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Isolation of Coagulase Negative *Staphylococcus* from Different Clinical Specimens

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Coagulase negative Staphylococcus (ConS) are essentially opportunists infections that are nearly always associated with abnormal circumstances especially the presence of foreign medical devices such as Intravascular catheters, CSF shunts, prosthetic heart valves, prosthetic joints, peritoneal dialysis catheters, haemodialysis shunts, and vascular grafts. These bacteria are pathogenic especially in immunocompromised patients and malignancies. ConS cause Bacteraemias of various severities. Blood, sputum, urine, wound swabs, pus and transthoracic aspirations were collected. Standard bacteriological procedures are done to identify and confirm it as ConS. Out of 184 wound swabs 43 were ConS (23.86%), out of 108 blood samples 25 were ConS (23.14%) out of 94 pus sample – 16 were ConS (17.02%). Out of 33 sputum sample 3 were ConS (9.09%) and out of 14 transthoracic aspirations 1 was Cons (7.14%). Among these *S.epidermidis* was most commonly isolated 55 out of 102 (53.92%) followed by *S. saprophyticus* 12 out of 102 (11.76%), *S.saccharolyticus* 10 (9.80%), *S. intermedius* 7 (6.86%), *S.capitis* 6 (5.88%), *S.haemolyticus* 5 (4.90%), *S.lugdunensis* 3 (2.99%), *S.hyicus, S.caseolyticus, S.Schleiferi, S. Cohnii* 1 each (1%). 32% of the strains were slime positive, 31% produced beta lactamase, 16% were Novobiocin resistant, Methicillin resistance was 29.41%.

Key words: Coagulase negative *Staphylococcus*; Slime production; Methicillin resistance.

Coagulase negative *Staphylococcus* (ConS) belonging to the normal human flora, cause a wide range of hospital acquired infections with increasing mortality and morbidity. Originally considered to be clinically insignificant contaminants in clinical specimens, in recent years ConS have been recognized as important agents of hospital acquired infection. According to Data of Centre's for Disease Control & National Nosocomial Surveillance system (NNIS) in fact ConS today belong to five most frequent cause of Nosocomial infections. Now ConS account for 40% of causes of Nosocomial Bacteraemias and are the second most frequent cause of surgical site infection in the ICU.

The types of infections associated with Coagulase negative staphylococci include the following. (Koneman)[1]; Nosocomial and community-acquired urinary tract infections ,Infections of indwelling devices (Joint prostheses, hemodialysis and cerebrospinal shunts, pacemakers, peritoneal dialysis catheters,

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prosthetic heart valves, intravenous catheters),Bacteraemias in compromised hosts (premature infants, patients with cardiovascular and neoplastic diseases, patients with hematologic malignancies, burns patients, trauma patients, transplant recipients, patients with congenital defects, Osteomyelitis (Post surgical infections, prosthesis associated infections, trauma associated infections) and Post surgical endophthalmitis.

In addition, transfusions of contaminated platelets with ConS have caused severe symptoms in recipient in which donor's venipuncture site acted as contaminant.

Foreign body infections by ConS are an important and growing problem in hospitals *Staphylococcus* isolated from human is based on the original work of Kloss and Schleifer (1935). Research in the ConS has lead to the identification of new species and subspecies, development of accurate, rapid and definitive identification system. Baird –Parker (1963)[2] applied modern taxonomic concept to the staphylococci. Six groups were defined within staphylococci by means of a member of biochemical and physiological characters. Kloss and Schleifer (1975)[3] recognized several new species of ConS by biochemical, chemical, physiological and morphological properties.

MATERIAL AND METHODS

The present study was conducted on various clinical samples obtained from patients of Basaveshwar Hospital attached to M.R. Medical College, Gulbarga, during the period of 1year (January to December) .

A total of 500 samples like blood sputum, pus, wound swab, urine and transthoracic aspiration were taken.

Inclusive Criteria

Among 500 samples strains of ConS grown in pure culture were included in the study.

Exclusive Criteria

Coagulase negative staphylococci isolated along with other bacteria (mixed growth) were excluded.

A total of 102 strains of ConS isolated and speciated from various samples. Following the isolation of ConS Gram's stain and Coagulase tests were conducted for confirmation. Important biochemical tests and culture method carried out for further speciation. They are as follows.

