JPAM
JOURNAL OF PURE AND APPLIED MICROBIOLOGY

RESEARCH ARTICLE   **OPEN ACCESS**

Prevalence of Coagulase Negative *Staphylococcus* and their Antibiotic Sensitivity Pattern from Various Clinical Samples

Dimple Raina[1]* , **Iva Chandola[1]** , **Nidhi Negi[2]**, **Vijay Kataria[1] and Reshmi Roy[1]**

[1]Department of Microbiology Shri Guru Ram Rai Institute of Medical and Health Sciences, Dehradun, Uttarakhand, India. [2]Governmentt Medical College Doon, Dehradun, Uttarakhand, India.

Abstract

Coagulase Negative *Staphylococci* (CONS) have in the current years been increasingly incriminated as important pathogens. CONS are frequently implicated in various nosocomial infections, particularly blood stream infections and prosthetic infections. The present study was carried out to study the Coagulase Negative *Staphylococci* isolated from various clinical specimens and to determine their antimicrobial susceptibility patterns. A total of 60 strains of CONS isolated in pure form or isolated twice from various clinical specimens were recruited for the study. All clinical specimens were processed as per the standard laboratory methods. Out of 83 culture positive specimens CONS spp. were isolated in 60 specimens. In the present study maximum isolates i.e 20% isolates were recovered from paediatric ward patients, followed by 18.3% isolates being isolated from patients admitted in ICU. Isolation of CONS was maximum from blood (45%), followed by pus (21.6%) and urine (20%). Predominance of *Staphylococcus haemolyticus* (25%) and *Staphylococcus warneri* (20%) was seen. Maximum resistance was seen for ampicillin (80%), followed by resistance for ciprofloxacin (74.3%), erythromycin (73.3%) and the minimum resistance was seen for tigecycline (5%). Methicillin resistant CONS isolates (MRCONS) were seen maximum in blood (p<0.05) followed by urine (p<0.05) and pus (p<0.05). CONS have now evolved as significant cause of nosocomial infections and are progressively showing increased resistance to beta-lactamase stable penicillins. Multidrug resistance patterns have also been reported in MR-CONS and these can act as a reservoir for drug resistance in hospitals. Various levels of glycopeptide resistance have also been reported in these isolates.

Keywords: Coagulase Negative Staphylococcus, antimicrobial susceptibility, tertiary care hospital, contaminant, Pathogen, *Staphylococcus haemolyticus*

*Correspondence: dr.dimpleraina@gmail.com

INTRODUCTION

The pathogenic role of Coagulase Negative Staphylococci (CONS) has been acknowledged since long but their increased pathogenicity and increased implications in many diseases has recently come to the fore. Genus *Staphylococcus* members that do not clot rabbit plasma are known as coagulase negative staphylococci.

These organisms are constituents of the commensal human flora and are generally considered as non-virulent. Increased cases of bacteremia related to indwelling devices have been attributed many a times to Coagulase Negative Staphylococci. In the last decade CONS have emerged as important pathogens. For instance other important infections due to Coagulase Negative *Staphylococcus* include central nervous system shunt infections, native or prosthetic valve endocarditis, urinary tract infections, and endophthalmitis[1].

The isolation of Coagulase Negative Staphylococci from blood cultures is a frequent occurrence which often presents the clinician with the difficult task of deciding if the culture reflects true bacteremia or contamination from the skin[2]. CONS have also become a serious problem due to associated methicillin resistance, leading to significant limitations in therapeutic options. Currently the resistance pattern of CONS to various antimicrobial agents particularly those isolated from hospitals is very high. More than 70% of the CONS strains isolated throughout the world are oxacillin resistant and vancomycin has become the drug of choice for the treatment of infections caused by such resistant isolates[3]. The distinction between oxacillin-susceptible and oxacillin- resistant strains is of utmost importance to curtail the injudicious use of vancomycin in the hospital setups and to keep it as a reserve drug for serious infections caused by oxacillin- resistant strains[4]. Hence the objective of our study was primarily to evaluate CONS species distribution and susceptibility patterns in various clinical specimens with a special emphasis on resistance patterns for methicillin and vancomycin in our hospital.

