Species distribution, Antibiotic sensitivity pattern and methicillin resistance of coagulate negative staphylococci isolated from various clinical samples at a tertiary care hospital

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Abstract
Introduction: Coagulate negative staphylococci (CoNS) which were formerly regarded as contaminants of clinical samples, these undoubtedly need more recognition as their pathogenic potential is being increasingly understood and can cause serious human infections. They are causing problems to clinicians because of their drug resistance. Susceptibility testing should be done in anolate considered to be a cause of infection because of the resistance of these organisms to a wide spectrum of antimicrobial agents.

Materials and Methods: Total One hundred strains of CoNS were isolated from 1486 clinical Samples processed (blood – 320, urine – 518, exudates – 522 and body fluids-126. Out of 100 CoNS were isolated from blood (35), urine (33), exudates (29), and body fluids (3) samples collected from both outpatients and inpatients of our Hospital. The organisms were identified and speciation was done by standard biochemical reactions. Antibiotic susceptibility testing was done by Kirby–Bauer disk diffusion method.

Results: Out of total 100 strains of CoNS isolated, majority were in the age group of 15-45 years (43%). Maximum isolates were from females 54(54%) than males 46 (46%). Maximum CoNS were isolated from blood (35%), followed by urine (33%), exudates (29%) and body fluids (3%). Most common species isolated was S. epidermidis (45%), followed by S. saprophyticus (22%), S. haemolyticus (13%), S. xylosus (5%), S. lugdunensis (4%), S. hominis (4%), S. capitis (4%) and S. cohnii (3%). Methicillin resistance was found in (61%) strains. Linezolid, Amikacin, Doxycycline, Gentamicin, Erythromycin, Norfloxacin and Ciprofloxacin were found to be the most effective antibiotics.

Conclusion: S. epidermidis was the predominant species isolated. The most effective antibiotics were Linezolid and Amikacin.

Keywords: S. epidermidis, Linezolid and Amikacin.

Introduction
Coagulate negative staphylococci (CoNS) were generally considered to be contaminants in the past having little clinical significance. Over the past two decades, however, these organisms have become recognized as important agents of human disease.¹ CoNS are opportunistic pathogens that cause infection in debilitated patients such as premature neonates, burn patients and end stage renal disease.² Literature available from the Western World has established CoNS as the most common organisms associated with late onset nosocomial sepsis in neonates, responsible for more than 50% of cases.³⁻⁶ The two most frequently isolated CoNS species in clinical samples are S. epidermidis and S. saprophyticus. Overall S. epidermidis is the predominant agent in nosocomial infection, bacteremia, UTI and surgical wound infection.⁷ In the reports of national survey, S. epidermidis has been remarkably quoted as primary nosocomial pathogen S. epidermidis has been implicated as the aetiologic agent in infections of wound, urogenital tract, respiratory tract, meninges, conjunctiva and skin.⁸⁻¹⁰ S. saprophyticus, a CoNS species, has been identified as a common cause of primary urinary tract infections, particularly in young women of child bearing age.¹⁰ Clinical studies, have indicated S. epidermidis, S. haemolyticus, S. warneri and S. hominis as the most prevalent CoNS in hospital infections.¹¹⁻¹² The majority of infections assumed to be caused by CoNS are a significant consequence of hospitalization.¹³ Nosocomial bacteraemia is most commonly caused by CoNS, so it is important to explore the sources of CoNS for prevention and management of infections.¹⁴ Multiresistant CoNS commonly colonizing the skin of hospitalized patients and hospital personnel, serves as a potential reservoir for antibiotic resistance genes that can transfer among CoNS and be acquired by S. aureus.¹⁵ Multiple antibiotic resistance is a common finding among clinical CoNS isolates indicating its potential pathogenicity.⁹ Resistance of these organisms to wide range of antimicrobial agents is well documented.¹⁶ Methicillin resistance among CoNS is particularly important due to cross resistance to virtually all betalactam agents and other anti microbial classes. Susceptibility testing should be done on any isolate considered to be a cause of infection because of the resistance of these organisms to a wide spectrum of antimicrobial agents.¹⁷ This type of study was not conducted so far in this Institute, an attempt was made to isolate and speciate CoNS from various clinical samples with their antibiogram.

Objectives
Speciation of CoNS, their antibiogram and methicillin resistance.