Tests for enzyme production

Oxidase, catalase, Coagulase (slide and tube), phosphatase, urease, Dnase, Tests for carbohydrate utilization: fermentation of glucose, sucrose, lactose, mannitol, trehalose, O/F test, Beta Lactamase production by Iodomeric method, Slime production on Congo red Media Antibiotic susceptibility testing by disk diffusion technique with special reference to Methicillin.

Slime production

Freeman *et al.,* (1989)[4]

Congo red medium

Composition

BHI broth -37g/L, Sucrose - 50g/L, Agar -10g/L, Congo red dye -0.8g/L

Method

Plates inoculated an incubated aerobically at 37^0 for 24hrs. Developments of black colonies with dry crystalline consistency were taken as positive. Non slime producers were pink in color.

Antibiotic sensitivity testing

All isolates were subjected to antibiotic susceptibility testing. Method used was disc diffusion by Kirby-Bauer using commercially available discs.

Colonies were inoculated into Peptone water and turbidity was observed as per Mac Farland tube No.5 the test cultures swabbed on the Muller Hinton agar and plates dried for not more than 15mnts. The discs were applied on to the surface of the agar. Two plates used for one isolate due to the increased number of discs. Results were read after incubation at 37^0C for 18 hrs and interpreted as per Kirby-Bauer char

RESULTS

The present study was conducted on 102 isolate, of Coagulase negative *Staphylococci*. Following are the results observed

Graph 1 show sex wise distributions of ConS Majority of the isolates were from females i.e., 50.98% and among males it was 49%. In this study incidence of ConS from clinical samples were more from females than from males.

Table 2 show distribution of ConS among different age groups. In this study majority of

the isolates were from the age groups between 16 to 30 years is 39%.

Speciation was done based on the biochemical reactions as enlisted in Table 4 diagram 4 and Novobiocin sensitivity pattern as enlisted in Table 4. The different species isolated are as follow *S.epidermidis* (55), *S. saprophyticus* (12), *S.saccharolyticus* (10), *S.intermedius* (7), *S.capitis* (6), *S.haemolyticus* (5), *S.lugdunensis* (3), *S.hyicus* (1), *S.caseolyticus* (1), *S.schleiferi* (1) and *S.cohnii (*1). Majority of the species among 102 isolates were *S.epidermidis* and *S.saprophyticus*. *S.epidermidis* was isolated mainly from pus and blood. *S.saprophyticus* from urine, *S.saccharolyticus* from wound discharge and *S.intermedius* from pus, sputum and blood.

Table 5 shows the incidence of species of ConS among the clinical samples. Out of 102 isolates, 55 were *S.epidermidis,* 12 were *S.saprophyticus,* 10 were *S.saccharolyticus*, 7 were *S.intermedius*, 6 were *S.capitis*, 5 were *S.haemolyticus*, 3 were *S.lugdunensis* and

Table 1. Distribution of ConS among the clinical specimens

No.	Specimen	Total No.	Total. No. of Cons	Percentage
1.	Wound Swab	184	43	23.36
2.	Blood	108	25	23.14
3.	Pus	94	16	17.02
4.	Urine	74	14	18.91
5.	Sputum	33	3	9.09
6.	Transthoracic aspiration.	14	1	7.14

Table 2. Age wise distribution of Cons

Age group	Wound swab	Blood	Pus	Urine	Sputum	Transthoracic aspiration	Total
0-5	6	12	5	3	0	0	25%
16-30	15	12	7	6	0	0	39%
31-45	15	1	1	4	1	1	22%
46-60	5	0	3	1	2	0	10%
61-85	2	0	0	0	0	0	1.9%

Table 3. Speciation based on biochemical reactions (Koneman)

Species	Oxidase	Urease	Phosphatase	Glucose	Maltose	Trehalose	Mannitol	Sucrose	No. of Isolates
S.epidermidis	-	+	+	+	+	-	-	+	55
S.saprophyticus	-	+	-	+	+	+	V	+	12
Saccharolyticus	-	V	V	+	-	-	-	-	10
S.intermedius	-	+	+	+	-	+	+	+	7
S.capitis	-	-	-	+	-	-	+	+	6
S.haemolyticus	-	-	V	+	+	+	V	+	5
S.lugdunensis	-	V	-	+	+	+	-	+	3
S.hyicus	-	+	+	+	-	+	-	+	1
S.caseolyticus	-	V	V	+	+	V	-	V	1
S.schleiferi	-	-	+	+	-	V	-	-	1
S.cohnii	-	-	-	+	V	+	+	V	1

