

Speciation and antibiogram of Coagulase Negative Staphylococci (CoNS) in a Tertiary Care Hospital

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Abstract

Introduction and Objectives: CoNS which were considered as contaminants with minimal significance, have emerged as important agents of human disease. The recognition of increasing antimicrobial resistance among CoNS isolates constitutes a potentially worrisome emerging characteristic. Hence, this study was done to speciate and determine the resistant pattern of CoNS.

Materials and Methods: 122 clinically significant CoNS isolates were obtained from various clinical samples between August 2016 to July 2017. These samples were processed conventionally.

Results: Of the 122 CoNS species isolated majority were from pus. Identification of CoNS showed *S.epidermidis* 70 (57.4%), as the most frequently isolated species. Antimicrobial susceptibility pattern of the isolates showed maximum resistance to Penicillin. Methicillin resistance was seen among 19 (15.6 %) isolates. But all the isolates showed 100% sensitivity to glycopeptides and Linezolid.

Conclusion: Meticulous identification, speciation and resistant pattern of the isolate is of paramount importance in clinical management of patients.

Keywords: Coagulase negative Staphylococci(CoNS), Contaminant, Anti microbial susceptibility pattern, Methicillin resistance.

Introduction

CoNS which were considered as contaminants with minimal significance, have emerged as important agents of human disease. Among the CoNS species, *S. epidermidis* is the most common organism, amounting from 50 to 80% of isolates causing Surgical site infections, prosthetic device associated infections, peritoneal dialysis related infections, cerebrospinal fluid shunt and ophthalmic infections.¹⁰ *S.saprophyticus* is the second most common cause of urinary tract infections accounting for 11-32% of UTI in female out patient. The recognition of increasing antimicrobial resistance among CoNS isolates constitutes a potentially worrisome emerging characteristic. Hence, this study was done to speciate and determine the resistant pattern of CoNS.

Aims and Objectives

1. To isolate and speciate clinically significant CoNS from various clinical samples.
2. To determine its antimicrobial susceptibility pattern as per CLSI guidelines.

Materials and Methods

122 clinically significant CoNS isolates were obtained from various clinical samples between August 2016 to July 2017. These samples were processed conventionally.

Inclusion Criteria:

Patients of both sex and all age groups.

Patients with infections like boil, folliculitis, cellulitis, abscesses osteomyelitis and implant associated infections.

Patients with pneumonia, sepsis and endocarditis.

Patients with burn wound, diabetic ulcer, non healing ulcer.

Patients with urinary tract infections.

Exclusion criteria:

Patients with gastro intestinal infections, CNS infections.

Identification of Staphylococcus species: Species identification of Staphylococcus will be done by studying the colony morphology on nutrient agar (small, circular, low convex, opaque colonies), Gram reaction of smear prepared from single isolated colony (Gram positive, cocci in cluster), Haemolysis on blood agar (beta hemolysis), Mannitol salt agar, Catalase test, Coagulase test, Modified oxidase test, Methyl red test, Voges proskauer test, Urease test, Oxidative fermentative test, DNase test, Phosphatase test. Speciation of CoNS was done based on Kloos and Schleifer and Koneman Classification.^{9,10} Antimicrobial Susceptibility testing was done based on CLSI guidelines.³

Identification of MRCoNS (Methicillin resistant Coagulase negative Staphylococci):

All isolates of CoNS were tested by using 30 microgram of cefoxitin by Disk Diffusion method for methicillin sensitivity as per CLSI guidelines.

Interpretation

S. lugdunensis: The zone diameter ≥ 22 mm for cefoxitin is considered as methicillin sensitive CoNS and zone diameter ≤ 21 mm is Methicillin resistant CoNS.

CoNS except S.lugdunensis: The Zone diameter, ≥ 25 mm for cefoxitin is considered as methicillin sensitive CoNS. The zone diameter, ≤ 24 mm for cefoxitin is considered as Methicillin resistant CoNS.

