoNS infection, accurate species identification and determination of antibiotic resistance are of paramount importance to treat CoNS infections. The aim of the study was to investigate species distribution and antimicrobial resistance pattern of CoNS isolated from clinical specimens at a tertiary care centre.

Material and method

The study was conducted at the Department of Microbiology in a tertiary care teaching hospital of Marathwada region of Maharashtra state, India. Study was done over a period of one year from January 2015 to December 2015. The study was approved by the institutional ethical committee.

CoNS isolated from different clinical samples such as pus, urine, blood, sputum, vaginal swab, wound swab, suction tip, pleural fluid and nasal swab were included. Same strain of CoNS isolated twice in pure culture from an infected site or body fluid was considered clinically significant. Samples were cultured on nutrient and blood agar plates for bacterial isolation. Plates were incubated aerobically overnight at 37°C for 48 hours [6]. Isolates were identified by colony morphology, Gram's stain, catalase and coagulase tests. Bacitracin susceptibility was performed to exclude micrococci and *Stomatococcu*s species [7]. The speciation of CoNS was done by ornithine decarboxylase, urease, mannose fermentation and novobiocin (5 µg) sensitivity tests [8,9].

The antimicrobial susceptibility of all the isolates was performed by Kirby-Bauer disc diffusion method using Muller-Hinton agar plates as per the recommendation of Clinical Laboratory Standard Institute guidelines [10]. *S. aureus* ATCC 25923 was used as a standard control strain for antimicrobial susceptibility testing.

Antimicrobial discs used were penicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), oxacillin (1 µg), erythromycin (15 µg), linezolid (30 µg), gentamicin (10 µg), and vancomycin (30 µg). The various antibiotic discs used were purchased from HiMedia Laboratories Private Limited, India.

Results

A total of 108 CoNS isolates were included in the study. Out of 108 isolates, 65 (60.2%) and 43 (39.8%) were from male and female patients respectively. Maximum numbers of isolates (44.4%) were from age group 21-40 years followed by 18.5% from age group 41-60 years [Table-1].

Majority of CoNS were from pus sample (44.4%) followed by urine (23.1%), blood (9.3%), suction tip (6.5%), sputum (4.6%), vaginal swab (3.7%) and, 2.8% each from wound swab, nasal swab and pleural fluid [Table-2]. The commonest CoNS species isolated was *S. epidermidis* 39 (36.1%) followed by *S. saprophyticus* 25 (23.1%), *S. hemolyticus 19* (17.6%), *S. hominis* 14 (13%) and *S. lugdunensis* 11 (10.2%) [Table-2]. Antimicrobial sensitivity test of the isolates showed maximum resistance to oxacillin (88%), ciprofloxacin (80%), penicillin (78.7%), amoxicillin-clavulanic acid (75%), erythromycin (60.2%) and ofloxacin (60.2%). None of the CoNS species showed resistance to vancomycin while only 9.3% were resistant to linezolid [Table-3].

Table-1: *Age and sex distribution (n=108)*

Age groups	Male Number	Female Number	Total n (%)
1 day-1 year	7	4	11 (10.2)
1-20 years	12	8	20 (18.5)
21-40 years	29	19	48 (44.4)
41-60 years	11	9	20 (18.5)
61-80years	6	3	9 (8.3)
Total	**65**	**43**	**108 (100)**

Table-2: *Distribution of CoNS and their species in different clinical samples (n=108)*

Sample	CoNS Number	*S. epidermidis* n (%)	*S. saprophyticus* n (%)	*S. hemolyticus* n (%)	*S. hominis* n (%)	*S. lugdunensis* n (%)
Pus	48	26 (54.2)	1 (2.1)	8 (16.7)	8 (16.7)	5 (10.4)
Urine	25	3 (12)	21 (84)	-	-	1 (4)
Blood	10	5 (50)	-	1 (10)	1 (10)	3 (3)
Suction tip	7	1 (14.3)	2 (28.6)	-	3 (42.9)	1 (14.3)
Sputum	5	1 (20)	-	4 (80)	-	-
V. Swab	4	2 (50)	1 (25)	1 (25)	-	-
P. fluid	3	-	-	2 (66.7)	-	1 (33.3)
Nasal swab	3	1 (33.3)	-	2 (66.7)	-	-
Wound swab	3	-	-	1 (33.3)	2 (66.7)	-
Total	**108**	**39 (36.1)**	**25 (23.1)**	**19 (17.6)**	**14 (13)**	**11 (10.2)**

Note: V. Swab – vaginal swab, P. Fluid – plural fluid.

