



Speciation of the Coagulase Negative *Staphylococci* Isolated from Various Clinical Samples and their Antimicrobial Resistance Pattern at a Tertiary Care Hospital

Authors

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Abstract

Background and Objectives: Increased frequencies of CoNS infection is being observed in various hospital setups and a need for better understanding of species distribution and antimicrobial susceptibility pattern prevailing among them has arisen. The rising incidence of bacteraemia along with the long term use of indwelling prosthetic devices is of great concern and demands further research. The study is done to speciate the Coagulase negative *Staphylococci* isolated from various clinical samples and to reveal the antimicrobial susceptibility pattern among them.

Method: The present study was conducted in the Department Of Microbiology and Department of Medical Laboratory Technology, Government Medical College Thiruvananthapuram. A total of 52 strains of Coagulase negative *Staphylococci* isolated as a sole agent from various clinical samples were included in the study. The speciation of CoNS was done based on the biochemical reactions.

Results & Conclusion: Out of 52 sample, 61.5% is male population and most of the samples were exudates and 27% of the patients were coming under the working age group. *Staphylococcus epidermidis* is recognized as the predominant species (58% of the total isolates). 19.2% were Methicillin resistant CoNS (MR-CoNS). 44.2% of all isolates shows induced Clindamycin resistance.

Keywords: CoNS, MR CoNS.

Introduction

Coagulase negative staphylococci (CoNS) are ubiquitously present on skin and were considered as laboratory contaminants and non-pathogenic commensals with relatively low virulence till recently. But now, the trend has changed and CoNS have emerged as one of the most important opportunistic pathogen. Since 1950, cases of CoNS associated infections have been reported with increasing frequencies.

These normal skin inhabitants turn out to be pathogenic when they gain entry into body which may result in CSF shunt infection, prosthetic

valve infection, prosthetic joint infection, vascular graft infection etc. The other infections associated with CoNS are eye & ear infection, skin abscess, infections associated with IV cannulae, peritonitis (in patients receiving peritoneal dialysis). One particular species (*Staphylococcus saprophyticus*) is associated with UTI in young sexually active females.

The predisposing factors for infections with CoNS are immunosuppression (cancer, renal transplant cases), breaches in natural mucocutaneous barriers, prior exposure to antibiotics and most frequently the presence of an indwelling device.

Device related infections are mainly due to their ability to adhere onto the polymer surfaces and subsequent Biofilm production. The Biofilm protect them from the antibiotics administered and also from the patient's own immune system.

According to National Nosocomial Infection Surveillance System (NNIS, 2001), there is a widespread occurrence of Methicillin resistant CoNS. Increased frequencies of CoNS infection is being observed in various hospital setups and a need for better understanding of species distribution and antimicrobial susceptibility pattern prevailing among them has arisen. The rising incidence of Bacteraemia along with the long term use of indwelling prosthetic devices is of great concern and demands further research.

Methodology

A cross sectional study was done in the Central Microbiology laboratory, Department of MLT, Government Medical college Hospital, Thiruvananthapuram. A total of 52 strains of Coagulase negative Staphylococci isolated as a sole agent from various clinical samples were included in the study.. The study was approved by Institutional Human Ethics Committee and Institutional Research Committee. The speciation of CoNS was done based on the following test procedures. Tube coagulase test. Slide coagulase test (Clumping factor), PYR Broth hydrolysis test, Novobiocin (5µgm) sensitivity test, Ornithine decarboxylase test, Alkaline Phosphatase test, Polymyxin B (300U) sensitivity test, Voges-Proskauer test, Fermentation of Trehalose, Mannitol

Results

Table 1: Sex Distribution of Positive Isolates

Gender	Frequency	Percentage
Male	32	61.5%
Female	20	38.5%

Table 2: Age Distribution of Positive Isolates

Age (in years)	Frequency	Percentage (%)
10 - 19	4	7.7
20 - 29	6	11.5
30 - 39	14	27
40 - 49	6	11.5
50 - 59	6	11.5
60 - 69	11	21
70 - 79	3	5.7
>80	2	3.7