MATERIAL AND METHODS

The current study was carried out in the department of Microbiology and Immunology at Shri Guru Ram Rai Institute of Medical and Health Sciences, Patel Nagar, Dehradun over a period of one year from October 2016 to December 2017. The clinical specimens were selected from patients attending outpatient departments (OPDs) and those who were admitted in the wards and ICUs of Shri Mahant Indiresh Hospital (SMIH), Patel Nagar Dehradun. A written informed consent was obtained from all patients who were part of the study. A total of 83 consecutive non repeated strains were isolated from deep wounds, blood samples, ear swabs, CSF, ascitic fluid, synovial fluid and urine samples. The isolates were considered clinically significant when isolated in pure culture from infected site or body fluid or if the same strain was isolated twice.

The specimens received from various OPDs, wards and ICUs were subjected to bacterial culture and sensitivity in the Central laboratory, Microbiology section of SMIH, Dehradun under all aseptic precautions. The processing and reporting was done as per the standard methods. Microscopic examination of the specimens was followed by their culture on MacConkey agar and 5% sheep blood agar (Himedia). The inoculated plates were incubated aerobically at 37°C for 18-24 hours in case of positive growth and in case no growth was observed, the plates were incubated further for 48 hours after which a negative growth report was given. Automated blood culture system (BACT / ALERT 3D BioMerieux) was used to detect growth in blood samples.

Importance of CONS bacteremia was based on isolation of CONS from blood on more than one occasion or from both received bottles and relevant clinical history of the patient. If two blood samples (we have paired bottle policy in the hospital) were submitted and only one beeped positive then the isolate was reported as a probable contaminant. However, if only one blood sample was submitted (especially in pediatric patients) clinical history was assessed and if there were no signs or symptoms of sepsis or no underlying risk factors were present, the isolate was reported as a probable contaminant. In the scenario of presence of any sign/symptoms of sepsis or any risk factors and the absence of any other apparent source of infection CONS were reported as significant in these cases. If two (or more) blood samples were positive for CONS

and the isolated strains had the same identities and antibiotic susceptibility patterns then they were considered pathogenic and were reported. The isolated CONS were identified on the basis of standard methods and any potentially clinically significant growth appearing on the culture media was reported based on quantity, feature of growth, source and site of specimens.

For primary identification basic micro-biological features and biochemical characteristics such as colony morphology, Gram staining, catalase and coagulase tests were taken into account. Auto analyzing system (VITEK-2 Biomerieux) was used to detect bacteria from clinical specimens and the final identification and antibiotic susceptibility testing was reported based on it however the results of VITEK-2 were corroborated manually by performing biochemical tests, Kirby-Bauer disk diffusion tests and E-test (for Vancomycin) in concordance with Clinical and Laboratory Standards Institute (CLSI) guidelines[5]. Speciation of Coagulase Negative *Staphylococcus* into various genomic species was done by using a battery of bio-chemical tests: Slide Coagulase test, Tube Coagulase test, Urease hydrolysis, Mannose utilization, Trehalose growth, Mannitol production, Lactose fermentation, Anaerobic growth, Ornithine decarboxylation and Novobiocin sensitivity as per standard protocol. In vitro antibiotic susceptibility testing of all isolated CONS was determined against antibiotics (µg); Cell-wall inhibitors like penicillin, ampicillin; betalactam- betalactamase inhibitor combination like amoxicillin-clavulanic acid, Cephalosporins: cefazolin, ceftazidime, ceftriaxone. Other drugs used were trimethoprim-sulfamethoxazole, oxacillin, gentamicin, erythromycin, clindamycin ciprofloxacin, levofloxacin, nitrofurantoin, linezolid, tetracycline and vancomycin. Reference strains of *Staphylococcus aureus* ATCC 25923 were used as quality controls during the performance of the tests.

Data was entered and analyzed on Microsoft Excel and interpreted by descriptive methods in terms of frequency distribution in percentages, proportions, rates ratios etc. Non parametric tests i.e., chi square and unpaired t-test were applied to ascertain significance of association.

