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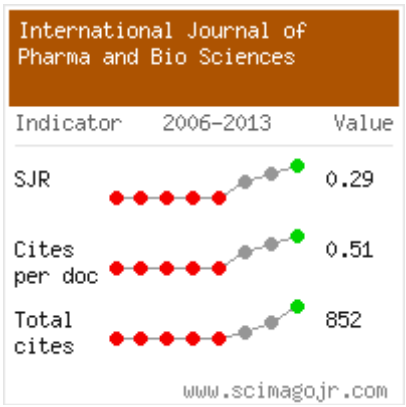

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COAGULASE NEGATIVE STAPHYLOCOCCI AMONG CLINICAL ISOLATES IN A TERTIARY CARE CENTRE

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ABSTRACT

Coagulase negative staphylococci are increasingly being reported as etiological agents in a nosocomial setup. Speciation of CoNS is not routinely done because of the practical difficulties as batteries of tests are needed to identify. Drug resistance is a very common observance among CoNS. Above all formation of biofilm makes these organisms more rigid towards the treatment. In the present study 100 CoNS isolated from various samples were speciated phenotypically, their resistance patterns were observed and the potential for the formation of biofilm in individual species is noted. The predominant species isolated in our study was S.epidermidis(43%). Methicillin resistance was noted in 52% of the total isolates. Biofilm production was detected in 40% of the total isolates.

KEY WORDS: CoNS, MRCoNS, Biofilms, Drug resistance

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INTRODUCTION

Coagulase negative staphylococci, previously considered as saprophytes with little pathogenic potential , are increasingly been reported as an opportunistic pathogens that cause infection in debilitated or compromised patients such as premature neonates, cancer patients, burn patients, end stage renal disease, renal transplantation cases and oncology patients, often by colonizing biomedical devices such as prosthesis, implants and intravascular lines.[1,2] Numerous species of CoNS have been recognized as pathogens. *S.epidermidis* is the CoNS species most frequently isolated from infections. *S.epidermidis* has been implicated as the etiological agent in infections of wound, urogenital tract, respiratory tract, meninges, conjunctiva and skin.[3,4] *S.saprophyticus* is second to coliforms as the most common cause of the acute urethral syndrome and UTI in women.[5,6] Although *S.epidermidis* accounts for most CoNS infections, many other species have been identified in association with human infection. CoNS species identification, which is still difficult for most clinical laboratories, is necessary in order to establish epidemiological trends, confirm treatment failures, or determine the cause of specific infections Multiple antibiotic resistance is a common finding among clinical CoNS isolates indicating its potential pathogenecity.[7] Resistance of these organisms to wide range of antimicrobial agents is well documented.[8] Methicillin resistance among CoNS is particularly important due to cross-resistance to all other β-lactam agents and agents of other anti-microbial classes like macrolides and fluoroquinilones.[9] Susceptibility testing should be done on any isolate considered to be a cause of infection because of the resistance of these organisms to a wide spectrum of antimicrobial agents.[10] A working knowledge of the biology and antimicrobial susceptibility of these organisms may be necessary to distinguish infecting from contaminating isolates and to device appropriate therapy[11] The purpose of the present study is to provide a current scenario, species distribution in clinical specimens determining their antimicrobial susceptibility pattern, mainly to screen for methicillin resistance, multi-drug resistant strains and biofilm formation.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, SVS Medical College and Hospitals, Mahabubnagar for a period of one year. A total 1530 samples were processed during the study to isolate 100 strains of CoNS in culture, grown either in pure culture or predominant form and are associated with symptomatic infection, which are considered clinically significant. Identification and speciation of CoNS was done by various tests like Catalase , Oxidase , Coagulase, Urease, Phosphatase ,Oxidation – Fermentation, Nitrate reduction , Ornithine decarboxylase and Carbohydrate fermentation for the sugars Glucose, Sucrose, Lactose, Mannitol, Maltose, Mannose, Trehalose and Xylose. The strains were identified according to the dichotomous key in Elmer Koneman et al. and in accordance with the International Code of Nomenclature of Bacteria.[12] Antibiotic Susceptibility Testing were done on Mueller-Hinton agar according to CLSI procedures. *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228 strains were used as control strains. Methicillin resistance was determined by Cefoxitin 30 µg disc. Plates were incubated at 370C for 24 hours with breakpoints of ≤24 mm and ≥25 mm as resistant and sensitive respectively (CLSI).[13] The following antibiotics were tested Ampicillin(10µg),Amoxyclav (20/10µg),Erythromycin(15µg), Ciprofloxacin (5µg), Gentamicin (10µg), Cephoxitin (30µg),Co-Trimoxazole (25µg),Linezolid (30µg), Novobiocin(5µg). For Vancomycin MIC testing was done to determine the susceptibility by E-test method. Biofilm production was detected in the Congo red agar medium. Congo red medium plates were inoculated and incubated aerobically at 37ºC for 24hrs. Developments of black colonies with dry crystalline consistency