Graph 1: Age Distribution of Positive Isolates

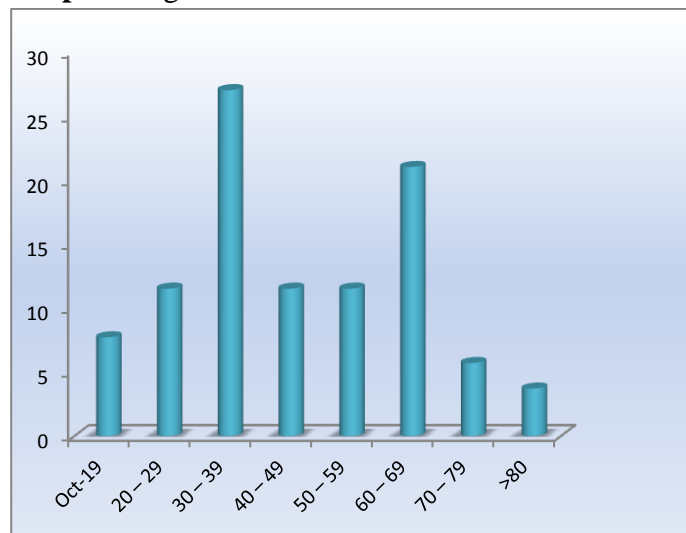
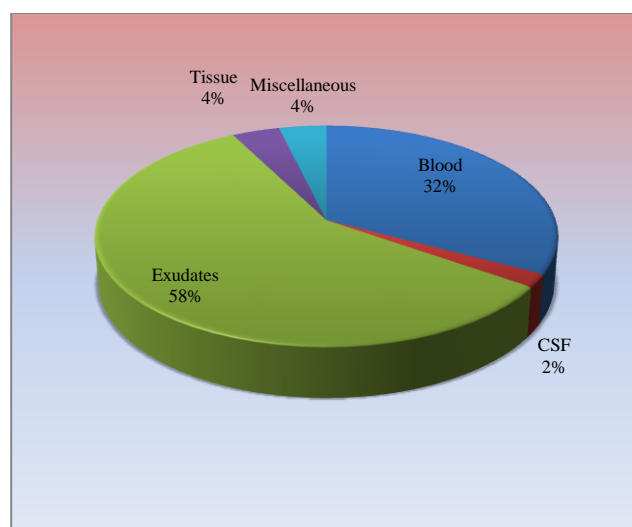


Table 3: Specimen Distribution of Positive Isolates

Specimen	Frequency	Percentage
Blood	17	32.7
CSF	1	1.9
Exudates	30	57.7
Tissue	2	3.8
Miscellaneous	2	3.8



Graph 2 showing specimen distribution

Table 4: Species Distribution of CoNS

Species	Frequency	Percentage %
<i>S. epidermidis</i>	30	58
<i>S. lugdunensis</i>	14	27
<i>S. haemolyticus</i>	8	15

Table 5: *S. Epidermidis* – Specimen Distribution

Specimen	Frequency	Percentage (%)
Blood	11	36.7
CSF	1	3.3
Exudates	17	56.7
Tissue	1	3.3
Miscellaneous	-	0

Table 6: *S. lugdunensis* – Specimen Distribution

Specimen	Frequency	Percentage (%)
Blood	7	50
CSF	-	0
Exudates	5	35.8
Tissue	1	7.1
Miscellaneous	1	7.1

Antibiotic Susceptibility Pattern

Antibiotic susceptibility testing was performed by disc diffusion method as per the Clinical Laboratory Standard Institute (CLSI) recommendations. Among the beta lactams, maximum resistance was observed against Penicillin (98.08%; n = 51) and resistance to Cefoxitin was seen in 19.2 % (n = 10) of the isolates. All the studied isolates were susceptible to Vancomycin.