RESULTS

On the basis of automated and the standard manual methods CONS strains were isolated from 60 clinical specimens during the study. Majority of the isolates were recovered from paediatric ward patients (20%), followed by patients admitted in ICU (18.3%), while least percentage of isolation was observed from Nephrology, ENT, Urology, & Gynaecology wards (Table 1). Highest percentage of Coagulase Negative *Staphylococcus* was from patients on antibiotic intake >72hours (31.66%) followed by the patients with ICU stay (18.33%) and least percentage from patients who were mechanically ventilated (1.67%) (Table 2). Out of 83 culture positive specimens Coagulase Negative *Staphylococcus* spp. were

Table 1. Ward wise distribution of Coagulase Negative *Staphylococcus* isolates (n=60)

Ward/ICU	No.of Isolates	Percentage
Paediatric ward	12	20%
ICU	11	18.3%
Surgery ward	10	16.6%
Medicine ward	6	10%
Orthopaedic ward	5	8.3%
Obstetric ward	3	5%
OPD	3	5%
Labour Room	3	5%
Neurology	3	5%
ENT	1	1.6%
Nephrology	1	1.6%
Urology	1	1.6%
Gynaecology	1	1.6%
Total	60	100%

Table 2. Isolation rate of CONS on the basis of pre-disposing factors (n=60)

Pre-disposing factors	No. of patients	Percentage isolation
Antibiotic intake >72hours	19	31.66%
ICU stay	11	18.33%
Post surgery	10	16.66%
IV/catheter/Tip	9	15%
Urinary catheterization >48hrs.	7	11.67%
Endotracheal intubation	3	5%
Mechanical ventilation >48hrs	1	1.67%

isolated in 60 specimens. Isolation of Coagulase Negative *Staphylococcus* was maximum from blood (45%), followed by pus (21.6%) and urine (20%) and minimum percentage was from CSF (5%) (Table 3). Table 4 Shows the species distribution of Coagulase Negative *Staphylococcus* isolates with predominance of *Staphylococcus haemolyticus*

Table 3. Specimen wise distribution of Coagulase Negative *Staphylococcus* isolates (n=60)

Specimen	No. of isolates	Percentage
Blood	27	45%
Pus	13	21.6%
Urine	12	20%
HVS	5	8.3%
CSF	3	5%
Total	60	100%

Table 4. Species Distribution of Coagulase Negative *Staphylococcus* isolates (n=60)

Species/subspp.	No. of Isolates	Percentage
S. haemolyticus	15	25%
S. warneri -hominis	12	20%
S. epidermidis	7	11.67%
S. simulans	6	10%
S. capitis	5	8.3%
S. cohnii cohnii s.s	5	8.3%
S. saprophyticus	4	6.67%
S. schleiferi	2	3.33%
S.cohnii	1	1.67%
S.lugdinensis	1	1.67%
S. schleiferi	1	1.67%
S.lentus s.s	1	1.67%
Total	60	100%

(25%) and *Staphylococcus warneri* (20%), and the least isolated were *Staphylococcus cohnii, Staphylococcus lugdinensis, Staphylococcus schleferi and Staphylococcus lentus*. High levels of resistance were seen for ampicillin (80%), followed by resistance for ciprofloxacin (74.3%), erythromycin (73.3%) and the minimum resistance was seen for tigecycline (5%). 15% isolates showed vancomycin resistance (Table 5). Methicillin resistant CONS isolates were seen maximum in Blood (p<0.05) followed by urine (p<0.05) and pus (p<0.05). Least methicillin resistance was found in the CSF samples (Table 6).

DISCUSSION

In our work maximum CONS isolates were obtained from paediatric ward patients (20%), whereas patients admitted in ICU accounted for 18.3% of the cases. Least percentage of isolation was observed from Nephrology, ENT, Urology & Gynaecology wards.

