ORIGINAL RESEARCH PAPER

Volume - 9 | Issue - 8 | August - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SPECIATION AND ANTIMICROBIAL SUSCEPTIBILITY OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM CLINICAL SAMPLES IN A TERTIARY CARE CENTRE

Microbiology

Dr. Aparna Aparajita* Senior Resident, Department of Microbiology, RIMS, Ranchi *Corresponding Author

Dr. Ashok Kumar Sharma Associate Professor, Department of Microbiology, RIMS, Ranchi

ABSTRACT

Introduction: Coagulase negative staphylococci are usually considered contaminants when isolated from clinical samples but have gained importance in causing various nosocomial infections in seriously ill and immunocompromised patients specially in those with indwelling device or prosthetic implants. Several commercial identification systems for identification of coagulase negative staphylococci are available but are still out of reach to most of the laboratories in developing countries. The present study aimed to identify these group of organisms by conventional methods with minimum number of tests which were simple, inexpensive and easy to perform. **Materials & methods:** The present study was a cross sectional study, conducted in the Department of Microbiology, RIMS, Ranchi from July 2017 to June 2018. A total of 111 consecutive, clinically significant strains of CoNS were isolated from various clinical samples which included blood, wound swab, pus, body fluids, sputum, urine etc. Identification was done using conventional microbiological methods. Antibiotic sensitivity testing was performed by Kirby Bauer disc diffusion method on Mueller-Hinton agar using a number of antibiotics. The results were read as the zone of inhibition as per CLSI guidelines. **Results:** Out of 657 staphylococci samples a total of 111samples were identified as coagulase negative staphylococci. S. epidermidis 55(49.5%) was the most common isolate in our study, followed by S. haemolyticus 27(24.3%), S. saprophyticus 11(9.9%), S. cohnii 9(8.1%), S. lugdunensis 5(4.5%), S. hominis 3(2.7%) and S. warneri 1(0.9%) respectively. Antibiotic susceptibility pattern of the isolates showed that most isolates were resistant to Ampicillin 107 (96.4%), followed by cefotaxime 104(93.7%), Erythromycin 95(85.6%), Ciprofloxacin 78 (71.2%), cefoxitin 39(35.2%) and Trimethoprim-sulfamethoxazole 67(60.3%). Sensitivity of the isolates to Tetracycline were 57(51.3%), gentamicin 53(47.7%) and Clindamycin 78(70.2%). Highest sensitivities of CoNS isolates were shown to Vancomycin and Linezolid (100%) followed by Netilmycin 98 (88.2%) and Ampicillin-sulbactam 89 (80.1%). **Conclusion:** The conventional methods of identification can be useful in identification of CoNS isolates even in the absence of automated systems. The rising resistance to various antibiotics is of prime concern therefore correct and timely identification along with appropriate antibiotic selection helps the clinicians to provide effective therapies thereby reducing the chances of development of resistance, patient morbidity and mortality.

KEYWORDS

Coagulase negative staphylococci, Speciation, Antibiotic Susceptibility.

INTRODUCTION

Coagulase negative staphylococci have been considered for long as non pathogenic contaminants. However, they have gained importance as opportunist pathogens causing infections in very young, old and immunocompromised patients. These organisms are now regarded as major cause of nosocomial bloodstream infections, urinary tract infections, skin and soft tissue infections, and indwelling device-related and prosthetic implants infection.(1) Currently there are 38 species of CoNS isolated from various human infections.(2) The important among them are Staphylococcus saprophyticus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus lugdunensis, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus warneri, Staphylococcus xylosus, Staphylococcus cohnii. (3) These group of organisms are difficult to treat because of the multidrug resistant pattern shown by them. Colonization of skin and mucous membrane of the inpatient by multidrug resistant CoNS strain and its transmission through hands of health care workers has led to increase in infection rates caused by these organisms.(4) CoNS account for 9% of nosocomial infections.(5) They show an increasing trend of resistance towards various commonly used antibiotics. There is increasing antimicrobial resistance limiting the therapeutic options. Human and animal origin CoNS harbor large reservoir of mobile genetic elements leading to resistance of a number of antibiotics such as beta lactams, aminoglycosides, quinolones, macrolides and tetracylines.(1) Identification and speciation of these group of organisms has been a difficult task as it need differentiation between being a contaminant or a pathogen causing infections. Several commercial identification systems for identification of coagulase negative staphylococci are available but are still out of reach to most of the laboratories in developing countries. The present study aimed to identify these group of organisms by conventional methods with minimum number of tests which were simple, inexpensive and easy to perform. The antibiotic susceptibility pattern of these organisms will help clinicians to prescribe appropriate antibiotics reducing patient morbidity and long term hospital stay thus decreasing financial burden on our tertiary care centre.

