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Characterization of CONS Isolated from Clinical Samples of Paediatric Cases in a Tertiary Care Hospital

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A B S T R A C T

Coagulase negative staphylococci (CoNS) were generally considered to be contaminants in the past having little clinical significance. Over the past two decades, these organisms have become recognized as important agents of human disease. *S. epidermidis* is the predominant agent in nosocomial infection, bacteremia, surgical wound and urinary tract infections. Their pathogenic potential is being increasingly understood and also causing problems to clinicians because of their drug resistance. Characterization of CoNS isolated from clinical samples of paediatric cases, their antibiogram and methicillin resistance. The study was conducted from December 2014 to August 2015. The organisms were identified and speciation was done by standard biochemical reactions. Antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method and following Clinical laboratory standards institute guidelines (2015). The maximum isolates were from female patients 21 (55%) than male patients 17 (45%). Most common age group affected was Neonate (1-28 days) (29%) in both sex followed by Infant (29 days -12 months) in female (13%) and in male (8%). The majority of CoNS species isolated were *S. epidermidis* 19 (50%), followed by *S. haemolyticus* 9 (24%), *S. xylosus* 3 (8%), *S. lugdunensis* 2 (5%), *S. hominis* 2 (5%), *S. cohnii* 2 (5%) and *S. saprophyticus* 1(3%). *S. epidermidis* was the predominant species isolated. The most effective antibiotics were Linezolid and Amikacin.

Introduction

Coagulase negative staphylococci (CoNS) were incriminated in life threatening blood stream nosocomial infection in critically ill newborn or patient receiving immunosuppressive therapy for malignant neoplasm, hematological malignancy and bone marrow transplant.3 The increased incidence of CoNS infections in neonates has occurred predominantly in low birth weight infants who are known to be immunologically immature.4 These mucocutaneous commensals can cause serious invasive infections in NICU patients.4 The two most frequently isolated CoNS species in clinical samples are *S. epidermidis* and *S. saprophyticus*. Overall *S. epidermidis* is the predominant agent in nosocomial infection, bacteremia, UTI and surgical wound infection.5 Many CoNS also produce lipases, proteases and other exoenzymes, which are

possibly implicated with the bacterial persistence and dissemination in the host tissue.6 Clinical studies, have indicated *S. epidermidis*, *S. haemolyticus*, *S. warneri* and *S. hominis* as the most prevalent CoNS in hospital infections.6,7

The majority of infections assumed to be caused by CoNS are a significant consequence of hospitalization.8 Nosocomial bacteraemia is most commonly caused by CoNS, so it is important to explore the sources of CoNS for prevention and management of infections. 9 This type of study was not conducted so far in this Institute, an attempt was made to isolate and speciate CoNS from various clinical samples with their antibiogram.

Characterzation of CoNS isolated from clinical samples of paediatric cases, their antibiogram and methicillin resistance.

Materials and Methods

This was an observational study and conducted at Department of Microbiology, S Nijalingappa Medical College and Hospital, Bagalkot from December 2014 to August 2015 after obtaining the Institutional Ethical Committee clearance. All clinical samples were collected under aseptic precautions and following standard clinical laboratory guidelines. The isolates were identified as CoNS by colony morphology, Gram stain, catalase test and coagulase test (slide and tube coagulase). Bacitracin (0.04 U) susceptibility was performed to exclude Micrococci and *Stomatococcus species*.8

The strains which were slide and tube coagulase negative were selected for further speciation. Speciation was done after reviewing the scheme of Kloos and Schleifer and Koneman, *et al.*, 7,8,9 The various biochemical tests used for speciation are as follows:

Ornithine decarboxylase test, Phosphatase test, Urease test, Nitrate reduction test and Carbohydrate fermentation test (Mannose, Mannitol and Xylose).

