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SPECIATION OF COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM CLINICAL SAMPLES WITH SPECIAL REFERENCE TO THEIR ANTIBIOGRAM

DR. J. NAGASUDHA RANI[1], DR. S. USHA VIDYA RANI [2,],DR. N. SUNEETHA [3] DR. T. KASTURI[4], DR. B. V. RAMANA,[5] DR. B. KAILASANADHA REDDY.[6]

1. Assistant professor Department of Microbiology, Sri Venkateswara Medical College, Tirupati (AP) India.
2. Assistant professor Department of Microbiology, Sri Venkateswara Medical College, Tirupati (AP) India
3. Assistant professor Department of Microbiology, Sri Venkateswara Medical College, Tirupati (AP) India.
4. Professor Department Microbiology, Sri Venkateswara Medical College, Tirupati (AP) India.
5. Associate Professor, Department of Microbiology, Sri Venkateswara Institute of Medical Sciences, Tirupati.
6. Professor Department of Microbiology, Sri Venkateswara Medical College, Tirupati (AP) India.

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Abstract: Most of CoNS infections were nosocomial and their antibiogram pattern showed multi-drug resistance along with methicillin. Objectives: To find out the species of Coagulase-negative Staphylococci and study its current susceptibility pattern to antibiotics in a tertiary care teaching hospital, S. V. R. R. G. G. Hospital and Govt. Maternity Hospital, Tirupati. Materials and Methods: 124 strains of CoNS isolated from various clinical samples were speciated and studied. The antimicrobial susceptibility test was performed for the isolates. Results: S. epidermidis was the predominant species (58.9%) among the different species of CoNS isolated, followed by S. saprophytic us (21.8%) and S. haemolyticus (15.3%).From pus and wound swabs (36/42), S. epidermidis was the predominant species. In urine samples, S. saprophytic us were the predominant species (26/40). From blood samples S. epidermidis and S. haemolyticus was the major species. Slime production was seen in 54.8% of the strains. High percentage of strains was resistant to Penicillin (93.5%) and Co-trimoxazole (92.3%). Low percentage of resistance was noticed to Aminoglycoside group of drugs i.e. Amikacin (31.5%) and 3rd generation Cephalosporin i.e. ceftriaxone (33.1%). Among 124 CoNS isolated 66.1% strains were Methicillin resistant and 33.9% strains were Methicillin sensitive. All strains were sensitive to Vancomycin. Conclusion: Regular surveillance of hospital associated infections and monitoring of antibiotic susceptibility patterns is required to reduce prevalence of Methicillin resistance among coagulase negative staphylococci.

Keywords: clinical isolates, coagulase negative staphylococci, identification, antibiotic susceptibility.

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INTRODUCTION

The coagulase negative Staphylococcus (CoNS) species as a group constituted a major component of the normal microbial flora of humans.[11] In the past, CoNA were generally considered to be contaminants having little significance.[5] Now the role of CoNS species in causing nosocomial infections has been recognized and well documented over the last two decades, especially for the species S. epidermidis.[11]The infection rate has been correlated with the increase in the use of prosthetic and indwelling devices and the growing number of immune-compromised patients in the hospitals.[10] CoNS are major cause of foreign body infections, by adhesion of bacteria to biomaterials.[11]

The need exists for accurate identifications of CoNS, so that precise delineation of the clinical disease produced by this group of bacteria and their determination of the etiologic agent can be done.[11] *S.epidermidis* has been documented as a pathogen, in numerous cases of bacteraemia, native and prosthetic valve endocarditis, in surgical wounds, in urinary tract infections, cerebrospinal fluid, prosthetic joint, peritoneal dialysis related infections, ophthalmologic and intravascular catheter related infections.[5,11]

S. saprophyticus is an important pathogen in human urinary tract infections, especially in young sexually active females and it is resistant to Novobiocin.[11] *S. haemolyticus* is another most frequently encountered CoNS species associated with human infections and has been implicated in native valve endocdarditis, septicaemia, peritonitis, urinary tract, wound, bone and joint infections.[11] Other CoNS species have also been implicated in a variety of infections – *S. capitis* has been implicated in endocarditis, septicaemia, cathter infections; *S. warneri* in endocarditis and osteomyelitis; *S. simulans* in septicaemia, osteomyelitis; *S. cohnii* in native valve endocdarditis and pneumonia; *S. xylosus* and *S. hominis* have been implicated in urinary tract infections.[11] Most of CoNS infections were nosocomial and their antibiogram pattern showed multidrug resistance along with methicillin. A high incidence of resistance to methicillin is seen in S. epidermidis that is Methicillin Resistance *Staphylococcus epidermidis* (MRSE).Methicillin resistance has now been detected in several other staphylococcal species including *S.haemolyticus, S.hominis, S.capitis, S.warneri, S.caprae, S.simulans, S.saprophyticus, S.xylosus, S.sciuri.*[2]

Thus CoNS will continue to be an infective agent in the future and studies on CoNS will be helpful in formulating and adopting specific antibiotic policies for treating CoNS infections and to restrict further emergence of drug resistant strains, in future.