Control strains used: MSSA *S.aureus* ATCC 29213. MRSA *S.aureus* ATCC 43300.

Results

A total of 122 CoNS species were isolated from various clinical samples. Maximum number of CoNS were isolated from pus, 97 (79.5 %) followed by urine, 17 (13.71%), blood, 7 (5.65%) and body fluids, 1 (0.81%). Identification of CoNS showed *S.epidermidis* 70 (57.4%), as the most frequently isolated species followed by *S.lugdunensis* 17 (13.71%), *S.saprophyticus* 16 (12.90%), *S.haemolyticus* 13 (10.48%), *S.schleiferi* 4 (3.23%) and *S.xylosus*

2 (1.61%). Age wise distribution of CoNS species was analysed, which showed 6 isolates were from less than 1 year, 23 isolates from 1-15 years of age group, 58 from 16-40 years of age group, 21 from 41-60 years of age group and 14 from, more than 60 years of age group. Antibiotic susceptibility pattern of the isolates showed maximum resistance to Penicillin. Methicillin resistance was seen among 19 (15.6 %) isolates out of 122. The department wise isolation of Methicillin Resistant CoNS was analyzed. It showed 6 were from surgery, 2 from medicine, 5 from ortho, 4 from OG and 2 from paediatric unit. Among the MRCoNS 12(17.1%) were *S. epidermidis*, 4(25%) were *S.saprophyticus*, 2 (15.38%) were *S.haemolyticus*, 1(5.88%) was *S.lugdunensis*. But all the isolates showed 100% sensitivity to Vancomycin and Linezolid.

Discussion

In the present study 122 CoNS were obtained from various clinical samples. The most common source was pus and wound swab, 97 (79.5 %) followed by urine 17 (13.72%) blood, 7 (5.65%) and body fluids, 1 (0.81%). In our study predominant isolates were from pus obtained from wound infection which is similar to studies conducted by R.Goyal et al⁷ Surekha et al.¹⁷ Pavithra DP¹³ and RajyalakshmiGunti.¹⁴ The most common species isolated in our study was *S.epidermidis* which is concordant with Surekha et al , R.Goyal et al, Shubra Singh et al,^{17,7,16} Surekha Y Asangi,¹⁷ et al, have documented in their study from Bellary, India, that the predominant CoNS species isolated were *S.epidermidis* (44.8%), followed by *S.Saprophyticus* (27.1) and *S.haemolyticus* (19.7%) among various clinical samples. In our study *S.saprophyticus* was the common isolate obtained from urine samples, which is similar to the studies conducted by Uma Chaudhary et al, Mohan et al, Kumari et al.^{19,12,11} In our study antibiotic susceptibility pattern showed maximum resistance to penicillin, which correlates with studies by Shubra Singh et al, and Golia S. et al.^{16,6} In our study Methicillin resistance was seen among 19 isolates (15.32%), which is similar to studies conducted by KL Shoba et al (14%),¹⁵ while various other studies by Amita V. Jain et al 60%,² Saroj Golia 66.4%,⁶ and Asanji et al 67.7%¹ showed higher percentage of resistance to methicillin. Methicillin resistance in CoNS was determined to be 67.5% and 52.83% in a study conducted by Yasar F. Koksai²¹ and U. Farooq et al respectively.²⁰ Dhanalakshmi, et al (2012),⁴ have stated that Cefoxitin disc diffusion test can be used as reliable screening methods for MRSA in their study from Karnataka, India. Gandham Pavani (2012)⁵ has concluded in his study from Andra Pradesh, India, that Cefoxitin method is a reliable method and can be used as a sole method for the detection of MRSA. In our study all the isolates were sensitive to Vancomycin in concordance with R Goyal et al.⁷ Sunil B. Bhamare. et al, in a study from Pune India have concluded that one strain out of 55 MRCoNS among various clinical samples was resistant to Vancomycin.¹⁸