Table 4. Novobiocin sensitivity pattern observed among ConS

Novobiocin	Sensitive	Resistant
	86%	16%

S.caseolyticus, *S.schleiferi S.hyicus, S.cohnii* were 1 each. Out of 43 samples of wound swabs 22 isolates were *S.epidermidis, S.saccharolyticus* 6 and *S.intermedius* 5 *S.capitis* 4, *S.haemolyticus* 2 and *S.lugdunensis, S.schleiferi, S.hyicus, S.cohnii* 1 each.

Table 5. Incidence of species of ConS in clinical samples

Species	Wound swab	Blood	Pus	Urine	Sputum	Transthoracic aspiration	Total
S.epidermidis	22	21	9	-	2	1	55
S.saprophyticus	-	-	-	12	-	-	12
S.saccharolyticus	6	-	3	1	-	-	10
S.intermedius	5	-	2	-	-	-	7
S.capitis	4	-	1	-	1	-	6
S.haemolyticus	2	2	1	-	-	-	5
S.lugdunensis	1	2	-	-	-	-	3
S.caseolyticus	-	-	-	1	-	-	1
S.schleiferi	1	-	-	-	-	-	1
S.hyicus	1	-	-	-	-	-	1
S.cohnii	1	-	-	-	-	-	1

Table 6. Production of Slime with regards to specimen

Specimen	Slime Positive	Slime Negative	Total No.
Wound swab	17	26	43
Blood	9	16	25
Pus	5	11	16
Urine	-	14	14
Sputum	1	2	3
Transthoracic aspiration	1	-	1
Total	33	69	102

Table 7. Production of Slime with regards to species

Species	Slime +ve	Slime –ve	Total
S.epidermidis	20	35	55
S.saprophyticus	-	12	12
S.saccharolyticus	6	4	10
S.intermedius	2	5	7
S.capitis	1	5	6
S.haemolyticus	2	3	5
S.lugdunensis	1	2	3
S.caseolyticus	-	1	1
S.schleiferi	1	-	1
S.hyicus	-	1	1
S.cohnii	-	1	1

Out of 25 samples of blood 21 were *S.epidermidis* and 2 *S.haemolyticus* and 2 *S.lugdunensis*. Out of 16 samples of pus, 9 were *S.epidermidis S.saccharolyticus* 3, *S.intermedius* 2, *S.capitis* and *S.haemolyticus* 1 each.

Out of 14 samples of Urine, majority of the isolates (12) were *S.saprophyticus* followed by *S.saccharolyticus* (1) and *S.caseolyticus* (1). The 3 sputum samples yielded 2 *S.epidermidis* and 1 *S.capitis*. One Transthoracic aspiration yielded *S.epidermidis.*

The special property of ConS, such as slime production is shown in Table 6 and table 7 respectively.

Table 6 and 7, respectively show production of slime with regards to specimen and species. Out of 102 isolates 37.28% gave slime positivity from pyogenic skin lesion, 36% from blood, 33% each from sputum and transthoracic aspiration. Maximum slime positivity was expressed by *S.epidermidis* i.e., 36.36% followed by 60% of *S.saccharolyticus*, 28% *S.intermedius*, 16% *S.capitis*, 40% *S.haemolyticus*, 33% *S.lugdunensis*, and 100% *S.schleiferi*. None of the isolates from urine gave positive slime reaction.

Table 8 shows Betalactamase production by ConS species with respect to the clinical samples. In this study, Betalactamase production was noticed more among *S.epidermidis* isolated i.e. 36%, blood .Other species producing Betalactamase were *S.saccharolyticus* 50%, *S.intermedius* 28%, *S.haemolyticus* 40% and *S.lugdunensis* 33%, *S.capitis* 16%, *S.schleiferi* 100% and majority of them were isolated from wound discharge.

Table 9 Show the antibiotic pattern of ConS We observed high incidence of resistance with Penicillin 51.96%, Ampicillin 50.98%, Gentamycin 47.05% and Cotrimoxazole 41.17%, followed by Cephalexin 38.23%, Tetracycline 35.29%, Chloromphenicol 24.50%, Erythromycin 23.52%, Ciprofloxacin 21.56% and Norfloxacin 1.96%. Oxacillin showed 29.41% resistance.