The antibiotic sensitivity testing was performed on Mueller-Hinton agar by the Kirby-Bauer disc diffusion method, The antibiotics included Amikacin(AK)), Amoxicillin-Clavulanate (AMC), Cotrimoxazole (COT), Ciprofloxacin (CIP), Doxycycline (DO), Erythromycin (E), Gentamicin (GEN), Linezolid (LZ) Norfloxacin (NX), Nitrofurantoin (NIT), Novobiocin (NV) and Cefoxitin10 (CX). Four to five colonies from 16 to 24 hours grown culture from an agar plate was suspended in peptone water. This is compared with 0.5 McFarland turbidity standards and inoculated onto Mueller Hinton Agar to get a confluent growth. Plates were incubated at 37oC for 18 to 24 hours. Zone of growth inhibition was measured.11

Detection of Methicillin resistance: Cefoxitin (CX-30µg) was used to identify methicillin resistant coagulase negative Staphylococci (MR-CoNS) 10and *Staphylococcus aureus* ATCC 25923 was used as control strain. A 0.5 Mc Farland suspension of the isolate was made and lawn culture done on MHA plate. Plates were incubated at 30ºC for 18 h and zone diameters were measured. An inhibition zone of $\geq$ 22 mm was considered as susceptible and $\leq$ 21 mm resistant for cefoxitin. The results of the test are interpreted as sensitive and resistant as per CLSI Guidelines (2015).10

Results and Discussion

The maximum isolates were from female patients 21 (55%) than male patients 17 (45%). Most common age group affected was Neonate (1-28 days) (29%) in both sex followed by Infant (29 days -12 months) in

female (13%) and in male (8%). The majority of CoNS were isolated from blood 32 (84%), followed by urine 5 (13%) and ascitic fluid 1 (3%).The majority of CoNS species isolated were *S. epidermidis* 19 (50%), followed by *S. haemolyticus* 9 (24%), *S. xylosus* 3 (8%), *S. lugdunensis* 2 (5%), *S. hominis* 2 (5%), *S. cohnii* 2 (5%) and *S. saprophyticus* 1(3%). 23 (61%) were methicillin resistant and 15 (39%) were methicillin sensitive. All the strains of CoNS were sensitive to Linezolid (100%), Amikacin (79%) and Doxycycline (76%).However they were resistant to Amoxycillin-Clavulanic acid 25 (66%), Cotrimoxazole 24 (63%) and Ciprofloxacin 17 (45%).

CoNS are the most frequently reported pathogen in blood stream infection, especially patient with indwelling or implanted foreign polymer bodies. CoNS species apparently changing the status from non-pathogens to opportunistic pathogens.[12] Simplicity and speed are very important in certain circumstances, e.g., for the identification of CoNS isolates from normally sterile body sites such as blood cultures, in which these isolates are the most common cause of bacteremia, as well as the most common blood culture contaminants. Repeated CoNS isolates from patients with invasive diseases should be identified and allow a comparison of the strains. On the other hand, species identification is a prerequisite for epidemiological studies.13 CoNS were responsible 11 % (35/320) of bacteremia cases in our study. The CoNS positive bacteremia found maximum 28(80%) in age group 0-14 years in our study, co-relate with study by Parashar [1]. The CoNS species *S. epidermidis* was most frequent isolate 15(43%); followed by *S. haemolyticus* 10(29%) and *S. xylosus* 3(9%) (Table 1–5).

Table.1 Showing Age wise and Sex wise distribution of CoNS (n=38)

Age Groups	Male	Female
Neonate (1-28 days)	**11**	**11**
Infant (29 days-12 months)	**3**	**5**
Toddler (1-3 years)	**1**	**0**
Pre-school (3-5 years)	**1**	**0**
School (6-10 years)	**1**	**2**
Adolescent (11-14 years)	**0**	**3**
Total	**17**	**21**

Table.2 Showing distribution of CoNS individual sample wise (n=38)

Samples	Number	Percentage
Blood	**32**	**84**
Urine	**5**	**13**
Ascitic fluid	**1**	**3**
Total	**38**	**100**

Table.3 Different species of CoNS from various clinical samples (n=38)