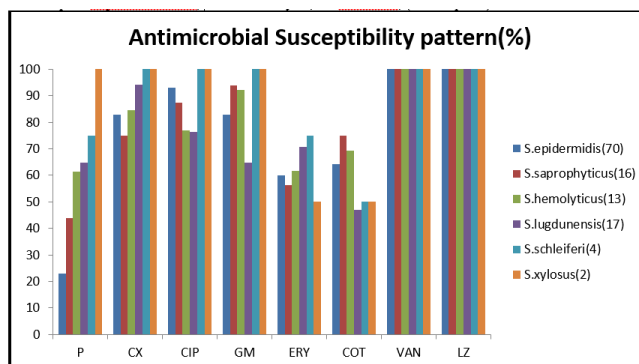
Table 1: Distribution of Clinically Significant CoNS species among various clinical samples

CoNS species	Pus and wound swab	Blood	Urine	Body Fluids	Total no of isolates
<i>S.epidermidis</i>	61	6	2	1	70
<i>S.saprophyticus</i>	1	-	15	-	16
<i>S.hemolyticus</i>	12	1	-	-	13
<i>S.lugdunensis</i>	17	-	-	-	17
<i>S.schleiferi</i>	4	-	-	-	4
<i>S.xylosus</i>	2	-	-	-	2
Total	97	7	17	1	122

Table 2: Antimicrobial Susceptibility pattern of CoNS species isolated

CoNS Species	P	CX	CIP	GM	ERY	COT	VAN	LZ
	S	S	S	S	S	S	S	S
<i>S.epidermidis</i> (70)	16	58	65	58	42	45	70	70
<i>S.saprophyticus</i> (16)	7	12	14	15	9	12	16	16
<i>S.hemolyticus</i> (13)	8	11	10	12	8	9	13	13
<i>S.lugdunensis</i> (17)	11	16	13	11	12	8	17	17
<i>S.schleiferi</i> (4)	3	4	4	4	3	2	4	4
<i>S.xyloso</i> (2)	2	2	2	2	1	1	2	2

Abbreviation: P-Penicillin, CX-Cefoxitin, CIP-Ciprofloxacin, GEN-Gentamicin, ERY-Erythromycin, COT-Trimethoprim/sulphamethaxazole, VAN-Vancomycin, LZ-Linezolid, (S-Susceptible)

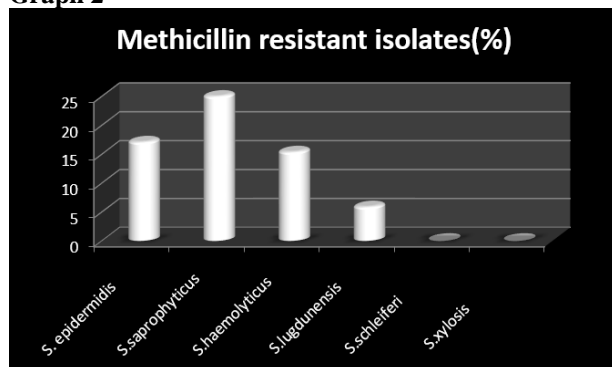
Graph 1**Table 3: Distribution of Methicillin resistance among CoNS species isolated**

CoNS species	Methicillin resistant isolates
<i>S. epidermidis</i> (70)	12 (17.1%)
<i>S.saprophyticus</i> (16)	4 (25%)
<i>S.haemolyticus</i> (13)	2 (15.38%)
<i>S.lugdunensis</i> (17)	1 (5.88%)
<i>S.schleiferi</i> (4)	0
<i>S.xyloso</i> (2)	0
Total: 122	19 (15.6%)

speciation and resistant pattern of the isolate is of paramount importance in clinical management of patients and also helps in epidemiological survey.

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Graph 2

Conclusion

With advancement of science intravascular and prosthetic devices are being used widely and frequently. This has led to increased incidence of nosocomial infections due to CoNS. Meticulous identification,

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