Materials and Methods
This was an observational study and conducted at Department of Microbiology, S Nijalingappa Medical College and Hospital, Bagalkot from December 2014 to August 2015 after obtaining the Institutional Ethical
Committee clearance. All clinical samples were collected under aseptic precautions and following standard clinical laboratory guidelines. The isolates were identified as CoNS by colony morphology, Gram stain, catalase test and coagulase test (slide and tube coagulase). Bacitracin (0.04 U) susceptibility was performed to exclude Micrococcil and Stomatococcus species.13

The isolates which were clinically significant, slide and tube coagulase negative were selected for further speciation. Speciation was done after reviewing the scheme of Kloos and Schleifer and Koneman, et al.12,14 The various biochemical tests used for speciation are as follows: Ornithine decarboxylase test, Phosphatase test, Urease test, Nitrate reduction test and Carbohydrate fermentation test (Mannose, Mannitol and Xylose).

The antibiotic sensitivity testing was performed on Mueller-Hinton agar by the Kirby-Bauer disc diffusion method. The antibiotics included Amikacin (AK), Amoxicillin-Clavulenate (AMC), Cotrimoxazole (COT), Ciprofloxacin (CIP), Doxycycline (DO), Erythromycin (E), Gentamicin (GEN), Linezolid (LZ) Norfloxacin (NX), Nitrofurantoin (NIT), Novobiocin (NV) and Cefoxitin (CX). Four to five colonies from 16 to 24 hours grown culture from an agar plate was suspended in peptone water. Culture from an agar plate was suspended in peptone water. Farland suspension of the isolate was made and lawn was done on MHA plate. Plates were incubated at 30ºC for 18 h. Nitrate reduction test and Carbohydrate fermentation test, Ornithine decarboxylase test, Phosphatase test, Urease test, Nitrate reduction test and Carbohydrate fermentation test (Mannose, Mannitol and Xylose).

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Results
The maximum isolates were from female patients (54%) than male patients (46%). Most common age group affected was 15-45 years (43%) followed by 0-14years (38%); 46-60 years (12%) and >60years (7%). Highest percentage among females were in the age group of 15-45 years 24(44.44%) and highest percentage among males were in the age group of 15-45 years 19(41.30%). The important clinically significant samples were blood, urine followed pus in our study. The majority of CoNS were isolated from blood 35(35%), followed by urine 33(33%), exudates 29(29%) [pus 28(28%) and ear discharge 1(1%)], and body fluids 3(3%) [ascitic fluid 2(2%) and pleural fluid1(1%)]. The majority of CoNS species isolated were S. epidermidis 45(45%), followed by S. saprophyticus 22(22%), S. haemolyticus 13(13%), S. xylosus 5(5%), S. lugdunensis 4(4%), S. hominis 4(4%), S. capitis 4(4%) and S. cohnii 3(3%). Maximum number of S. epidermidis were isolated from urine 18(54.55%) followed by blood 15(42.86%) and exudates 10(34.48%), 30(30%) were novobiocin resistant. Majority of novobiocin resistant strains were isolated from urine 14(42.42%) followed by exudates 10(34.48%) and blood 5(14.29%). 61(61%) were methicillin resistant and 39(39%) were methicillin sensitive. The methicillin resistance in CoNS isolates was found as follows: S. epidermidis 26(42.62%), S. saprophyticus 15(24.59%), S. haemolyticus 8(13.11%) and S. xylosus, S. hominis each 3(4.92%); S. lugdunensis, S. capitis and S. cohnii each 2(3.28%). All the strains of CoNS were sensitive to Linezolid (100%), Amikacin (78%) and Doxycycline (77%). However they were resistant to Amoxicillin-Clavulanic acid 65(65%), Cotrimoxazole 55(55%) and Ciprofloxacin 45(45%).

Discussion
Coagulate Negative Staphylococci form a part of normal flora, more over if CoNS isolated along with another organism, its pathogenic potential may be totally neglected. Hence it is necessary to speciate CoNS and understand the pathogenic potential of individual CoNS.19 Repeated isolation or pure growth of isolate from sterile or infected site was considered clinically relevant.20 CoNS are the most important cause of life threatening blood stream infection in some European countries and mucocutaneous commensals can cause serious invasive infections in NICU patients.9 CoNS species in causing nosocomial infections attention has now been focused on them because of their apparently changing status from non pathogens to opportunist pathogens.21 Simplicity and speed are very important in certain circumstances, e.g., for the identification of CoNS isolates from normally sterile body sites such as blood cultures, in which these isolates are the most common cause of nosocomial bacteremia, as well as the most common blood culture contaminants. Repeat CoNS isolates from patients with invasive diseases should be identified to allow a comparison of the strains. On the other hand, species identification is a prerequisite before typing procedures for epidemiological studies are undertaken.22