Table 5. *In vitro* activity of various antimicrobial agents against CONS (n=60)

Antibiotics	Sensitive (%)	Resistant (%)
Novobiocin	51(85%)	9(15%)
Ciprofloxacin	13(21.6%)	47(74.3%)
Gentamycin	47(78.3%)	13(21.6%)
Erythromycin	16(26.6%)	44(73.3%)
Cefoxitin	32(53.3%)	28(46.6%)
Ampicillin	12(20%)	48(80%)
Clindamycin	45(75%)	15(25%)
Levofloxacin	24(40%)	36(60%)
Doxycycline	51(85%)	9(15%)
Vancomycin	51(85%)	9(15%)
Tigecycline	57(95%)	3(5%)

Table 6. Clinical specimens showing isolation rates and Methicillin resistance pattern (n=60)

Specimen	No. of isolates (%)	Methicillin sensitive CONS isolates (%)	Methicillin resistant CONS isolates (%)	P value
Blood	27(45%)	14 (43.7%)	13 (46.4%)	P<0.05
Pus	13(21.6%)	10 (31.2%)	3 (10.7%)	P<0.05
Urine	12(20%)	8 (25%)	4 (14.2%)	P<0.05
HVS	5(8.3%)	0	5 (17.8%)	-
CSF	3(5%)	0	3 (10.7%)	-
Total	60	32 (53.33%)	28 (46.67%)	p<0.05

This is in variance with studies by Bhatt P, Jayanthy R.S, Roopa C in which the maximum number of isolates were obtained from ICU (48.7%), Medical wards (28%), surgical ward (58%) respectively[6,7,8]. All these studies point to the plethora of places in the hospitals that can be colonized by these organisms.

Maximum Coagulase Negative *Staphylococcus* isolates were recovered from blood (45%) samples and 44.4% of these blood samples were from the paediatric age group which explains the recovery of CONS isolates mostly from paediatric patients in our study.

Highest percentage of Coagulase Negative Staphylococci were isolated from patients on antibiotic intake >72hours (31.66%) followed by the patients with ICU stay (18.33%) and least percentage from patients who were on mechanical ventilation (1.67%). Prior exposure to antibiotics as one of the predisposing factors for CONS infections has been reported by Piette A and Agvald-Ohman C in their respective studies[9,10]. In fact host factors leading to serious patient infections with Coagulase Negative Staphylococci have been well characterized. These factors include any compromise in natural mucocutaneous barriers either due to any trauma or inflammation, prior exposure to antibiotics, and immunosuppression. Prolonged ICU stay leading to increased resistance, decreased clonal diversity, endogenous dissemination and cross-transmission of multi resistant CONS has been reported in a Swedish study by Agvald-Ohman[10].

Isolation of coagulase negative *staphylococcus* was maximum from blood (45%), followed by pus (21.6%) and urine (20%) and minimum percentage from CSF (5%).

The results are in concurrence with a study by Bhatt P in which 34.7% of the CONS isolates were from blood samples[6]. Similar results have also been reported from a study by Parashar S wherein 45.95% of the isolates have been reported from blood specimens[11]. Out of 27 CONS isolates from positive blood cultures 44.4% were found in neonates indicating CONS infection as an important cause of neonatal bacteremia correlating with other studies conducted by Simonsen KA et al and Roy I et al.[12,13]. The increased rate of isolation from the blood specimens in our study could be due to a higher number of blood samples tested for CONS and testing of more patients with predisposing factors (in situ medical devices or immunocompromised status), although the level of contamination may also be high in BSI. In the current study the isolation of CONS isolates from blood samples were clinically correlated and interpreted as pathogen only when they were isolated from paired blood samples from two peripheral veins. In case of patients on intravascular catheters the CONS isolates recovered from Catheter tips were clinically correlated and interpreted as significant only if the simultaneously collected peripheral blood sample yielded the same isolate.

Staphylococcus haemolyticus (25%) constituted the predominant species followed by *Staphylococcus warneri* (20%). *Staphylococcus epidermidis* however constituted 11.67% of the isolated species.