MATERIALS AND METHODS

The present study was a cross sectional study, conducted in the Department of Microbiology, RIMS, Ranchi from July 2017 to June 2018 . The study was approved by Institutional Ethical Committee. A total of 111 consecutive, clinically significant strains of CoNS were isolated from various clinical samples which included blood, wound swab, pus, body fluids, sputum, urine etc. Identification was done using conventional microbiological methods. Isolates were considered clinically significant when isolated in pure culture from infected sites, body fluids or if same strain was isolated twice from blood culture within 5days. Isolates grown in mixed culture with more than 2 types of organisms were excluded from the study. The tests that were simple, inexpensive and easy to perform were selected from the scheme of Kloos and Schleifer to identify the CoNS species. The isolates were identified by colony morphology, gram staining, catalase test, coagulase test, both slide and tube coagulase, hemolysis using blood agar, and growth on Mannitol salt agar. The tests done for speciation included susceptibility to Novobiocin, Voges Proskauer, nitrate reduction, urease test, ornithine and arginine decarboxylase, resistance to polymyxin B, acid production from maltose, xylose, lactose, sucrose, fructose, mannitol and mannose.

Antibiotic sensitivity testing was performed by Kirby Bauer disc diffusion method on Mueller-Hinton agar using a number of antibiotics namely, Ampicillin(10µg), cefotaxime(30µg), cotrimoxazole(1.25/23.75µg), ciprofloxacin(5µg), ampicillin-sulbactam(10/30µg), netilmycin(30µg), tetracycline(30µg), erythromycin(15µg), gentamicin(10µg), cefoxitin(30µg), clindamycin(2µg), vancomycin(30µg). Al the antibiotic discs were obtained from Himedia laboratories Pvt. Ltd. The results were read as the zone of inhibition as per CLSI guidelines.(6)

Table-1 : Identification of CoNS by simple scheme using 6 tests (S-Sensitive, R-Resistant, V- variable)

Species	Slide coagulase test	Tube coagulase test	Ornithine Decarboxylase test	Urease test	Mannose fermentation test	Novobiocin sensitivity

S. epidermidis	-	-	+	+	+	S
S. haemolyticus	-	-	-	-	-	S
S. saprophyticus	-	-	-	+	-	R
S. cohnii	-	-	-	+	-	S
S. hominis	-	-	-	+	+/-	V
S. warneri	-	-	-	+	-	S
S. lugdunensis	+	-	+	+	+	S

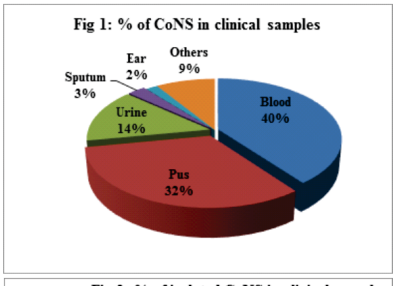
Table 2. Summary of additional tests used for the identification of CoNS spp.