In the light of the above, the present study has been undertaken.

MATERIAL AND METHODS:

124 strains of coagulase negative staphylococci (CoNS) isolated from various clinical samples namely pus and wound swabs from stitch abscesses, infected compound fractures, burns etc. Urine from urinary tract infections, catheterized patients and young sexually active women; blood from septicaemia, PUO and endocarditis patients; and swabs from ear, conjunctiva , and cervix; and urinary catheter, I. V. cannula tips at S. V. R. R. G. G. Hospital and Govt. Maternity Hospital, Tirupati, during the period from May 2013 to May 2014.

CoNS isolated in pure and predominant growth in mixed cultures by repeatedly collected samples were taken for the study. They are isolated and characterized by standard methods, by doing Gram's Staining cultured on Blood Agar and Nutrient Agar. The strains isolated were first identified by colony morphology, Gram staining, catalase test, slide coagulase and tube coagulase test. All the CoNS strains speciated based on various biochemical tests.

All the CoNS strains were kept for antibiogram by Kirby-Bauer disc diffusion method as per national committee of clinical laboratory standards.[10] Various antibiotics tested were Penicillin, Erythromycin, Amikacin, Cephalexin, Ceftriaxone, Ciprofloxacin, Amoxy-clav, Co-trimoxazole, Oxacillin and Vancomycin. Nitrofurantoin is used for urinary samples.

RESULTS:

124 strains of CoNS isolated from various clinical samples were studied. All these strains were isolated and identified and were subjected to antimicrobial susceptibility tests. The more number of CoNS was isolated from the pus and wound swabs (33.9%) followed by urine (32.3%) and blood (21.8%). CoNS was isolated more among femle patients (61.3%) than male patients (38.7%). Incidence of CoNS was high in 21-30 years age group (42.7%) than other age groups.

Table 1: Specimen-wise isolation of CoNS

S. No.	Specimen	No. of CoNS	Percentage (%)
1	Pus and Wound swabs	42	33.9%
2	Urine	40	32.3
3	Blood	27	21.8
4	IV Cannula & Urinary Catheter tips	8	6.5
5	Cervical swabs	3	2.4
6	Conjunctival swabs	3	2.4
7	Aural swabs	1	0.8
	Total Number	124	100%

TABLE 2 Species incidence of CoNS

S. No.	Name of species	Total	Percentage (%)
1	S. epidermidis	73	58.9
2	S. saprophyticus	27	21.8
3	S. haemolyticus	19	15.3
4	S. hominis	2	1.6
5	S. xylosus	2	1.6
6	S. warneri	1	0.8
	Total	124	100

S. epidermidis was the predominant species (58.9%) among the different species of CoNS isolated followed by *S. saprophyticus* (21.8%) and *S. haemolyticus* (15.3%) *S. hominis* and *S. xylosus* were isolated in 2 cases each and *S. warneri* was isolated from one specimen. S. epidermdis was the predominant species isolated from pus & wound swabs (36/42). S. saprophyticus was the predominant species isolated from urine samples (26/40).

From blood culture *S. epidermidis* and *S. haemolyticus* were the major species. 96 of 124 strains (77.4%) were sensitive to Novobiocin (5µg) and the rest 28 strains (22.6%) were resistant. 52 strains (41.9%) producded haemolysis. 59(47.6%) strains are phosphatase positive, 57 (78%) were *S. epidermidis* and 2 were *S. xylosus*. 68 strains (54.8%) produced slime. Slime production was more among the strains of *S. epidermidis*. Out of 73, 55(75.3%) strains are slime positive followed by *S. haemolyticus*. Out of 19, 11(57.9%) and only 2 strains of *S. saprophyticus* are slimepositive. Out of 27 only 2(7.4%) produced slime. Other strains had not produced slime.