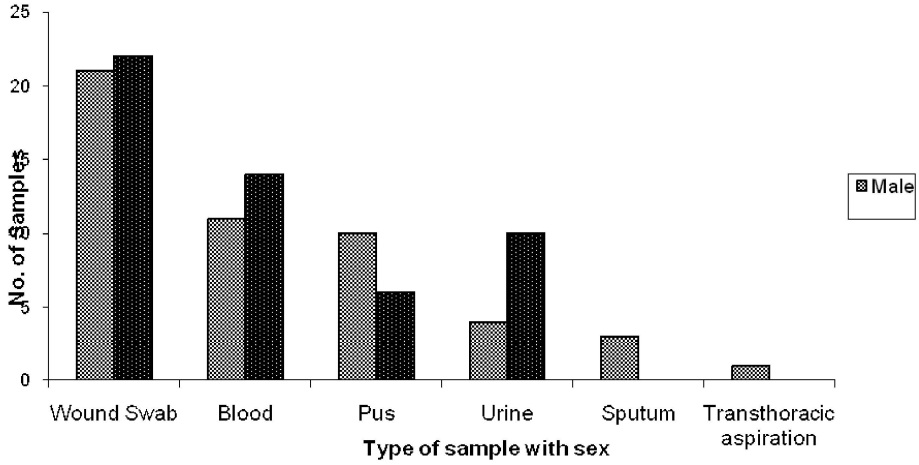
Out of 53 Penicillin resistant isolates, maximum were from wound swab i.e. 29, followed by pus 9, blood 8. Out of 30 Oxacillin resistant strains 13 were from wound swab, 6 from pus, 5 from blood.

Table 8. Production of Beta lactamase among clinical isolates of ConS

Species	Wound swab	Blood	Pus	Urine	Sputum	Transthoracic aspiration	Total
S. epidermidis	10	5	3	-	1	1	20
S. saprophyticus	-	-	-	-	-	-	-
S. saccharolyticus	4	-	1	-	-	-	5
S. intermedius	2	-	-	-	-	-	2
S. capitis	1	-	-	-	-	-	1
S. haemolyticus	-	1	1	-	-	-	2
S. caseolyticus	-	-	-	-	-	-	-
S. schleiferi	1	-	-	-	-	-	1
S.hyicus	-	-	-	-	-	-	-
S.cohnii	-	-	-	-	-	-	-
S.lugdunensis	1	-	-	-	-	-	1

Table 9. Antibiotic resistance pattern of ConS

Antibiotic	Total. No.		Percentage
	Sensitive	Resistance	
Penicillin	49	53	51.96
Oxacillin	72	30	29.41
Novobiocin	86	16	15.68
Ampicillin	50	52	50.98
Chloramphenicol	77	25	24.50
Erythromycin	78	24	23.52
Gentamycin	54	48	47.05
Norfloxacin	100	2	1.96
Tetracycline	66	36	35.29
Co-trimoxazole	60	42	41.17
Ciprofloxacin	80	22	21.56
Cephalexin	63	39	38.23



Graph 1. Sex wise distribution of Cons

DISCUSSION

Coagulase negative *Staphylococcus* is often considered as commensals on skin and mucosa. Because of this they are considered as nonpathogenic. During past 10 years, ConS have been recognized as major cause of septicemia in patients with various implanted medical devices. Varieties of clinical conditions have been attributed to ConS such as bacterial endocarditic, Osteomyelitis, mastitis, CAPD peritonitis, septicemia, wound infection and UTI.

We have studied 102 isolated of ConS from different clinical samples during the period of 1 year's i.e., Jan to Dec. In our study prevalence of ConS among wound swab is (23.36%) i.e., 43 out of 184. In blood were (23.14%) i.e. 25 out 108. Our study correlates with this study Seema Bansal *et al.,*[5] who reported prevalence of ConS from 22.7% to 24.3% other Indian studies have reported incidence of ConS in late onset neonatal septicemia varies from 2.8% to 24% out 74 sample from urine 14 were ConS which account for 18.91% . Out study correlates with Paed *et al.,*[6] were prevalence was 16% out 33 sputum sample 3 were ConS, out of 14 transthoracic aspiration 1 was ConS