Table 7: Antibiotic Susceptibility pattern of Isolates

Antibiotic	Sensitive		Resistant	
	Frequency	Percentage	Frequency	Percentage
Penicillin	1	1.92	51	98.08
Gentamicin	26	50	26	50
Cephalosporin 1 st Generation	27	52	25	48
Erythromycin	23	44.2	29	55.7
Amikacin	47	90.4	5	9.6
Vancomycin	52	100	0	0
Cefoxitin	42	80.8	10	19.2
Clindamycin	43	82.6	9	17.3

Table 8: Antibiotic susceptibility pattern of *S. epidermidis*

Antibiotic	Sensitive		Resistant	
	Frequency	Percentage	Frequency	Percentage
Penicillin	1	3.3	29	96.6
Gentamicin	17	57	13	43
Cephalosporin 1 st generation	18	60	12	40
Erythromycin	13	43	17	57
Amikacin	28	93.4	2	6.6
Vancomycin	30	100	0	0
Cefoxitin	24	80	6	20
Clindamycin	25	83.4	5	16.6

Graph 3: Antibiotic susceptibility pattern of *S. epidermidis*

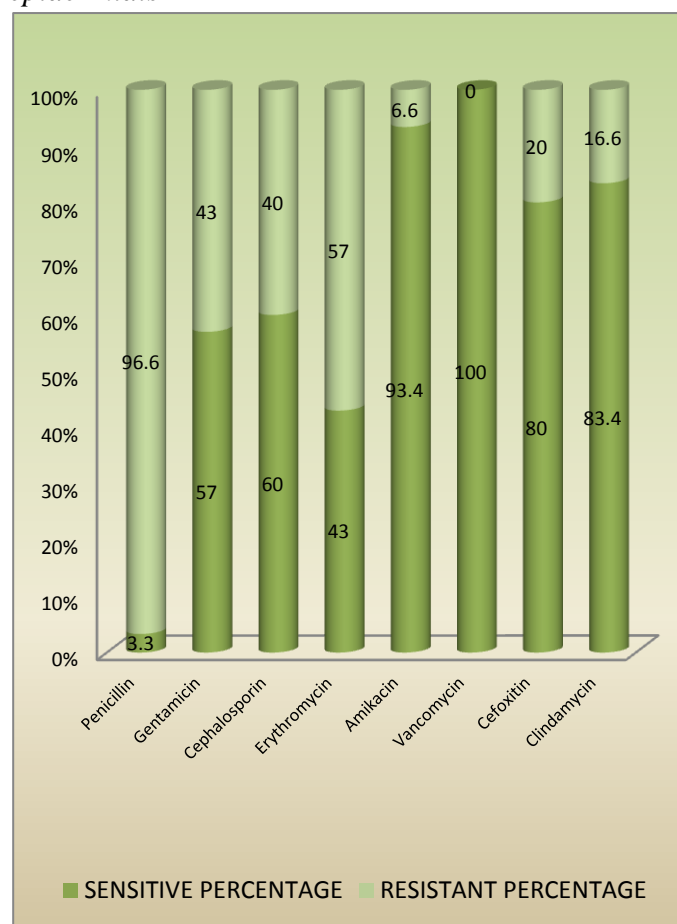


Table 9: Methicillin Resistance in CoNS

Species	<i>S.epidermidis</i>	<i>S. lugdunensis</i>	<i>S. haemolyticus</i>
Frequency of Isolate	30	14	8
Frequency of MR Cons	6	3	1
Percentage	20	21	12.5

Table 10: Macrolide Resistance in CoNS

	<i>S.epidermidis</i>		<i>S.lugdunensis</i>		<i>S. haemolyticus</i>	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
iMLS _B PHENOTYPE(23)	14	61%	4	18%	5	21%
cMLS _B PHENOTYPE(6)	3	50%	3	50%	-	0%

Discussion

Among the 52 isolates of CoNS, most of them were from exudates followed by blood, tissue, catheter tips and CSF (57.7%, 32.7%, 3.8%, 3.8%, 1.9% respectively). Majority of the patients were male and coming under the working age group of 30 – 39.