were taken as positive. Non slime producers were pink in colour.

RESULTS

A total of 100 Coagulase Negative Staphylococci were isolated in different samples during the study period and included for further processing. Among the total CoNS isolates, majority were obtained from exudates constituting 37% of total isolates, followed by blood 35% and urine 28%. Majority of isolates were in age group of 31-50yrs (40%) maximum isolates were from males (55%) than females (45%). Nine different species of CoNS were isolated and identified. *S.epidermidis* was the predominantly isolated species (43%) followed by *S.saprophyticus* (16%), *S.haemolyticus* (13%) and *S.hominis*(12%) These four species together constitute more than 2/3 of the total isolates. Majority of the *S.epidermidis* species were recovered from blood and exudates constituting 44% and 42% respectively.Whereas *S.saprophyticus* was predominantly isolated from urine (75%). *S.haemolyticus* was predominately isolated from blood culture specimens indicating its potential bacteraemia. (61.5%).

DISTRIBUTION OF CoNS SPECIES IN VARIOUS CLINICAL SPECIMENS

Species	Urine	Exudates	Blood	Total
S.epidermidis	6 (14%)	18 (42%)	19 (44%)	43
S.saprophyticus	12 (75%)	2(12.5%)	2(12.5%)	16
S.haemolyticus	2 (15%)	3(23%)	8(61.5%)	13
S.hominis	5(41.6%)	4(33.3%)	3(25%)	12
S.capitis	1 (20%)	2(40%)	2(40%)	5
S.cohinii	1 (25%)	3(75%)	0	4
S.warneri	0	3(100%)	0	3
S.lugdunensis	1 (33.3%)	2(66.6%)	0	3
S.xylosus	0	0	1(100%)	1

Resistance pattern of various CoNS species to different antibiotics

S. no	Name	Amp	Amc	E	Cip	Gen	Cot	Cx	Va	Lz
1	*S. epidermidis*	100%	60%	46.5 %	49%	42%	70%	56%	0%	0 %
2	*S.saprophyticus*	100%	75%	62.5 %	68.75%	56.25 %	87.5 %	50%	0%	0 %
3	*S. heamolyticus*	100%	77%	92%	61.5%	77%	85%	69%	0%	0 %
4	*S.hominis*	100%	42%	42%	33%	67%	50%	42%	0%	0 %
5	*S. capitis*	100%	40%	20%	20%	40%	20%	40%	0%	0 %
6	*S.cohinii*	100%	75%	50%	50%	50%	50%	50%	0%	0 %
7	*S. warneri*	100%	33%	33%	66%	33%	33%	33%	0%	0 %
8	*S.lugdunensis*	100%	100 %	67%	67%	67%	100 %	33%	0%	0 %
9	*S. xylosus*	100%	0%	0%	0%	100 %	100 %	0	0%	0 %

SUSCEPTIBILITY PATTERN OF CoNS SPECIES TO METHICILLIN (Cefoxitin)

Species	MRCoNS	Percentage
S.epidermidis	24	56
S.saprophyticus	8	50
S.haemolyticus	9	69
S.hominis	5	42
S.capitis	2	40
S.cohinii	2	50
S.warneri	1	33
S.lugdunensis	1	33
S.xylosus	0	0

Methicillin resistance was noted in all most all species. Resistance was noted highest among S. haemolyticus (69%) followed by S.epidermidis (56%), S.saprophyticus (50%). All the species were sensitive to linezolid and vancomycin(MIC range from 0.19 -1.5mcg/ml)