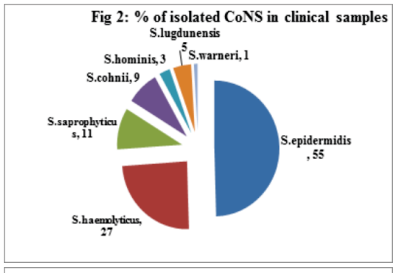
Spp	Urease	Xylose	Sucrose	Trehalose	Maltose	Mannitol	lactose	Mannose	PO4	Nitrate	Hemolysis
S.epidermidis	+	-	+	-	+	-	+/-	+/v	+	+/v	-/v
S. hominis	+	-	+	+/-	+	-/+	+/-	-/+	-	+/v	-/v
S. hemolyticus	-	-	+	+	+	+/-	-/+	-	-	+/-	+/v
S. warneri	+	-	+	+	+/-	+/-	-/+	-	-	-/v	-/v
S. capitis	-	-	+/-	-	-	+	-	+/v	-	+/-	-/v
S. saprophyticus	+	-	+	+/-	+	+/-	+/-	-	-	-	-
S. cohnii	-/+	-	-	+	v/+	+/v	-/+	+/v	-/+	-	-/v
S. xylosus	+	+	+	+	+/v	+/v	+/-	+/v	+/-	+/-	-/v
S. simulans	+	-	+	+/-	-/v	+/-	+	+/-	v/-	+	v/-

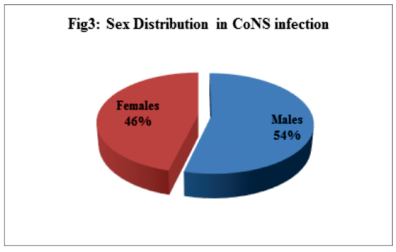
RESULTS

Out of 3398 clinically significant samples 657(19.3%) were identified as Staphylococci . Out of 657 samples 111 were identified as coagulase negative Staphylococci. Maximum number of coagulase negative Staphylococci were isolated from blood 44 (39.6%), pus 36 (32.4%), urine 16 (14.4%), sputum 3(2.7%), ear 2 (1.8%) and others 10 (9%). (Fig 1). Identification of coagulase negative Staphylococci showed S. epidermidis 55 (49.5%) to be the most common isolate in our study, followed by S. haemolyticus 27 (24.3%), S.saprophyticus 11 (9.9%), S. cohnii 9 (8.1%), S. lugdunensis 5 (4.5%), S. hominis 3(2.7%) and S. warneri 1(0.9%) respectively. (Fig 2).

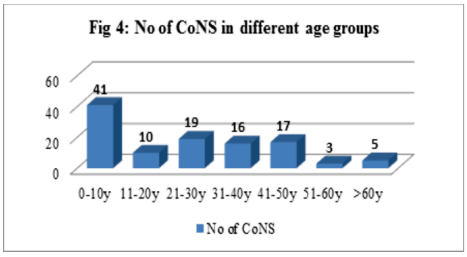
Table 3: Frequency of clinically significant CoNS in different clinical samples







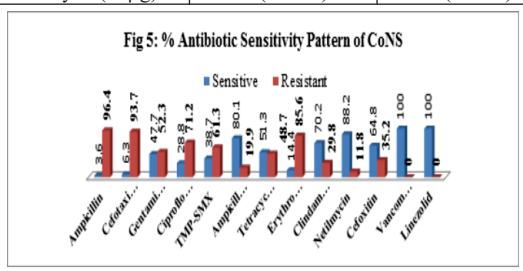
Males 60 (54%) were found to be infected more than females 51 (45.9%).(Fig 3.). Age wise distribution was analysed which showed 41 were from less than 10 yrs, 10 isolates were from 11-20 yrs age group, 19 isolates were from 21-30 years age group, 16 were from 31-40 yrs. age group, 17 were from 41-50 yrs age group, 3 from 51-60 years age group and 4 were more than 60 yrs (Fig 4).

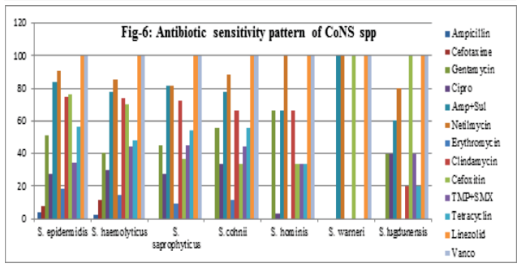


Antibiotic susceptibility pattern of the isolates showed that most isolates were resistant to Ampicillin 107(96.4%), followed by cefotaxime 104(93.7%), Erythromycin 95(85.6%), Ciprofloxacin 78(71.2%), cefoxitin 39(35.2%) and Trimethoprim-sulfamethoxazole 67 (60.3%).(Table-3). Sensitivity of the isolates to Tetracycline were 57 (51.3%), gentamicin 53 (47.7%) and Clindamycin 78 (70.2%). Highest sensitivities of CoNS isolates were shown to Vancomycin and Linezolid (100%) followed by Netilmycin 98 (88.2%) and Ampicillin sulbactam 89 (80.1%).(Fig5,6)

