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Characterization of Coagulase Negative *Staphylococci* Isolated From Urine Samples in a Tertiary Care Hospital

T.S. Kumudini[1*] and Shivakumar S. Solabannavar[2]

[1]Department of Microbiology, Vijayanagar Institute of Medical Sciences (VIMS), Bellary-583104, India
[2]Department of Microbiology, S. Nijalingappa Medical College and Hospital, Navanagar, Bagalkot, India

**Corresponding author*

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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. Characterization of CoNS isolated from urine samples, their antibiogram and methicillin resistance. The study was conducted from December 2014 to August 2015. We received 518 urine samples, 33 strains of CoNS were isolated. 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). From urine culture, 33/518 (6%) were obtained as CoNS isolates. From CoNS isolates, majority was in the age group of 0-14 years 25(76%). Maximum isolates were from females 20(60%) than males 13(40%). The most common species isolated was *S. epidermidis* 18(55%), followed by *S. saprophyticus* 14(42%) and *S. lugdunensis* 1(3%). Methicillin resistance was found 61% of strains. Linezolid (100%), Amikacin (79%), Doxycycline (76%), Nitrofurantoin (67%), Gentamicin (61%), Erythromycin and Norfloxacin (each 58%) and Ciprofloxacin (55%) were found to be the most effective antibiotics. *S. epidermidis* was the predominant species isolated and more susceptible antibiotics were Linezolid and Amikacin.

Introduction

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. These mucocutaneous commensals can cause serious invasive infections in NICU patients.1 Clinical studies, have indicated *S. epidermidis*, *S. haemolyticus*, *S. warneri* and *S. hominis* as the most prevalent CoNS in hospital infections.2 Methicillin resistance

among CoNS is particularly important due to cross resistance among betalactam agents and other anti-microbial classes. Susceptibility testing should be done and considered to be a cause of infection due to their resistance to wide spectrum of antimicrobial agents.3

Objectives

Characterization of CoNS isolated from urine samples, 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. Clean catch midstream urine was collected in a sterile wide mouth container.4

The isolates were identified as CoNS by colony morphology, Gram stain, catalase test and coagulase test (slide and tube coagulase). The strains which were slide and tube coagulase negative were selected for further speciation. Speciation was done after reviewing the scheme of Kloos, Schleifer and Koneman *et al.*, 4,5,6 The various biochemical tests used for speciation are as follows: Ornithine decarboxylase test, Phosphatase test, Urease test, Novobiocin susceptibility 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 Cefoxitin7 (CX).

D etection of Methicillin resistance: Cefoxitin (CX-30µg) was used to identify methicillin resistant coagulase negative Staphylococci (MR-CoNS) 7 and *Staphyloccus aureus* ATC C 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).7

Results and Discussion

From urine culture, 33/518 (6%) were obtained as CoNS isolates. From CoNS isolates, majority was in the age group of 0-14 years 25(76%). Maximum isolates were from females 20(60%) than males 13(40%). The most common species isolated was *S. epidermidis* 18(55%), followed by *S. saprophyticus* 14(42%) and *S. lugdunensis* 1(3%). Methicillin resistance was found 61% of strains. The following antibiotics; Linezolid (100%), Amikacin (79%), Doxycycline (76%), Nitrofurantoin (67%), Gentamicin (61%), Erythromycin and Norfloxacin (each 58%) and Ciprofloxacin (55%) were found to be most effective drugs in our study.

Coagulase Negative Staphylococci form a part of normal flora and CoNS isolated along with another organism, its pathogenic potential may be neglected. Hence it is necessary to speciate CoNS and understand the pathogenic potential of individual CoNS.8 Over the last two decades, especially for the species *S. epidermidis*. *S. saprophyticus* is an important pathogen in human urinary tract infections, especially in young sexually active females

and it is resistant to novobiocin. *S. haemolyticus is* another most frequently encountered CoNS species associated with human infections and has been implicated in septicemia, peritonitis, urinary tract, wound, bone and joint infections.9 *S. saprophyticus* is a well-documented pathogen causing primary acute urinary tract infections. Uroepithelial tissue, tropism and production of urease contributed to bladder tissue invasion.4