Biofilm production in CoNS

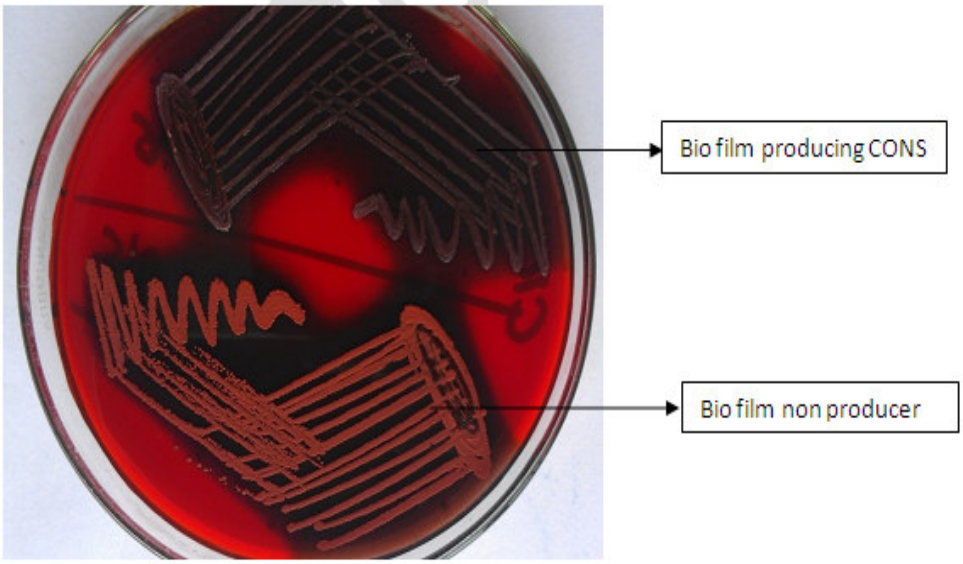
Out of 100 CoNS isolated 40% CoNS were biofilm producers and 60% were non biofilm producers. Of 40 Biofilm producers 37 were Methicillin resistance. Three isolates were MSCoNS in which two isolates were from *S.epidermidis* and one from *S.saprophyticus*.

Biofilm productions in each species of CoNS

S.No	Species	Total Isolates	Biofilm producers	Percentage
1	*S. epidermidis*	43	22	51
2	*S.saprophyticus*	16	6	37.5
3	*S. heamolyticus*	13	5	38.4
4	*S.hominis*	12	2	16.6
5	*S. capitis*	5	1	20
6	*S.cohinii*	4	2	50
7	*S. warneri*	3	1	33.3
8	*S.lugdunensis*	3	1	33.3
9	*S. xylosus*	1	0	0

The most common species producing biofilm was S.epidermidis 22 (51%), followed by S.haemolyticus 5 (38.4%) and S.saprophyticus 6 (37.5%).

Biofilm production on Congo red agar



Novobiocin resistance(*S.saprophyticus*)