Majority of the isolates were from pyogenic skin lesions such as wound swab and abscess. Out 102 ConS were 59 (57.84%), followed by blood 25 (24.50%), Urine 14 (13.72%), sputum (3%) and Transthoracic aspiration (1%). Most of the isolates from wound swabs were *S.epidermidis* 22 out of 55 (40%). *S.epidermidis* from blood was 21 out of 55 (38%) from pus 9 out 55 (16%), sputum 2 out of 55 (3.6%) transthoracic aspiration 1 out 55 (1.8%). *S.saprophyticus* was 12 (100%). *S.intermedius* was isolated only from pyogenic skin lesion (100%). *S.saccharolyticus* was isolated mainly from pyogenic skin lesion (90%) and from urine (10%) *S.capitis* was isolated from pyogenic skin lesion (83.35%) and 1 from sputum (16.66%) *S.haemolyticus* was isolated from pyogenic skin lesions (60%) and from blood (40%). *S.lugdunensis* was isolated equally from wound (33.33%), blood (66.66%) and *S.caseolyticus* (100%) from urine *S.schleiferi*, *S.hyicus* and *S.cohnii* were all isolated only from wound swab (100%each).

Out of 59 ConS isolates from pyogenic skin lesion majority of the species were *S.epidermidis* (52.54%) followed by *S.saccharolyticus* (15.25%), *S.intermedius* (11.86%), *S.capitis* (8.5%), *S.haemolyticus* (5%), *S.lugdunensis* (1.6%), *S.schleiferi*, *S.hyicus*, *S.cohnii* (1.6% each). Out of 25 ConS isolates from blood 84% were *S.epidermidis*, *S.haemolyticus* and *S.lugdunensis* contributing 8% each. Out of 14 urine samples 85% were *S.saprophyticus*, 7% *S.saccharo* lyticus and 7% *S.caseolyticus*. Of 3 isolates from sputum, 66%

were *S.epidermidis* and 33% were S.capitis. One isolate from transthoracic aspiration was *S.epidermidis* (100%).

In our observation the ConS isolated from the pyogenic skin lesions were often chronic gaping postoperative wounds and slough was not healthy. They were also collected from burns and chronic Osteomyelitis in debilitated patients where immunosupperssion and long term exposure to various antibiotics was thought of. However neutropenia was not observed in all but percentages of hemoglobin was found to be subnormal along with poor general condition. The wound swabs were also taken from patients operated for carcinoma penis with chronic non healing wound.

The blood samples were from new born and children between the age group 0-15 years, adults between 16-30 years (50%) where clinical diagnosis of septicemias was done.

We observed that ConS were isolated more frequently among the age group of 16-30 years 40% of both sexes. And among this age group the incidence was high in females 27 (69%) than males 12 (30.7%). Out of 102 ConS isolates 52 were from females and 50 were from males.

Vijayalakshmi *et al.,* (1980)[7] reported 24% of isolation of *S.epidermidis* from blood, 24% from pyogenic skin lesions, 12% from urine. Martin *et al.,* (1996)[8] reported *S.epidermidis* as the principal cause of Bacteraemias. They reported an increase from 8% of 26% in Nosocomial Bacteraemias.

Gillepsie *et al.,* (1977)[9] reported UTI in young women due to *S.saprophyticus*. Out of 124 patients S. saprophyticus was isolated from urine of students <26 years (28%), venerology department 24% in <26years of age and 2% in > 26 years of age group. Jayanthi Pathak (1994)[10] reported 21.6% of ConS from wound infection and 18.8% from urine

Lisa A. *et al.,* (2002).[11] Conducted a study on prevalence of ConS in pediatric intensive care unit and showed that prevalence was 21.3%.

Freeman *et al.,* (1987)[12] reported cons Bacteraemias .A five fold increase in the number of Nosocomial infection associated with low birth weight babies with positive blood cultures of Cons.

Khadilkar (1995)[13] reported that infants of birth weight less than 1000gm are at highest risk for Nosocomial infection especially with Cons.