In the present study, out of 100 CoNS isolated, majority was from females 54(54%) than males 46(46%). Most common age group affected was 15-45 years (43%) followed by 0-14years 38 (38%); 46-60 years 12(12%) and >60 years 7(7%). Highest percentage among females were in the age group of 15-45 years 24(44.44%) and highest percentage among males were in the age group of 15-45 years 19(41.30%). The important clinically significant samples were blood, urine followed pus in our study. The majority of CoNS were isolated from blood 35(35%), followed by urine 33(33%), exudates 29(29%) [pus 28(28%) and ear discharge 1(1%)], and body fluids 3(3%) [ascitic fluid 2(2%) and pleural fluid1(1%)]. The majority of CoNS species isolated were S. epidermidis 45(45%), followed by S. saprophyticus 22(22%), S. haemolyticus 13(13%), S. xylosus 5(5%), S. lugdunensis 4(4%), S. hominis 4(4%), S. capitis 4(4%) and S. cohnii 3(3%). Maximum number of S. epidermidis were isolated from urine 18(54.55%) followed by blood 15(42.86%) and exudates 10(34.48%), 30(30%) were novobiocin resistant. Majority of novobiocin resistant strains were isolated from urine 14(42.42%) followed by exudates 10(34.48%) and blood 5(14.29%). 61(61%) were methicillin resistant and 39(39%) were methicillin sensitive. The methicillin resistance in CoNS isolates was found as follows: S. epidermidis 26(42.62%), S. saprophyticus 15(24.59%), S. haemolyticus 8(13.11%) and S. xylosus, S. hominis each 3(4.92%); S. lugdunensis, S. capitis and S. cohnii each 2(3.28%). All the strains of CoNS were sensitive to Linezolid (100%), Amikacin (78%) and Doxycycline (77%). However they were resistant to Amoxicillin-Clavulanic acid 65(65%), Cotrimoxazole 55(55%) and Ciprofloxacin 45(45%).

In the present study out of 100 CoNS isolated, majority was from females 54(54%) than males 46(46%). Most common age group affected was 15-45 years 43(43%) followed by 0-14 years 38 (38%); 46-60 years 12(12%) and >60 years 7(7%). Highest percentage among females were in the age group of 15-45 years 24(44.44%) and highest percentage among males were in the age group of 15-45 years 19(41.3%). The above findings correlated with the study of Kumari N et al. (2001) who reported majority of CoNS isolates from females 32(54.1%) than males 27(45.9%).15-45 years age group had highest percentage among females 20(62.4%) while 46-60 years age group had highest percentage among males 8(29.60%).21

In the present study out of 100 CoNS isolated, majority was from blood 35(35%), followed by urine 33(33%), exudates 29(29%) and body fluids 3(3%). The below mentioned workers, Sewell CM et al. (1982) -43%24 Shrikhande S et al. (1996) -43%25 and Fule RP et al. (1996)- 40.68%26 have reported majority of isolates from...
exudates and Deighton MA et al.(1988),27 Ieven M et al. (1995),28 Usha MG et al., (2013)29, Murad Ehsan et al(2013),30 Bhamare S et al.,(2014)31 have reported majority of isolates from blood and exudates.

In the present study, majority of isolates from urine were S. epidermidis 18(54.55%) and S. saprophyticus 14 (42.42%) similarly from blood S. epidermidis 15(42.86%) and S. haemolyticus 10(28.57%). [Table 3] This is in correlation with Sarathabu R et al, (2013).30 Out of all the CoNS isolated, S. epidermidis was the common species from all samples and from urine where S. saprophyticus was most common in the present study. Other species isolated were S. haemolyticus, S. xylosus, S. lugdunensis, S. hominis, S. capitis, and S. cohnii in the present study.

Methicillin resistant CoNS (MR-CoNS) most notably S. epidermidis, S. haemolyticus, S. hominis are major MR-CoNS and the main colonizers of the anterior nares and human skin. Methicillin resistant staphylococcal strains have acquired and integrated into their genome the staphylococcal cassette chromosome mec (SCCmec), which carries the methicillin resistance (mecA) gene, and other antibiotic resistance determinants.31 The methicillin resistant strains are heterogeneous; each population contains both methicillin susceptible and methicillin resistant organisms. The methicillin resistant organisms grow more slowly and prefer lower temperatures and a more hypertonic environment, which necessitates the use of special procedures to enhance detection in susceptibility tests.32 The percentage of MR-CoNS isolated from clinical specimens by different workers varies from 13.8% to 82.8%.

In the present study, out of 100 CoNS isolated, 61(61%) were methicillin resistant This is in correlation with other workers such as Singh M et al 52% (2015),32 Marsik FJ et al. 64.1% (1982),33 Golia S et al 66.4% (2015)19 and Koksal F et al. 67.5% (2009).34 In the present study, highest number of isolates were sensitive to Linezolid (100%) and Amikacin (78%) and Doxyecycline (77%) [Table 6]. This is similar to above mentioned workers.