This is in concurrence to a study by Bouchami O et al. who have reported *Staphylococcus haemolyticus* as the commonest species (38%) as has been observed in our study (14). Maximum number of these isolates (22.22%) have been reported from blood and catheter specimens. Similar reports have also been reported by Cuevas et al and D'Azevedo PA et al.[15,16]. However, this is in variance to studies by Roopa C, Asangi YS, Kavitha Y, wherein *Staphylococcus epidermidis* constituted the predominant species; 50.8%, 44.8% and 46.84% respectively[8,17,18]. The maximum number of CONS isolates (45%) were derived from blood and the maximum number of *Staphylococcus haemolyticus* isolates (29.62%) were also from blood whereas 14.8% of the *Staphylococcus haemolyticus* isolates were isolated from pus; this could explain the increased figures of *Staphylococcus haemolyticus* in our study. Most of the studies have reported *Staphylococcus haemolyticus* as the second most frequently isolated CONS strain from human blood cultures however in our study it was the predominant isolate. It is also the second most frequent cause of nosocomial infections in patients on medical devices[19,20]. However its increasing role in various infections as suggested by our study warrants further studies to assert its role as a major nosocomial pathogen.

For ampicillin 80% isolates showed resistance, for ciprofloxacin (74.3%) and this was

followed by erythromycin (73.3%). Minimum resistance was seen for tigecycline (5%). Cefoxitin resistance was seen in (46.6%) isolates.

High levels of ampicillin resistance have also been reported in a study by Asangi S et al wherein 94.8% isolates showed resistance to Penicillin and 88.5% isolates showed resistance to Ampicillin[17]. Similar results have also been observed by Singh S et al wherein the antibiotic susceptibility patterns showed maximum resistance to Penicillin and ampicillin (80%)[21].

In our work the maximum sensitivity was seen for tigecycline (95%) followed by vancomycin and doxycycline (85%). Although many studies showed 100% sensitivity to vancomycin our study shows 15% resistance of isolates to vancomycin. 15% resistance to vancomycin has also been observed in a study by Jain N[22]. As per the reports available from the world 3-11 percent CONS isolates have decreased susceptibility to vancomycin[23,24]. Such patterns of reduced susceptibility to vancomycin have also been reported by Diekema DJ et al. and Natoli S in their respective studies[25,26]. Factors like indiscriminate use of glycopeptides and beta lactams, frequent hospitalizations and concomitant pneumonia act as important risk factors for the development of resistance to glycopeptides[27]. According to the CDC recommendations the spread of vancomycin resistance can be prevented by prescribing vancomycin only when CONS is isolated from multiple blood cultures. Vancomycin should not be the drug of choice when only one in a series of blood cultures is CONS positive[28].

Methicillin resistant CONS (MRCONS) isolates were isolated maximum from the Blood (46.42%). The overall MRCONS isolation stood out to be 46.67% as compared to 53.33% sensitive CONS isolates. The P value was also found to be statistically significant (<0.05). In a study conducted by Sharma V, the prevalence of methicillin resistance among CONS in their hospital was 52% which is in concurrence with our findings[29]. However, in another study conducted By Hajera M from Hyderabad the prevalence of methicillin resistance among CONS isolates was 26.31%[30]. CONS isolates showing methicillin resistance pose serious challenge to the clinicians limiting the options to glycopeptide antibiotics like Vancomycin and Teicoplanin[31].

The isolation of CONS and their antibiotic susceptibility patterns should be reported with caution and seriousness in clinical practice and clinical epidemiology as the increasing prevalence of multidrug resistant CONS (particularly methicillin resistant CONS) has serious implications in not only limiting the therapeutic options but can also act as a reservoir for drug resistance genes.

CONCLUSION

Coagulase Negative Staphylococci have in the recent times emerged as potent nosocomial pathogens and the reasons are multifactorial however of particular mention are the factors like increased use of intravascular devices and an augmentation in the number of hospitalized patients particularly who are immune compromised. Although vancomycin is still the reasonable choice for the treatment of severe infections due to multi-resistant CONS, there is a need for surveillance of nosocomial isolates of CONS for resistance to glycopeptides. We conclude that individual exposure to glycopeptides and β-lactams, in association with a history of multiple hospitalization and exposure to medical devices, plays a pivotal role as risk factors for the development of glycopeptide resistance. On the basis of our statistical analysis, we are also confident to suggest that it is desirable to implement the antibiotic restriction policy suggested by the CDC for the high-risk patients, not only to prevent the spread of CONS glycopeptide-resistant bacteremia, but also to reduce the mortality rate, duration of hospitalization, and cost of hospital care.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ETHICS STATEMENT