Vancomycin E strip

DISCUSSION

In the present study, 100 CoNS isolates were obtained from various clinical specimens. The most common source of CoNS in our study was exudates (37%) followed by blood [35%], and urinary isolate [28%] , similar pattern of distribution was observed in study conducted by R Goyal et al[14] and Surekha et al[15] where CoNS were pre-dominantly isolated from wound infection contributing 38% and 33% respectively. In other studies conducted by Shubra Singh et al[16] and Uma Choudhary et al[17], CoNS were most commonly isolated from blood samples accounting for 60% and 45% respectively. In our study, the most common species isolated was *S.epidermidis* (43%). Studies done by Surekha et al[15], R Goyal et al[14] have reported similar findings with 43% and 41% isolation rate of this organism respectively which was the pre dominant organisms. The second most common isolate in our study was *S. saprophyticus* accounting for 16% of the total isolates, this correlates well with the studies conducted by Mohan et al (15.6%) [19] and R Goel et al (16.6%) [14], Shubra Singh et al (14%) [16] where this is the second most common organism isolated after *S. epidermidis*. In our study *S. saprophyticus* was predominantly isolated from urine samples accounting for 75%, which is similar to the studies conducted by Uma Choudhary et al[17] and Mohan et al[19] where *S. saprophyticus* is isolated mostly from urine specimens contributing 100% and 78% respectively. Third most common organism isolated is *S. haemolyticus* 13%, which is similar to the studies conducted by R Goyal et al[14], Surekha et[15], Shubra Singh et al[16], Shashidhar et al [20] with 14.7%, 19.7%, 12% and 18% respectively. Out of 13 isolates of *S. haemolyticus*, 8 were isolated from blood. Other species *S.hominis* and *S.capitis* also contributed significantly with the isolation rate of 12% and 5% respectively which is similar to the studies by R Goyal et al[14], Shrikande et al[18], Shubra Singh et al[16] where the isolation of these species was 14.7% and 1.9%, 8.7% and 2%, 6%and 4% respectively The remaining species like *S.cohinii*, *S.warneri*, *S.lugdunensis*, *S.xylosus* have a minor contribution together accounting to about 10% of the total isolates. In the present study maximum resistance was observed towards Ampicillin(100%), Erythromycin(63%), Amoxyclav(62%), Co–Trimoxazole(59%), Gentamicin(53%), Cefoxitin (52%), Ciprofloxacin(51%) .All isolates were sensitive to Linezolid and Vancomycin. Shashidhar et al 2012[20] has highlighted, that half of the strains showed resistance to methicillin, which is similar to our finding (52%). Methicillin resistant has been reported with wide variations in other studies. Amita V Jain et al [21] observed 60% where as KL Shoba et al[22] observed 14% resistant to Methicillin. In our study resistant to Erythromycin was 53% which is comprable to the study conducted by Shubra singh et al where resistant to Erythromycin was 54%. In fluoroquinolones, Ciprofloxacin showed resistance of 51%. Similar resistant pattern for Ciprofloxacin were observed in studies conducted by Shubra singh et al[16] 54% and Manikanda et al 40% [23]. In our study highest resistant was shown by *S.haemolyticus* with about 69% being resistant to Cefoxitin (MR CoNS). Similar resistant pattern was observed by Shubra Singh et al[16], A Choudhury et al[24], Surekha et al[15] where Methicillin resistance in *S.haemolyticus* was 76%, 68%, 90% respectively. Multi drug resistance was also commonly observed in this species. Majority of the isolates were resistant to Erythromycin 92%, Aminoglycosides-Gentamicin 72% and Fluoroquinolones-Ciprofloxacin 67.5%.

In our study, *S.saprophyticus* being the most common urinary pathogen showed resistant to majority of the antibiotics used for treating UTI which include Co-trimoxazole 87.5%, Ciprofloxacin 69% and Amikacin 50% rendering all these antibiotics ineffective. S.epidermidis, the most common isolate in our study showed significant percentage of resistant to Cefoxitin 56%. Study conducted by Surekha et al[15] and Kumari et[25] al also showed similar findings with a Methicillin resistance of 66% and 65% respectively. In the present study, out of 100 CoNS isolated 40 strains were Biofilm

producers. Out of 40 biofilm positive strains *S.epidermidis* was the most common CoNS species producing Biofilm (55%) followed by *S.saprophyticus* 6 (15%), *S.haemolyticus* 5 (12.5%).This finding correlate with other workers such as Seetha KS[2] et al and Mohan U et al.[19] The factor determining the pathogenicity of CoNS has now been well defined and found to be extracellular slime which is important in the colonization of foreign body. [26] Slime production by *S.epidermidis* strains is associated with symptomatic infections.[27] A significant association between the ability of the isolate to produce slime and its propensity to cause a disease has been found in other studies also.[28] Biofilm production has also been shown to produced predominantly by Methicillin resistance strains.[29] The test for slime production may have an important application in deciding the pathogenicity of the strains of CoNS and should be done routinely in a diagnostic laboratory.[2]

CONCLUSION

To conclude, the clinical significance of coagulase negative staphylococci is increasing day by day with the majority of the species causing potential infections especially as nosocomial pathogens. Proper identification of the species along with the surveillance for the antibiotic susceptibility pattern is very important in the proper management of the patients and are also useful for the epidemiological purpose.

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