



“SPECIATION AND ANTIBIOTIC SENSITIVITY TEST OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM SIR T HOSPITAL, BHAVNAGAR, INDIA.”

Microbiology

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ABSTRACT

Coagulase negative staphylococcus (CoNS) is normal flora of skin. CoNS are opportunistic bacteria. They cause infections in immunocompromised patients and in patients with indwelling urinary catheters, cardiac valves, pace makers and artificial joints. CoNS are now being increasingly recognized as pathogens. **Aim:** To identify CoNS species, their distribution and antibiotic sensitivity pattern from various clinical samples. **Method:** Total 287 CoNS isolated from various clinical samples such as blood, urine, pus, sputum, catheter tip, miscellaneous. Various tests used for species level identification. Antibiotic Sensitivity testing would be done by using Kirby Bauer Method. **Result:** In this study, total 1171 samples were examined. Out of them, 748 samples produce positive cultures. Among this 748 positive culture, 287 CoNS were isolated, out of 287 CoNS 164 CoNS in blood, 55 in urine, 54 in pus, 18 in catheter tip, 16 in sputum, 35 in miscellaneous. Out of 287 CoNS species, *S. epidermidis* was most commonly isolated species (164), followed by *S. saprophyticus* (55), *S. haemolyticus* (41), *S. lugdunensis* (12), *S. schleiferi* (5), *S. hominis* (3), unidentified species (7), the antibiotic susceptibility pattern against commonly used antibiotic showed multidrug resistance with more than 90% resistance to penicillin and no strains was resistance to Vancomycin. The methicillin Resistance was 4% among all isolates of CoNS. **Conclusion:** Study suggest increasing pathogenic potential of CoNS as well as emerging of drug resistance amongst them, that necessitates the need to adopt simple laboratory procedure to identify CoNS species and understand definitive therapy for CoNS isolates from various clinical samples.

KEYWORDS

Coagulase negative staphylococci (CoNS) species, identification, antibiotic susceptibility pattern.

INTRODUCTION:

Coagulase negative staphylococcus (CoNS) is normal flora of skin. CoNS are Gram positive cocci that occur in grape like clusters. Previously they were generally considered contaminant having little clinical significant but now being increasingly recognized as pathogens (1,2,3). they CoNS are opportunistic bacteria. They cause infections in debilitated or immunocompromised patients and in patients fitted with urinary catheters, cardiac valves, pace makers and artificial joints particularly blood stream infections and infections related to prosthesis. They were incriminated in life threatening blood stream infection in critically ill newborn (4).

Multidrug resistant CoNS strains are common. It was therefore planned to study the species identification and antibiotic sensitivity pattern of CoNS isolated from various clinical specimens received in the Microbiological Laboratory. The present study aimed to identified the most clinical isolates of CoNS by minimum number of tests and sufficient to discriminate between the species. The test which were simple, inexpensive and, easy to perform selected from the scheme of Kloos and Schleifer for identification of CoNS species (1,2).

MATERIALS AND METHODS:

The present study is carried out on various clinical specimens obtain from the patients admitted in Sir-T Hospital Bhavnagar. Coagulase negative staphylococci, isolated in various clinical specimens like urine, pus, blood and miscellaneous, are include in the study. The specimens were processed following standard guidelines. All specimens were transported as soon as collected to the microbiological laboratory for immediate processing. Aerobic cultures were performed using blood agar and MacConkey's agar with subsequent overnight incubation at 37°C. Identification of the bacteria were done on the basis of Gram's stain. All the strains, either slide or tube coagulase

negative, were further identified by scheme developed in our laboratory after reviewing references (1,2,5,6) including scheme of kloos & schleifer identification of species of CoNS. Test used are urease, Ornithine decarboxylation, alkaline phosphates and fructose, mannose sugar fermentation tests.

Antibiotic sensitivity test was done by the disc diffusion test (Kirby-Bauer method) using Mueller-Hinton agar following Clinical Laboratory Standards Institute (CLSI) guidelines (7).

Anti-microbial Drugs used such As Penicillin, Erythromycin, Azithromycin, Tetracycline, levofloxacin, Clindamycin, Ciprofloxacin, co-trimoxazole, Amikacin, Gentamycin, Linezolid, Cefoxitin, Teicoplanin, Vancomycin, Methicillin, Polymixim B, Novobiocin, By using Kirby Bauer disc diffusion method.