Staphylococcus epidermidis was recognised as the predominant species (58% of the total isolates). Goyal R *et al.*, Sheik *et al.*, have documented similar findings with 41% and 46% of isolation rates respectively^(1,2). Senger *et al* documented a large proportion of *Staphylococcus epidermidis* (68% of the total CoNS isolates) in their study⁽³⁾. The second most common isolate in our study was *S lugdunensis* accounting for 27% of the total isolates. The third most common organism isolated was *S haemolyticus* (15%).

In the present study, majority of the isolates are resistant to Penicillin (98%) followed by Erythromycin (55%), Gentamicin (50%) Cephalosporin (48%), Cefoxitin (19.2%) and Clindamycin (17%), Amikacin (9.6 %) Vancomycin(100%) Methicillin resistance(MR CoNS) (19.2%) was shown by 10 isolates and all are sensitive to Vancomycin.

Among the macrolide resistant *Staphylococcus epidermidis* strains, the cMLS_B phenotype and iMLS_B phenotype were demonstrated in 10% and 47% of the isolates respectively. Among *S haemolyticus* isolates, none of them reported as cMLS_B phenotype, where as iMLS_B phenotype was appeared in 62.5% of the isolates. Similarly

Gheradi *et al* reported MS_B phenotype in 38% of isolates of *S epidermidis* and 80% of isolates of *S haemolyticus* and cMLS_B phenotype was observed only in 28.5% of isolates of *S epidermidis*. Similarly, Brzychczy-Wloch M *et al.*, who researched the CoNS isolated from invasive infection in very low birth neonates, reported a higher percentage of MS_B phenotype among *S haemolyticus* strains (82%). The study documented 43% and 40% of cMLS_B and MS_B phenotypes among *S epidermidis* respectively⁽⁴⁾. Consistent with the previous reports, Methicillin resistance and multidrug resistance were found to be high among the studied isolates. 19.2% of the studied isolates showed Methicillin resistance which is in accordance with the previous reports from different countries including India.

The methicillin resistant isolates were found to be multi drug resistant. 20% and, 21% of methicillin resistance was observed in the isolates of *S epidermidis* and *S. lugdunensis* respectively. In this study, methicillin resistance was predominantly observed in *S epidermidis* followed by *S lugdunensis*.

Higher rates of Methicillin resistance have been reported from different countries in a study by Koksai *et al.*,⁽⁵⁾ Khan *et al.*,⁽⁶⁾ and Shan M U⁽⁷⁾ *et al.*, with 67.5%, 93%, and 40% resistance rates respectively.. The present study shows 100% sensitivity to Vancomycin which is similar to studies done by Khadri⁽⁸⁾, Rajadurai pandi⁽⁹⁾, Anuprabha⁽¹⁰⁾.

S. epidermidis was isolated from CSF sample of a 32 year old male patient in Neurosurgery ICU, with post hypothalamic glioma excision. The CSF sample was taken from peritoneal ventricular shunt. The patient was afebrile with and total WBC count was 12000. This isolate was only sensitive to Vancomycin and Clindamycin. Bio-film production was positive for this isolate by tube method. *S. lugdunensis* was isolated from an infected implant, which was removed by surgery and was negative for bio-film production. It was sensitive for all drugs except Penicillin, Erythromycin, Clindamycin. *S. epidermidis* was isolated from a case of carcinoma of breast with multiple ulcers in stomach wall and it was resistant to Penicillin and Gentamicin.

The first reports of Vancomycin resistance and Linezolid resistance was documented among CoNS. which suggests that there is an alarming threat of the transmission of the resistance to other virulent pathogens such as *S aureus*.

CoNS have been considered as normal skin inhabitants with relatively low virulence. But they turn out to be pathogenic once entered into the body and are able to cause a variety of infections in humans. Moreover, by acting as a reservoir of resistant genes, they promote the development of antibiotic resistance in other virulent pathogens. The hospital strains are mostly multidrug resistant and thus limits the therapeutic options in the treatment of CoNS related infections. For these reasons, CoNS demand more recognition as pathogens and must be addressed earnestly. Increased frequencies of CoNS infection is being observed in various hospital setups and a need for differentiating the pathogenic strains of CoNS from the non-pathogenic one has arisen.

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