TABLE 3 Antibiotic susceptibility pattern of CoNS

S. No.	Antibiotics	Disc conc.	Sensitivity (%)	Resistance (%)
1	Penicillin	10 units	8 (6.5)	116 (93.5)
2	Erythromycin	15 µg	58 (46.8)	66 (53.2)
3	Amikacin	30 µg	85 (68.5)	39 (31.5)
4	Ciprofloxacin	5 µg	71 (57.2)	53 (47.9)
5	Cephalexin	30 µg	41 (25)	93 (75)
6	Ceftriaxone	30 µg	83 (66.9)	41 (33.1)
7	Amoxy-clav	30 µg	61 (49.2)	63 (50.8)
8	Co-otrimxaole	25 µg	9 (7.3)	115 (92.7)

9	Oxacillin	1 µg	42 (33.9)	82 (66.1)
10	Vancomycin	30 µg	124 (100)	0
11	*Nitrofurantoin	300 µg	27 (67.5)	13 (32.5)

***Used only for 40 urine isolates.

All strains were sensitive to Vancomycin. Most of the strains were resistant to Penicillin and Co-trimoxazole. Majority of the strains were sensitive to Amikacin (68.5%) and Ceftriaxone (66.9%) In case of Amoxy-clav 61 strains were found to be sensitive and 63 strains were found to be resistant 82 (66.1%) are resistance to methicillin. Almost equal percentage of resistance to methicillin was seen in S. epidermidis (78%) and S. haemolyticus (78.9%) species. Least resistance was seen in S. saprophyticus (33.3%) species.

S. epidermidis was resistant to Penicillin (93%), Co-trimoxazole (90.4%), Oxacillin (78.1%), Cephalexin (73.9%) and Erythromycin (58.9%). S. haemolyticus was resistant to Penicillin (100%), Co-trimoxazole (94.7%), Cephalexin (89.5%), Erythromycin (78.9%), Oxacillin (78.9%) and Ciprofloxacin (58.4%). S. saprophyticus shows least resistance among all species. Maximum number that is 95 out of 124 CoNS strains showed resistance to 3 drugs or more than 3 (76.6%). 76(61.3%) out of 124 strains are resistance to 5 drugs or more than 5.

DISCUSSION:

Most of CoNS infections were nosocomial and their antibiogram pattern showed multidrug resistance along with methicillin. CoNS species in causing nosocomial infections Attention has now been focused on them because of their apparently changing status from non-pathogens to opportunistic pathogens.[2,11.]

In this study 124 CoNS were isolated from various clinical specimens and analysed results were discussed below in detail and correlating with others' study. In the present study majority of strains isolated were from pus and wound swabs (33.9%), followed by urine (32.3%), blood (21.8%). The remaining were from IV cannula and urinary catheter tips (6.5%), cervical swabs (2.4%), conjunctival swabs (2.4%) and aural swabs (0.8%). shows S. epidermidis (58.9%) was the predominant species isolated from all the specimens followed by S. saprophyticus (21.8%) mainly isolated from urine samples and S. haemolyticus (15.3%) from blood samples. S. hominis and S. xylosus were isolated in 2 cases each and S. warneri was isolated from one specimen.

U. Mohan and N. Jindal *et al* at Amritsar in 2001[9] isolated 192 CoNS from urine (48.4%), pus (17.7%), drain tips/cather tips/I. V. cannulas (14.5%), blood (4.7%), skin and conjunctival swabs (2.1%). In their study predominant species were S. epidermidis (82.3%). S. saprophyticus was

the second important species (15.6%) isolated mostly from urine specimens. Only two other species of CoNS were identified as S. cohnii (1) and S. haemolyticus.[59]

In this study specimen collection and species isolation were in close relation with our study studies. Surekha. Y. Asangi (2011) [21] *S.epidermidis* (43,44.8%), *S.saprophyticus* (26,27.1%), *S.haemolyticus* (19,19.7 %), *S.lugdunensis* (2,2.1%), *S.warneri* (2,2.1%), *S.cohnii* (1,1%), and others(3,3.1%). Species isolation was in close relation with above studies.

Rosana B. R. Frraira et al in Brazil in 2003[20]studied 152 CoNS isolated from blood (47.4%), nostrils (15.8%), surgical wounds (10.5%), urine (7.9%), catheter tips (2.5%) and other sites (15.8%). *S. epidermidis* (51.3%), *S. haemolyticus* (23%), *S.hominis* (5.9%) and S. *saprophyticus* (5.2%).[75] MG Usha, DC Shwetha(2013) et al[12]studied 102 CONS isolates, 54 were from blood samples, 32 from pus samples, 2 from throat swabs, 12 from urine samples and one each from the urine catheter tip and gastric lavage samples. *S. epidermidis* was the most frequently isolated (32%), followed by *S.hemolyticus* (18%), *S. lugdunensis* (12%), *S. hominis* (10%), *S. saprophyticus* (8%) in their study *S.hemolyticus* was predominant species because blood samples are more.,

In the present study of 124 strains, 40 strains were isolated from urinary samples. Of them 26 (65%) were identified as *S. saprophyticus* and 11 (27.5%) as *S. epidermidis*. U. Mohan et al (2001)[9]studied 192 strains. S. saprophyticus (15.6%) was the second important species, isolated mostly from urine specimens[59]. The present study correlated well with the U. Mohan *et al.,* study.