RESULT:

The study yield that 287 CoNS from various clinical samples such as blood, urine, pus, sputum, catheter tip, miscellaneous, total 1171 various type of samples taken out of this 748 samples produce positive culture and 423 samples has negative culture, among this 748 positive culture 287 CoNS where isolated, out of 287 CoNS, 109 CoNS in blood, 55 in urine, 54 in pus, 18 in catheter tip, 16 in sputum, 35 in miscellaneous.

out of 287 CoNS among species, *S. epidermidis* was most commonly isolated species (164), followed by *S. saprophyticus* (55), *S. haemolyticus* (41), *S. lugdunensis* (12), *S. schleiferi* (5), *S. hominis* (3), unidentified species (7) **table-1** the antibiotic susceptibility pattern against commonly used antibiotic showed multidrug resistance with more than 90% resistance to penicillin and no strains was resistance to Vancomycin. The methicillin Resistance was 4% among all isolates of CoNS. **table-2**

Table No-1 Frequency Of Clinically Significant CoNS In Different Clinical Samples

	Blood (38 %)	Urine (19.16%)	Pus (18.%)	Cath. Tip (6.2%)	Sputum (5.57%)	MICS (12.9%)	TOTAL
S.Epidermidis	83 (76.14%)	15(27.27%)	30(55.55%)	5(27.27%)	8(50%)	23(65.71%)	164(57.14%)
S.Saprophyticus	4(3.66%)	30(54.54%)	6(11.11%)	9(50%)	3(18.75%)	3(8.57%)	55(19.16%)
S.Haemolyticus	11(10.09%)	6	12(22.22%)	3(16.66%)	3(18.75%)	6(17.14%)	41
S.Lugdunensis	3(2.75%)	3(5.45%)	3(5.55%)	-	2(12.5%)	1(2.85%)	12
S.Schleiferi	2(1.83%)	-	1(1.85%)	-	-	2(5.71%)	5(1.70%)
S.Hominis	2(1.83%)	-	-	1(5.55%)	-	-	3
Unidentified	4(3.66%)	1(1.81)	2(3.7%)	-	-	-	7(2.4%)
Total	109	55	54	18	16	35	287 (100%)

Table No-2: Showing Antibiotic Susceptibility Pattern Of Cons

Drug	Sensitivity	Resistance
Penicillin	2%	98%
Erythromycin	12%	88%
Azithromycin	22%	78%
Tetracycline	40%	60%
Levofloxacin	40%	60%
Clindamycin	46%	54%
Ciprofloxacin	49%	51%
Co-trimoxazole	50%	50%
Amikacin	69%	31%
Gentamycin	70%	30%
linezolid	95%	5%
Cefoxitin	96%	4%
Teicoplanin	97%	3%
Vancomycin	100%	0%

DISCUSSION:

Many laboratories not identified clinical isolates of CoNS to the species level as they are Considered normal flora of healthy skin in addition to identification of CoNS species by reference method of kloos and schleifer scheme is cumbersome, time Consuming and used expensive reagents, not available in most clinical laboratories[1,2]. Several commercial kits ,automated instruments are available for identification of CoNS species but still out of reach of most laboratories as well as not economical in developing country (1,2,6) The species identification is important in monitoring the reservoir and distribution of CoNS involved in nosocomial infection and determination of etiologic agent (2,4,8).

Various clinical samples were followed for a period of 1 year from June 2017 to June 2018 at GMC Bhavnagar. Total 287 CoNS isolated in this study. Present scheme conveniently identified the most frequently encountered clinical isolate as *S.Epidermidis* 164/287 (57.14%) from all samples. The *S. saprophyticus* 55/287 (19.16 %) is next common species followed by *S. Heamolyticus* 41 (14.28%) found in this study similar to Author Mohan u et al [9] and Goyal et al [10], but differ from other author like s. parashar et al [11], M.levan et al [12]. Finding may be affected by difference in sample size and distribution of species in different geographical location. Maximum isolate of *S.saprophyticus* 30/55 (54.54%) found from urine samples. Out of 287 CoNS maximum isolates were from blood 109 (38%) followed by urine 55 (19.16%), pus 54 (18.8), catheter tip 18 (6.27%), sputum 16 (5.57%) and from MISC 35 (12.19%). Though the Pathogenicity of obtained CoNS is not confirmed either by history taking or by any means.