Out of 102 stains of ConS isolated from various clinical samples, majority gave positive slime test. The slime are extracellular polysaccharides, variously termed as capsule, slime or glycocalyx, appear to be significant virulence factors from some strains of Staphylococci. Capsules and microcapsules of S.aureus are the best defined of the Staphylococcal exopolysaccharides. They are relatively firmly attached to the cell wall, have definite external boundaries, are antiphagocytic and are typically composed of N-acetyl amino sugars and N-acetyl aminohexuronic acids. The distinction between capsule and slime is not always clear cut and glycocalyx may be used as more general term to refer to exopolysaccharides loosely associated with the cell and imparting viscosity to the culture medium. Much recent interests in *S.epidermidis* slime was stimulated by the reports of Christensen and Peters *et al.,* Slime production is often assessed by staining with a cationic dye. A crude slime preparation has been shown to reduce lymph proliferative responses of mononuclear cells to polyclonal stimulators and interfere with granulocytic function and has antiphagocytic effect of slime. As testing of slime production has been claimed to be useful marker for clinically significant infection with ConS, it was included in our studies. Instead of the conventional method used by Christensen *et al.,* where liquid cultures made of Trypticase soya broth were used, we preferred a solid media used by D.J. Freeman *et al.,* (1989) which incorporated high concentration of brain heart infusion broth with sucrose and Congo red as dye. The slime strains produced black colonies. H The Congo red is believed to have stained the exopolysaccharides of the cell. The method was more sensitive detecting weak slime producers and colonies were viable. In the method used by Christensen *et al.*,, in our study was found to be varying with each batch of media and failed to detect weak slime producers.

Majority of the ConS isolated from pyogenic skin lesions showed slime positivity. Out of 59 isolates 22 (37.28%) showed positive slime reaction. Out of 25 isolates from blood 9 (36%), 1 out of 3 sputum isolates (33.3%) and 1 (100%)

transthoracic aspiration produced slime.

S.epidermidis formed a major species in producing slime (36.36%) followed by *S.saccharolyticus* 60%, *S.intermedius* (28.57%) and *S.haemolyticus* 40%, *S.capitis* (16.66%), *S.lugdunensis* (33.33%) and *S.schleiferi* (100%) each. We observed that production of slime increased chronicity of the disease with multiple drug resistance compelling the patient for a longer hospital stay. Due to resistance of drugs used routinely, higher antibiotics were used costing more money for the patients.

Klee man *et al.,* (1993)[14] Published results of species identification on 500 ConS isolates recovered from specimens *S.epidermidis* accounted for (64.5%) ,*S.haemolyticus* (13.4%), S.hominis (7.4%) ,S.warneri (40%), *S.lugdunensis* (2.8%), S.simulan (2.4%) and *S.capitis* (2%).

Pirkko Kotilainen (1990)[15] reported ConS isolation from blood of 64 patients and 53% of them showed slime production. David Davenport *et al.,* (1986)[16] reported 106 cases of ConS isolation from various samples. Out of his 51% showed slime positivity. Makhija S.K. *et al.,* (1995)[17] reported slime producing ConS from clinical specimens. Out of 101 specimens, slime produce ranged from 20% to 66%. Parija *et al.,*(2004)[18]. Compared two methods for detection of slime production by Cons, tube method and spectrophotometric method.102 ConS isolates were subjected for 12 antibiotics. Maximum isolates produced Betalactamase, 51.96%. These Penicillin resistant strains were subjected to Methicillin sensitivity using Oxacillindisc and 29.41% of resistance of Oxacillin observed. These were followed by resistance of Ampicillin 50.98%.

Gentamycin 47.05 %, Cotrimoxazole 41.17%, Cephalexin 38.23% and Tetracycline 35.29% were resistant. However 77% were sensitive to Chloromphenicol, 80% to ciprofloxacin and 78% to Erythromycin.

70% of ConS isolated from pyogenic skin lesions showed resistance to Penicillin, followed by Oxacillin 63.3% Ampicillin 65%, Gentamycin 57%, Co-trimoxazole 58.8%, Cephalexin 63.6% and Tetracycline 60%. 14% of ConS from blood showed resistance to Penicillin followed by Oxacillin 16%, Gentamycin 28.9%, Co-trimoxazole 26%, and Ampicillin 22.5%, Tetracycline 23.3% and Cephalexin 18%. 11.7% of Gentamycin resistance was encountered from urine followed Cephalexin 12%. 7% Sputum isolates showed resistance to Penicillin, Ampicillin, Cotrimoxazole and Tetracycline. One isolate of transthoracic aspiration showed resistance to 7 drugs.