The precise reason for UTI by S. saprophyticus remains obscure. Special predilection of S. saprophyticus for production of urinary tract infection has been attributed to its urease positivity.

Out of 124, 96(77.4%) were Novobiocin sensitive and 28 (22.8%) were Novobiocin resistant. CoNS resistant to Novobiocin were found more among the isolates from urine samples. S. epidermidis, S. haemolyticus, S. hominis and S. warnerii were Novobiocin sensitive. S. saprophyticus and S. xylosus were Novobiocin resistant. Slime not only helps the organism in colonization of host tissue, but also protects from phagocytosis and from the action of antibiotics. This property has well been studied in S. epidermidis isolated from indwelling medical devices.

The present study slime production was studied on Congo red agar media. Slime production was more among the strains of *S. epidermidis* (75.3%) followed by *S. haemolyticus* (52.6%). Only two strains (7.4%) of *S. saprophyticus* produced slime. Maximum number that is 95 out of

124 CoNS strains showed resistance to 3 drugs or more than 3 (76.6%). 76(61.3%) out of 124 strains are resistance to 5 drugs or more than 5.

Dieghton MA, Franklin JC et al in Australia in 1998[19] studied 275 CoNS out of which 63% were *S. epidermidis*. Slime production was studied on congo red agar media. It was deducted in half the strains of *S. epidermidis, S. haemolyticus* and *S. saprophyticus* and rare in other species. Most *S. haemolyticus* strains and apporoximately half of the *S. epidermidis* strains were resitant to 5 or more antibiotics.[17] U. Mohan[9] studied 192 strains. Slime production was exhibited by 77 (48.7%) of S. epidermidis and only 8 (26.6%) of *S. saprophyticus*. Niranjan Nayak studied (2000)[17] studied CoNS from extra ocular infections. He found a positive association between slime positivity and multi drug resistant, A significant association was found between slime production and multiple antibiotic resistance. Present study correlated well with the results of Deighton and U. Mohan Niranjan Nayak *et al,.*

The present study shows antibiotic susceptibility tests to 124 CoNS strains. Antibiotic susceptibility tested against 10 commonly used antibiotics. All strains were sensitive to vancomycin. Most of the strains were resistant to Penicillin (93.5%) and Co-trimoxazole (92.7%).Majority of the strains were sensitive to Amikacin (68.5%) and Ceftriaxone (66.9%).In case of Amoxy-clav 49.2% strains were sensitive and 50.8% strains were found to be resistant.Out of 124, 82 strains (66.1%) were methicillin resistant and 42 strains (33.4%) were methicillin sensitive.

U. Mohan *et al.,* studied antibiotic susceptibility against commonly used antibiotics and found multi drug resistance with more than 90% resistance to penicillin, more than 50% to cephalexin and ciprofloxacin and more than 20% to methicillin. In their study the sensitivity is showing more to amikacin i.e. (71.3%) and cefotaxime (68.2%) and no resistance to vancomycin [59].

Goyal R, Singh N P *et al*., (2006)[13]The antibiotic susceptibility pattern revealed no resistance to vancomycin with 89% resistant to ampicillin followed by cefotaxime (59%), cloxacillin (25%), erythromycin (23%), ciprofloxacin (29%) and gentamicin (20%). MG Usha, DC Shwetha et al In 2013[12] showed that 56% of the isolates were MRCONS. Majority of the CONS species were resistant to ampicillin and amoxyclav (89% each), followed by ceftriaxone (52%), cotrimoxazole (46%), cefotaxime (32%), gentamicin (25%) and amikacin (21%). None of the CONS species showed resistance to vancomycin.

Rosana B. R. Ferrira *et al*[20] in Brazil studied 152 strains, out of which 103 strains (67.8%) were oxacillin resistance. The present study is in agreement with the above studies. Because of the frequency of drug-resistant strains, meaningful staphylococcal isolates should be tested for antimicrobial susceptibility to help in the choice of systemic drug

CONCLUSION:

From the data presented, it is clear that CoNS can cause a number of human infections and should no longer be accounted as a non-pathogen. Always alert to this fact and carry out the necessary investigations required for the isolation, identification and antibiogram study of such strains from clinical specimens. In the recent past 'Teicoplanin' a glycopeptide antibiotic related to vancomycin has been made available in India. Its indiscriminate use can lead to development of vancomycin resistant CoNS strains. The recommendation is to reduce the use of this drug. Therefore, it is important for clinical laboratories to distinguish between methicillin susceptible and methicillin resistant CoNS strains to control the unnecessary use of vancomycin in hospitals. Any effort made to prevent its emergence will be of great help to humanity, and all such efforts are to be encouraged..

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