Table 3: Antibiotic susceptibility pattern of Coagulase Negative Staphylococci

Antibiotics(µg)	Coagulase negative Staphylococci (CoNS)	
	Sensitive % (S%)	Resistance% (R%)
Ampicillin(30µg)	4(3.6%)	107(96.4%)
Cefotaxime(30µg)	7(6.3%)	104(93.7%)
Gentamicin(30µg)	53(47.7%)	58(52.3%)
Ciprofloxacin(5µg)	33(28.8%)	78(71.2%)
TMP-SMX(1.25/23.75µg)	44(39.6%)	67(60.3%)
Ampicillin+sulbactam (10/30µg)	89(80.1%)	22(19.9%)
Tetracycline(30µg)	57(51.3%)	54(48.7%)
Erythromycin(15µg)	16(14.4%)	95(85.6%)
Clindamycin(2µg)	78(70.2%)	33(29.8%)
Linezolid(30µg)	111(100%)	-
Cefoxitin(30µg)	72(64.8%)	39(35.2%)
Vancomycin(30µg)	111(100%)	-
Netilmycin(30µg)	98(88.2%)	13(11.7%)





DISCUSSION

In the present study 111 coagulase negative staphylococci species were obtained from various clinical samples. The most common source was blood 44 (39.6%) followed by pus 36 (32.4%), urine 16 (14.4%). In our study the predominant sample from which coagulase negative staphylococci were isolated was blood which is different from studies done by R Goyal et al., (7), Surekha et al., (8), Pavithra DP (9), and Rajyalakshmi Gunti (10).

The most common species isolated in the study was S. epidermidis which is in concordance to the study done by R Goyal et al., Surekha et al., Shubra Singh et al., (7,8,11). Surekha Y Asangi et al. , have documented in their study from Bellary, India, that the predominant CoNS species isolated were S. epidermidis (44.8%), followed by S. saprophyticus (27.1%) and S. haemolyticus (19.7%) among various clinical samples whereas in the present study S. epidermidis accounted for 49.5% followed by S. haemolyticus 24.3% and S. saprophyticus 9%. S. saprophyticus was the most common isolate obtained from urine accounting for 45.4% similar to studies conducted by Uma Chaudhary et al., Mohan et al., Kumari et al., (12,13,14). Most of the studies showed S. epidermidis to be the most common isolate.

In the present study the age group mostly affected by the coagulase negative Staphylococcus was found to be less than 10years which is quite different from studies done by other authors where maximum patients were in the age group of more than 40 years. Studies done by Golia et al.,(15) and Asangi et al.,(12) showed that 38.8% and 39.5% of patients infected by CoNS were more than 40 years of age. In a study done by David et al.,(16) 85.7% of the patients that were affected by CoNS were more than 40 years of age.

As in the present study most patients infected by coagulase negative Staphylococci were less than 10 years of age it may be attributed to the fact that the immunocompromised state or patients with weak immune systems as occurs in children are more predisposed to infections caused by these group of organisms.

In the present study antibiotic susceptibility pattern showed maximum resistance to ampicillin (96.4%) which is in good concordance to study done by Singh et al., and Golia S. et al., (11,15). However, the resistance to cefoxitin (35.2%) and gentamicin (47.7%) were less as compared to Golia et al., (66.4%) and Asangi et al., (67.7%) but that for gentamicin was high as compared to report given by Golia (24.6%).

CONCLUSION

Previously considered as contaminants the coagulase negative staphylococci have been responsible for various infections. They are more of opportunist pathogens causing infections in immunocompromised. The conventional methods of identification can be useful in identification of various species of these isolates even in the absence of automated systems. The rising resistance to different antibiotics is of prime concern therefore correct and timely identification along with appropriate antibiotic selection helps the clinicians to provide effective therapies thereby reducing the chances of development of resistance. There is a need to formulate effective antibiotic policy in every hospital and surveillance of antimicrobial sensitivity is a must to help the clinicians prescribe appropriate antibiotics, stop the injudicious and indiscriminate use of antibiotics and save higher antibiotics for use only in critically ill patients thus reducing the morbidity of patients as well as financial and patient load on the hospitals thus benefitting the society, state and nation.