Species of CoNS	No.	%
S. epidermidis	19	50
S. haemolyticus	9	24
S. xylosus	3	8
S. lugdunensis	2	5
S. hominis	2	5
S. cohnii	2	5
S. saprophyticus	1	3
Total	38	100

Table.4 Showing resistance to Methicillin (n=38)

Methicillin Sensitive		Methicillin Resistant	
No.	%	No.	%
15	39	23	61

Table.5 Showing Antibiogram of CoNS (n=38)

Antibiotic	Sensitive		Resistant	
	No.	%	No.	%
Amikacin	30	79	8	21
Amoxicillin-Clavulanate	13	34	25	66
Cotrimoxazole	14	37	24	63
Ciprofloxacin	21	55	17	45
Doxycycline	29	76	9	24
Erythromycin	22	58	16	42
Gentamicin	24	63	14	37
Linezolid	38	100	0	0
*Norfloxacin	3	60	2	40
*Nitrofurantoin	3	60	2	40

* For urinary isolates (n=5)

The CoNS isolates in other studies, 35% Sardar *et al.,* [14], 32% Kavita *et al.,* [15], 23% according to Jayanthi *et al.,* [16], 22% Nagasugha *et al.,* [12], 14% Golia *et al.,* [17] and our study 11%. In our study, the common species were *S. epidermidis* and *S. haemolyticus* (43% & 29%) respectively. Similarly in other studies by Nagasudha *et al.,* [12](59% &15%), Jayanthi *et al.,* [16] (56% & 30%), Golia *et al.,* [17] (46% & 20%) and Sardar *et al.,* [14](43% & 13%).

Moreover the conventional identification methods are accurate and employ a large battery of biochemical reactions, which often give variable results and all the tests are generally not available in most of the routine diagnostic laboratories. The commercial identification methods including automated systems are not economical to use for all CoNS isolates.18 In our study resistant to methicillin was 63% similar to other studies by Aher *et al.,* [19] -78%, Nagasuda *et al.,* [12] -66%, Golia *et al.,* [17] -66% and Sardar *et al.,* [14] -52%. Methicillin resistant CoNS isolate, *S. epidermidis* 43% and *S. haemolyticus* 29%. In the present study higher number of isolates was sensitive to linezolid 100%, amikacin and doxycycline 77%, gentamicin 63%, erythromycin 60% and ciprofloxacin 54%. The antibiogram of our study is similar to other studies, Aher *et al.,* [19], Kavita *et al.,* [15], Golia *et al.,* [17], Jayanthi *et al.,* [16], Nagasudha *et al.,* [12], and Sardar *et al.,* [14]. Methicillin resistant CoNS (MR-CoNS) most notably *S. epidermidis, S. haemolyticus, S. hominis* are major MR-CoNS and main colonizers of the anterior nares and human skin. Methicillin resistant staphylococcal strains have acquired and integrated into their genome, the staphylococcal cassette chromosome *mec* (SCC*mec*),which carries the methicillin resistance (*mec*A) gene and other antibiotic resistance determinants.20 The methicillin resistant organisms grow more slowly and prefer lower temperatures and more hypertonic environment, which necessitates the use of special procedures to enhance detection in susceptibility tests.21 Accuracy and promptness in the detection of methicillin resistance are of key importance in ensuring correct antibiotic treatment in infected patients and control of methicillin resistant staphylococci in the hospital environment.22

CoNS are one of the most common infectious agent responsible for blood stream infection, especially in neonates with low birth weight or prematurity. Therefore suggest that CoNS strain should not discard as contaminants and should be identified up to species level with their antibiogram to decrease mortality and morbidity. It is important to monitor antibiotic consumption and resistance trends of staphylococci, especially with infection control measures to prevent emergence and spread of multi-resistant bacteria within the hospital environment.23Further studies are needed with well characterized standardized strains for the development of simple, inexpensive, accurate and more reliable schemes for characterization of clinically significant CoNS.

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