The CoNS isolates in our study is 6% and other studies, 32% Nagasugha *et al.*, [9], 28% Sardar *et al.,*[10], 20% according to Golia *et al.,*[8] and 18% Jayanthi *et al.,*[11]. In our study, the common species were *S. epidermidis* and *S. saprophyticus* (55% & 42%) respectively. Similarly in other studies by Nagasudha *et al.*, [9](59% & 22%), Jayanthi *et al.*, [11] (56% & 7%), Golia *et al.*, [8] (46% & 28%) and Sardar *et al.*, [10](43% & 16%) (Table 1–7).

Table.1 Showing Age wise and Sex wise distribution of CoNS isolates

Age groups	**Male**		**Female**		**Total**	
In Years	No.	%	No.	%	No.	%
0-14	10	76	15	75	25	76
15-45	1	8	3	15	4	12
46-60	1	8	1	5	2	6
>60	1	8	1	5	2	6
Total	**13**	**100**	**20**	**100**	**33**	**100**

Table.2 Showing different species of CoNS isolates

Species of CoNS	**No.**	**%**
S. epidermidis	18	55
S. saprophyticus	14	42
S. lugdunensis	1	3
Total	**33**	**100**

Table.3 Showing Novobiocin sensitivity

Novobiocin	**No.**	**%**
Sensitive	19	58
Resistant	14	42
Total	**33**	**100**

Table.4 Showing Novobiocin sensitivity species wise

Species of CoNS	**Novobiocin Sensitive**		**Novobiocin**		**Total**	
	No.	%	No.	%	No.	**%**
S. epidermidis	18	95	0	0	18	**55**
S. saprophyticus	0	0	14	100	14	**42**
S. lugdunensis	1	5	0	0	1	**3**
Total	**19**	**100**	**14**	**100**	**33**	**100**

Table.5 Showing resistance to Methicillin

No. of CoNS isolated	Methicillin Sensitive		Methicillin Resistant	
	No.	%	No.	%
33	**13**	**39**	**20**	**61**

Table.6 Showing Methicillin resistance species wise

Species of CoNS	Methicillin Sensitive		Methicillin Resistant		Total	
	No.	%	No.	%	No.	%
S. epidermidis	8	62	10	50	18	**55**
S. saprophyticus	4	30	10	50	14	**42**
S. lugdunensis	1	8	0	0	1	**3**
Total	**13**	**100**	**20**	**100**	**33**	**100**

Table.7 Showing Antibiogram of CoNS

Antibiotic Discs	Sensitive		Resistant	
	No.	**%**	**No.**	**%**
Amikacin	26	79	7	**21**
Amoxicillin-Clavulanate	12	36	21	**64**
Cotrimoxazole	12	36	21	**64**
Ciprofloxacin	18	55	15	**45**
Doxycycline	25	76	8	**24**
Erythromycin	19	58	14	**42**
Gentamicin	20	61	13	**39**
Linezolid	100	100	0	**0**
Norfloxacin	19	58	14	**42**
Nitrofurantoin	**22**	**67**	**11**	**33**

In our study resistance to methicillin is 61% and similar to other studies by Aher *et al.,* [12] -78%, Nagasuda *et al.,* [9] -66%, Golia *et al.,* [8] -66% and Sardar *et al.,* [10] -52%. The following antibiotics Linezolid (100%), Amikacin (79%), Doxycycline (76%), Nitrofurantoin (67%), Gentamicin (61%), Erythromycin and Norfloxacin (58%) and Ciprofloxacin (55%) were found to be most effective in our study. The antibiogram of our study is similar to other studies by Golia *et al.,* [8], Nagasudha *et al.,* [9], Sardar *et al.,* [10,] Jayanthi *et al.,* [11], Aher *et al.,* [12] and Kavita *et al.,* [13].

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.14

S. epidermidis was the predominant species isolated and more susceptible antibiotics were Linezolid and Amikacin. Therefore suggest that CoNS strain should not discard as contaminants and identified up to species level with their antibiogram to decrease morbidity in urinary cases.

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