REFERENCES

1. Singh S, Dhawan B, Kapil A, Kabra SK, Suri A, Sreenivas V, et al. Coagulase-negative staphylococci causing blood stream infection at an Indian tertiary care hospital: Prevalence, antimicrobial resistance and molecular characterisation. Indian J Med Microbiol. 2016;34:500–5. [PubMed] [Google Scholar]
2. Borriello SP, Murray PR, Funke G. Topley & Wilson's Microbiology & Microbial Infections Bacteriology. 10th ed. London: Hodder Arnold; 2005. [Google Scholar]
3. Tille PM. Bailey & Scott's Diagnostic Microbiology. 13th ed. Missouri: Elsevier; 2014. [Google Scholar]
4. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clin Microbiol Rev. 2014;27:870–926. [PMC free article] [PubMed] [Google Scholar]
5. Kloos WE, Bannarman TL,. Update on clinical significance of coagulase negative staphylococci. Clin. Microbial Rev 1994; 7:117-140
6. Clinical Laboratory Standards Institute (CLSI) guidelines. Performance Standards for antimicrobial susceptibility testing: 22nd informational supplement. CLSI document M100-S22 Clinical and Laboratory Standards institute, Pennsylvenia; Wayne 2012
7. Goyal R, Singh NP, Kumar A, Kaur I, Singh M, Sumita N, et al. Simple and economical methods of speciation and resistotyping of clinically significant coagulase negative staphylococci. Ind J Med Microbiol 2006; 24: 201-204.
8. Surekha Y Asangi, Mariraj J, Satyanarayana M S, Nagabhushan, Rashmi. Speciation of clinically significant Coagulase Negative Staphylococci and their antibiotic resistant patterns in a tertiary care hospital.Int J Biol Med Res. 2011;2(3):735-739.
9. Pavithra DP, DivyaP,Clinically significant coagulase negative staphylococci speciation and their antibiogram patterns in tertiary care hospital, Indian Journal of Basic and Applied Medical Research; September 2015: Vol.-4, Issue- 4, P.358-63.
10. Rajyalakshmi Gunti, DurgaraniArava, RajasekharKoppada, Speciation of Coagulase Negative Staphylococci and Their Antibiogram, IOSR Journal of Dental and Medical Sciences (IOSR-JDMS).Volume 15, Issue 1 Ver. X (Jan. 2016),PP 28-31.
11. Shubhra Singh, Gopa Banerjee. Simple method for speciation of clinically significant coagulase negative staphylococci and its antibiotic sensitivity/resistance pattern in NICU of a tertiary care centre. Biomedical Research. 2008; 19(2): 97-101.
12. Uma Choudhary, BharatiArora, Madhu Sharma. Theprevalence of methicillin resistant coagulase- negative Staphylococcus in a Tertiary Care Hospital in North India. J Infect Dis Antimicrob Agents 25:13-7,(2008)15.18.
13. Mohan U, Jindal N, Agarwal P. Species distribution and antibiotic sensitivity pattern of coagulase negative Staphylococci isolated from various clinical specimens. Indian J Med Microbiol 2002;20(1):45-46
14. Kumari N, Rai A, Jaiswal CP. Coagulase negative Staphylococci as causative agent of UTI-Prevalence and Resistance status in IGIMS,Patna. Indian J PatholMicrobiol 2001;44:415-19.
15. Golia S, Telsang DB, Kamath B AS, Tiwari D. Speciation of clinically significant coagulase negative staphylococci and their antibiotic resistant patterns in a tertiary care hospital. Int J Res Med Sci 2015;3:1242.
16. Larry M. Baddour, David L. Comparison of microbiologic characteristics of pathogenic and saprophytic coagulase-negative staphylococci from patients on continuous ambulatory peritoneal dialysis. Diagnostic Microbiology and Infectious Diseases. 1986, 5(3): 197-205.