Richardson J.F And Marples *et al.,* (1982)[19] reported 532 clinical isolates of ConS They reported 80-85% of *S.epidermidis* being resistance to Penicillin, 73% resistance of Oxacillin, 33% to Gentamycin, 34% to Erythromycin, and 30% to Tetracycline. The resistance to Gentamycin in ConS is apparently a recent origin.

REFERENCES

1. Koneman E.W, Allen SD, Janda WM, Schreckenberger PC. Color Atlas and textbook of diagnostic microbiology 5thed. San Francisco Lippen Cott. 1997
2. Baird-Parker A.C.: A classification of micrococci and Staphylococci based on physiological and biochemical tests. *J. Gen, Microbiol.* 1963; **30**: 409-27.
3. Kloss WE, Schleifer KH. Simplified scheme for routine identification of human *Staphylococcus* species. *J. Clin. Microbiol*., 1975;1:82-88
4. Freeman D.J., F.R. Falkiner, C.T. Keane. Now method for detecting slime production by Coagulase negative *Staphylococcus*. *Journal of Clinical Pathology*, 1989; **42:** 872-874.
5. Seema Bansal, Amita Jain, Jyotsna Agarwal, Malik G.K. Significance of Coagulase negative staphylococci in neonates with late onset septicemia. *Indian J. Pathol Microbiology.* 2004; **47**(4): 586-588.
6. Paed *et al., Staphylococcus* and urinary pathogen. *Journal of Clinical pathology,* 1977; **30**: 472.
7. Vijayalakshmi N. L.N, Mohapatra, and R.A. Bhujwal. *et al.,* Biological characters and antimicrobial sensitivity of *S.epidermidis* isolate from human source. *Indian Journal of Medical Research.* 1980; **72**: 16-22.
8. Martin. M.A., Pfaller and R.P. Wenzel. Coagulase negative staphylococcal Bacteraemias. *Ann. Intern. Med*.1989; **110**: 9-16.
9. Gillepsie W.A. Margarel A.Sellin, Patricia Gill, *et al.,* UTI in young women with special

reference to *S.saprophyticus*. 1977; 31.348.

10. Jayanthi Pathak. Usha Udgaonkar. R.D.Kulkarni *et al.,* Study of coagulase negative staphylococcal and their incidence in Human Infections. *Indian J. Med. Microbial* 1994: **12**(2) 90-95.
11. Lisa A, Grohskopf, Ronda L, Sinkowitz. Cochran *et al.,* .A national point prevalence survey of pediatrics intensive care unit acquired infections in the United States. *The journal of pediatric*, 2002; **140**(4): 432-438.
12. Freeman J., Leclair J.H, Epstein M.F. Goldman N. D.A, "Coagulase negative *Staphylococcus* Bacteraemias in the changing neonates intensive care unit population, is there an epidemic. *JAMA.* 1987; **258**: 2548-2552.
13. Khadilkar V, Tudehope D, Fraser S. A prospective study of Nosocomial infection in a neonatal intensive care unit. *J. Pediatrics child health* 1995; **5**: 387-9.
14. Klee man K.T., Bannerman T.C, Kloss W.E. Species distribution of coagulase negative staphylococcal isolates at a community hospital and implication for selection of staphylococcal identification procedures. *J. Clin. Microbiol*. 1993; **31**: 1318-1321.
15. Prikko Kotilainen *et al.,* Associations of ConS slime Production and adherence with the development and outcome of adult septicemias. *Journal for clinical Microbiology*. 1990; **28**(12): 2779-2785.
16. David S. Davenport, R. Michael Massanari, Michael A. Pfaller, *et al.,* Usefulness of a test for slime production as a marker for clinically significant infections of Coagulase negative *Staphylococcus*. *The journal of infections Diseases*, 1986; **153**(2).
17. Makhija S.K, Jalgankar. S.V, Kher M.M. Slime producing staphylococci from clinical specimens a simple diagnostic test. *Indian J. Pathal Microbiol* 1995; **38**: 159-61.
18. Karthik S., Bhattacharya S. Harish B.N. Parija S.C. Detection of Slime production by Coagulase negative *Staphylococcus*. An assessment of two methods. *Indian J. Pathol Microbial* 2004; **47**(1): 85-89.
19. Richardson J.F and R.R. Marples *et al.,* Changing resistance to antimicrobial drugs and resistance typing in clinically significant strains of *S.epidermidis*. *Journal of Clinical Microbiology*. 1982; **15**: 475-484.