The study protocol was approved by the Ethical Committee Shri Guru Ram Rai Institute of Medical and Health Sciences.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

REFERENCES

1. Asante J et al. Review of clinically and epidemiologically relevant Coagulase Negative Staphylococci in Africa. *Microbial Drug Resistance*. 2020;00(00):1-20. https://doi.org/10.1089/mdr.2019.0381
2. Sidhu SK, Malhotra S, Devi P, Arpandeep K. Significance of coagulase negative *Staphylococcus* from blood cultures: persisting problems and partial progress in resource constrained settings. *Iran J Microbiol*. 2016;8(6):366-371.
3. Alicem T et al. In vitro susceptibility to methicillin, vancomycin and linezolid of staphylococci isolated from bloodstream infections in eastern Turkey. *Braz J Microbiol*. 2014;45(3):829-833. https://doi.org/10.1590/S1517-83822014000300010
4. Souza Antunes AL, Secchi C, Reiter KC, Rodrigues Perez LR, Peixoto de Freitas AL, d'Azevedo PA. Evaluation of oxacillin and cefoxitin disks for detection of resistance in coagulase negative Staphylococci. *Mem Inst Oswaldo Cruz, Rio de Janeiro*. 2007;(6):719-723. https://doi.org/10.1590/S0074-02762007005000078
5. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty – Seventh Informational Supplement. CLSI Document M100-S27. Wayne: CLSI; 2017.
6. Bhatt P, Tandel K, Singh A, Mugunthan M, Grover N, Sahni AK. Species distribution and antimicrobial resistance pattern of Coagulase-negative Staphylococci at a tertiary care centre. *Med J Armed Forces India*. 2016;72(1):71-74. https://doi.org/10.1016/j.mjafi.2014.12.007
7. Jayanthi RS, Jomy J. Phenotypic Characterization of Clinically Significant Coagulase Negative Staphylococci and their susceptibility pattern in a tertiary care hospital. *Int J Curr Microbiol App Sci*. 2015;4(4):647-652.
8. Roopa C, Biradar SK. Incidence and Speciation of Coagulase Negative *Staphylococcus* Isolates from clinically relevant specimens with their antibiotic susceptibility patterns. *Int J Curr Microbiol Appl Sci*. 2015;4(9):975-980.
9. Piette A, Verschraegen G. Role of Coagulase Negative Staphylococci in human disease. *Vet Microbiol*. 2009;134:45-54. https://doi.org/10.1016/j.vetmic.2008.09.009
10. Agvald-Ohman C, Hjelmqvist BH, Hedin G, Struwe J, Edulund C. ICU stay promotes enrichment and dissemination of multi resistant Coagulase Negative Staphylococcal strains. *Scand J Infect Dis*. 2006;38:441-447. https://doi.org/10.1080/00365540600561751
11. Parashar S. Significance of Coagulase Negative Staphylococci with Special Reference to Species Differentiation and Antibiogram. *Ind Med Gaz*. 2014;CXLVII (7):255-258.
12. Simonsen KA, Anderson-Berry AL, Delair SF, Dele H. Early-Onset Neonatal Sepsis *Clin Microbiol Rev*. 2014;27(1):21–47. https://doi.org/10.1128/CMR.00031-13
13. Roy I, Jain A, Kumar, Agrawal SK. Bacteriology of neonatal septicemia in a Tertiary care hospital of north india. *Indian J Med Microbial*. 2002;20(3):156-159.
14. Bouchami O, Achour W, Hassen B. Species distribution and antibiotic sensitivity pattern of Coagulase Negative Staphylococci other than *Staphylococcus epidermidis* isolated from various clinical specimens. *Afr J Microbiol Res*. 2011;5(11):1298-1305. https://doi.org/10.5897/AJMR11.112
15. Cuevas O et al. Evolution of the antimicrobial resistance of *Staphylococcus* spp. in Spain: five nationwide prevalence studies, 1986 to 2002. *Antimicrob. Agents Chemother*. 2004;48:4240-4245. https://doi.org/10.1128/AAC.48.11.4240-4245.2004
16. D'Azevedo PA et al. Oxacilin-resistant Coagulase Negative Staphylococci (CONS) bacteremia in a general hospital at Sao Paulo city, Brazil. *Braz J Microbiol*. 2008; 39(4):631-635. https://doi.org/10.1590/S1517-83822008000400006
17. Asangi SY, Mariraj J, Sathyanarayan MS, Nagabhushan R. Speciation of clinically significant Coagulase Negative Staphlococci and their antibiotic resistance pattern in a tertiary care hospital. *Int J Biol Med Res*. 2011;2:735-9.
18. Kavitha Y, Mohiddin Shaik K. Speciation and Antibiogram of Clinically Significant Coagulase Negative Staphylococci. *International Journal of Health Sciences & Research*. 2014;4(12):157-161.
19. Barros E M, Ceotto H, Bastos MCF , dos Santos KRN, Giambiagi-deMarvala M. *Staphylococcus haemolyticus* as an important hospital pathogen and carrier of methicillin resistance genes. *J Clin Microbiol*. 2012;50(1):166-168. https://doi.org/10.1128/JCM.05563-11
20. Daniel B, Saleem M, Naseer G, Fida A. Significance of *Staphylococcus haemolyticus* in Hospital Acquired Infections. *J Pioneer Med Sci*. 2014;4(3):119-125.
21. Singh S, Banerjee G, Agarwal SK, Kumar M, Singh RK. Simple method for speciation of clinically significant Coagulase Negative Staphylococci and its antibiotic sensitivity/resistant pattern in NICU of tertiary care centre. *Biomedical Research*. 2008;19(2):92-96.
22. Jain N, Mathur P, Mishra MC. Trend of vancomycin susceptibility of staphylococci at a level 1 trauma centre of India. *Indian J Med Res*. 2013;138(6):1022–1024.
23. Singhal R, Dhawan S, Mohanty S, Sood S, Dhawan B, Das B. Species distribution & antimicrobial