Table-3: *Species wise antibiotic resistance pattern of isolated CoNS*

Antibiotics	*S. epidermidis* N=39	*S. saprophyticus* N=25	*S. hemolyticus* N=19	*S. hominis* N=14	*S. lugdunensis* N=11	Total N=108
	Resistant n (%)	Resistant n (%)	Resistant n (%)	Resistant N (%)	Resistant n (%)	Resistant n (%)
Penicillin	33 (84.6)	23 (92)	17 (89.5)	6 (42.9)	6 (54.5)	85 (78.7)
Amox – Calv	31 (79.5)	24 ((96)	18 (94.7)	5 (35.7)	3 (27.3)	81 (75)
Oxacillin	35 (89.7)	22 (88)	18 (94.7)	11 (78.6)	9 (81.8)	95 (88)
Erythromycin	25 (64.1)	20 (80)	9 (47.4)	5 (35.7)	6 (54.5)	65 (60.2)
Gentamycin	19 (48.7)	14 (56)	6 (31.6)	2 (14.3)	3 (27.3)	44 (40.7)
Amikacin	11 (28.2)	7 (28)	6 (31.6)	5 (35.7)	4 (36.4)	33 (30.6)
Ciprofloxacin	38 (97.4)	22 (88)	13 (68.4)	8 (57.1)	6 (54.5)	87 (80.6)
Ofloxacin	29 (74.4)	18 (72)	9 (47.4)	5 (35.7)	4 (36.4)	65 (60.2)
Ceftazidime	20 (51.3)	16 (64)	7 (36.8)	4 (28.6)	0	47 (43.5)
Linezolid	5 (12.8)	3 (12)	2 (10.5)	0	0	10 (9.3)
Clindamycin	12 (30.8)	7 (28)	3 (15.8)	4 (28.6)	1 (9.1)	27 (25)
Vancomycin	0	0	0	0	0	0
Novobiocin	0	25 (100)	0	7 (50)	0	32 (29.6)

Note: Amox – Calv: amoxicillin-clavulanic acid

Discussion

CoNS formerly considered as contaminant bacteria have now emerged as a major cause of nosocomial infections. CoNS are the common agents of nosocomial bloodstream infections as well as other type of infections. Factors helpful in identification of true infections by CoNS include repeated isolation of same strain of CoNS in pure culture from infected site or specimen over the course of an infection plus presence of clinical evidence of infection [11,12]. Recent studies have shown that CoNS are one of the important causative agents of human infection, especially in immunocompromised patients, premature newborns and patients with indwelling medical devices [13]. In our study, majority of CoNS were isolated from male patients (60.2%). Similar findings are also reported by Usha et al and Asangi et al [14,15]. On the other hand, Goudarzi M et al found maximum number of CoNS from female patients [16].

In this study, out of 108 isolates, most of the isolates were from pus (44.4%) and urine (23.2%). The results differ from studies by Bhatt P et al and Parashar [17,18]. In their studies maximum numbers of CoNS were isolated from blood samples. This difference could be due to types of patients and hospital settings.

In the laboratory, staphylococci other than *S. aureus* are reported as CoNS without speciation. As various species of CoNS are associated with different diseases, CoNS should be identified to the species level by simple, reliable and inexpensive method [19]. In the present study we have identified CoNS species by slide and tube coagulase, ornithine decarboxylase, urease, mannose fermentation and novobiocin sensitivity tests. These tests are inexpensive and affordable and can be practiced in most of the diagnostic laboratories.

In the present study, *S. epidermidis* constituted the predominant species (36.1%) followed by *S. saprophyticus*, *S. hemolyticus* , *S. hominis* and *S. lugdunensis*. This is in concurrence with other reported studies from India and adjoining region [20-22]. Those studies have reported *S. epidermidis* as the most common species (41% - 46.84%) among the isolated CoNS. The second most common species in our study was *S. saprophyticus* which is similar to other studies who also found *S. saprophyticus* as second most common species [7,23].

In the present study, majority of the isolates showed resistance to oxacillin, ciprofloxacin, amoxicillin-clavulanic acid, penicillin, erythromycin and ofloxacin. So, these antibiotics could not be recommended for empiric treatment of CoNS. All the isolates in our study were sensitive to vancomycin and only 9.3% were resistant to linezolid. Similar results were also observed by others [24-26]. Vancomycin and linezolid are the most effective drugs in treating infections caused by CoNS species.

Prolonged hospital stays, widespread antibiotic use and the ability of CoNS to form multi-layered bio-films on artificial surfaces are the potential causes of high resistance rate to multiple antimicrobial agents especially for the species that are isolated from catheter tips and blood cultures. This high resistance to multiple antimicrobial agents poses a significant challenge in the clinical management of infections caused by CoNS.

Therefore, continued surveillance, prudent use of antibiotics and emphasis on infection prevention measures in hospitals are imperative in curbing the rise of antibiotic resistant CoNS strains. In addition to increased vigilance, advanced diagnostic approaches and an understanding of the antibiogram profiles are essential for effective clinical management and the prevention of CoNS associated infections in healthcare settings.

Conflict of interest*:*

The author declares no conflict of interest.

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