Various studies have been carried out for the isolation of CoNS proving their role as pathogens. Isolation rate of CoNS from blood:- Phatak et al isolated 4.71% [13], Sharma et al isolated 46.33% [14], Shrikhande et al isolated 23.68% [15] and Marsik et al [16] 29.89%, Goyal et al [10] have reported 14.7% CoNS from blood and Choudhary et al [17] reported the incidence as 45%. Priyanka et al [18] reported the incidence as 42.72%, in our study we have isolated 38% of CoNS from blood.

In present study, 19.16% CoNS were isolated from urine. Phatak et al [13] have reported 18.66% CoNS from urine. Priyanka et al [18] have reported 28.16 %, Gill [19] has reported 6.78% and Gemell [20] has isolated 53% CoNS from urine. Mohan et al [9] have reported 56% isolates from urine whereas Choudhary et al [17] have reported 20% isolates from urine. Goyal et al [10] have reported the incidence to be 28.4%.

Gill and colleagues [21] and Sundaram et al [22] have isolated 17.4% and 20.1% CONS from pus respectively. Incidence of CoNS from pus was reported to be 24.33% by Sharma et al [84], Choudhary et al [87] have reported the incidence to be 25% whereas Phatak et al [13] 36.7%. In present study we had got 18.8% isolates from pus. In present study, 6.27 % CoNS were isolated from catheter tips. Phatak et al [13] have reported 11% CoNS from catheter tips, Goyal et al [10] have reported 4% from catheter tips, Priyanka et al [18] have reported 5.83 %, and Sharma et al [14] have 5.7%.

Our study showed 287/748 (38%) CoNS positive Bacteremia out of all positive culture, Out of 109 CONS isolates from positive blood culture, 54 (50%) were found in neonates indicates that CoNS infection is an important cause of Neonatal Bacteremia. This may be

due increases use of central venous catheters, mechanical ventilation, and parenteral nutrition and with exposure to other invasive skin or mucosa breaching procedure. It is also observed that CoNS infection were more common in UTI and post-operative wounds patient.

Antibiotic susceptibility testing showed multidrug resistance and variability in sensitivity pattern similar to other studies [9], [10], [11]. Our study showed maximum resistance to penicillin(98%), similarly to other study [10],[14],[18] followed by Erythromycin(88%), Azithromycin(78%), Roxithromycin(73%), ampicillin(72%), oxacilline (71%), medium resistance drug are Tetracycline(60%), Levofloxacin(60%), Cephalaxin(55%), Clindamycin(54%), Ciprofloxacin(51%), Co-trimaxazole(50%), Cefotaxime(45%), less resistance seen in Gentamycin(30%), linezolid(5%), Teicoplanin (3%), No resistance were observed against vancomycin, The variability in susceptibility pattern of CoNS observed by different authors [9],[23] reflects the different protocol being used for antibiotic therapy in various hospitals. The variability in susceptibility pattern of CoNS observed by different authors [2],[3] reflected the different protocol being used for antibiotic therapy invarious hospital. Additionally antibiogram of most commonly encountered species *S.epidermidis* may vary in different geographical location.

CONCLUSION:

CoNS have emerged as a potential pathogen. CoNS have become the 3rd cause of nosocomial bloodstream infections as a result of the combination of increased use of intravascular devices and an increase in number of immunocompromised patients. Our study showed that *S. epidermidis* & *S. saprophyticus* were the more common isolates identified. 57.14% of blood samples showed *S. epidermidis* and 19.16% of urine samples of female patients who were sexually active showed *S. saprophyticus*. Out of all positive culture, CoNS are found most commonly in blood samples, among these, 50% CoNS isolated from critically ill pediatric patients (NICU).

Though the Pathogenicity of obtained CoNS is not confirmed either by history taking or by any means, The present study showed that CoNS are multidrug resistance, especially to penicillin group of antibiotic, maximum were sensitive to Linezolid, Teicoplanin, Vancomycin. MRCoNS are emerging nosocomial pathogens and every effort should be made for prevention and control of its infection. Effective hospital infection control measures and minimization of risk factors should be done by regular surveys of health care providers. Detection and treatment of CoNS carriers should be undertaken to prevent transmission of CoNS from one patient to another.

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