susceptibility of Coagulase Negative Staphylococci in a tertiary care hospital. *Indian J Med Res.* 2006;123:569-70.

24. Center KJ, Reboli AC, Hubler R, Rodgers GL, Long SS. Decreased vancomycin susceptibility of Coagulase Negative Staphylococci in a neonatal intensive care unit: Evidence of spread of *Staphylococcus warneri. J Clin Microbiol*. 2003;41:4660-4665. https://doi.org/10.1128/JCM.41.10.4660-4665.2003

25. Diekema DJ et al. Survey of Infections Due to *Staphylococcus* Species: Frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific Region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis*. 2001;32(2):114-32. https://doi.org/10.1086/320184

26. Natoli S et al. Characterization of Coagulase Negative Staphylococcal isolates from blood with reduced susceptibility to glycopeptides and therapeutic options. *BMC Infect Dis.* 2009;9:83. https://doi.org/10.1186/1471-2334-9-83

27. Tacconelli E et al. Glycopeptide resistance among Coagulase Negative Staphylococci that cause bacteremia: epidemiological and clinical findings from a case-control Study. *Clin Infect Dis.* 2001;33(10):1628-1635. https://doi.org/10.1086/323676

28. Centers for Disease Control and Prevention (CDC). Recommendations for preventing the spread of vancomycin resistance Recommendations of the hospital infection control practices advisory committee (HICPAC). *MMWR Morb Mortal Wkly Rep*. 1995;44:1-13.

29. Sharma V, Jindal N, Devi P. Prevalence of methicillin resistant coagulase negative staphylococci in a tertiary care hospital. *Iran J Microbiol*. 2010;2(4): 185-188.

30. Hajera M, Mustafa M, Sreenivasa Reddy C, Naidu N, Kiran Kumar V, Jayasimha Rayalu D. Prevalence and antibiotic susceptibility pattern of methicillin resistant Staphylococci from a tertiary care hospital in Hyderabad. *International Journal of Plant, Animal and Environmental Sciences*. 2014;4(2):65-69.

31. Becker K, Heilmann C, Peters G. Coagulase Negative Staphylococci. *Clin Microbiol Rev*. 2014;27(4):870-926. https://doi.org/10.1128/CMR.00109-13