Variability in the antibiotic susceptibility pattern of CoNS has been observed by various workers which positively reflect the different protocols and panels of antibiotics being used in different hospitals and differences in the geographical locations from where these isolates have been obtained.35

Conclusion
However, the increase in the implication of CoNS as significant nosocomial pathogens with a high rate of resistance to antimicrobial agents has underlined the need for species identification which is important in monitoring the reservoir and distribution of CoNS involved in infections and determining the aetiological agent.36 Despite the introduction of various antimicrobial agents, antibiotic resistance is increasing day by day. It is more prevalent in developing countries due to their misuse.37 It is important to monitor antibiotic consumption and resistance trends of nosocomial staphylococci, especially with infection control measures to prevent emergence and spread of multi-resistant bacteria within the hospital environment.38

Conflict of Interest: None.

References
1. Washington WC, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, Koneman’s Color Atlas and Textbook of Diagnostic Microbiology. 6thed. Philadelphia; Lippincott Company JB: 2006.
2. Seetha KS, Santosh PK, Shivananda PG. Study of coagulase negative staphylococci isolated from blood and CSF cultures. Indian J Pathol Microbiol 2000;43(1):41-5.
3. Singh S, Banerjee G, Agarwal SK, Anuradha R, Piyush T, Kumar M et al. Prevalence of Mec A Gene positive CoNS in NICU of a Tertiary care Hospital. Biomed Res 2009;20(2):94-8.
4. Yue QU, Andrew JD, Taghirid SL, Suzanne MG, Margaret AD. Antibiotic susceptibility of Coagulase Negative Staphylococci isolated from very low birth weight babies: comprehensive comparisons of bacteria at different stages of biofilm formation. Ann Clin Microbiol Antimicrob 2010;9(16):1-12.
5. Aher CS. The isolation pattern, species distribution and antibiotic susceptibility profile of coagulase negative staphylococci: emerging opportunistic pathogens. Jt J of BMed Adv Res 2014;5(1):23-5.
6. Nicolle LE, Hoban SA and Harding GKM. Characterization of coagulase negative staphylococci from urinary tract specimens. J Clin Microbiol 1983;17(2):267.
7. Goudarzi M, Seyedjavadi SS, Goudarzi H, Boromandi S, Ghazi M, Azad M, et al. Characterization of coagulase negative staphylococci isolated from hospitalized patients in Tehran. Iran J Para Scie 2014;5(2):44-8.
8. Almeida RJ, Jorgensen JH. Use of Mueller Hinton agar to determine Novobiocin susceptibility of coagulase negative staphylococci. J Clin Microbiol 1982;16(6):1155-6.
9. Borriello SP, Murray PR and Funke G. Tolepy and Wilson’s Microbiology and Microbial infections. Bacteriology 2,10th edition, London; Arnold: 2005.
10. Crass BA, Bergdoll MS. Involvement of coagulase negative staphylococci in toxic shock syndrome. J Clin Microbiol 1986;23:43-5.
11. Keim LS, Torres-Filho SR, Volli Silva P, Teixeira L.A. Prevalence, aetiology and antibiotic resistance profiles of coagulase negative staphylococci isolated in a teaching Hospital. Braz J Microbiol 2011;42:248-55.
12. Kloos WE, Bannerman TL. Update on clinical significance of coagulase negative staphylococci. Clin Microbiol Rev 1994;7:117-40.
13. Kloos WE, Schleifer KH. Simplified scheme for routine identification of human Staphylococcus species. J Clin Microbiol 1975;1(1):82-9.
14. Ehsan MM, Memon Z, Ismail MO, Fatima G. Identification and antibiotic susceptibility pattern of coagulase negative staphylococci in various clinical specimens. Pak J Med Sci 2013;29(6):1420-24.
15. Archer GL, Climo MW. Antimicrobial susceptibility of coagulase negative staphylococci. Antimicrob Agents Chemother 1994;38:2231-37.
16. Gaikwad SS, Deodhar LP. Study of coagulase negative staphylococci in clinical specimens. J Postgrad Med 1983;29:162-4.
17. Woods GL, Hall GS, Rutherford I, Pratt KJ, Knapp CC. Detection of methicillin resistant Staphylococcus epidermidis. J Clin Microbiol 1986;24(3):349-52.
18. Hajera M, Mustafa M, Sreenivas R, Naidu NV, Kumar KV, Jayasimha RD et al. Prevalence and antibiotic susceptibility pattern of methicillin resistant staphylococci from a tertiary
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Species distribution, Antibiotic sensitivity pattern and methicillin resistance in coagulase negative staphylococci isolated from various clinical specimens by standard bacteriological techniques. Indian J Basic Applied Med Res 2014;3(2):560-64.

29. Ieven M, Verhoeven J, Pattyn SR, Goossens H. Rapid and economical method for species identification of clinically significant coagulase negative staphylococci. J Clin Microbiol 1995;33:1060-63.

30. Bhamare S, Kongre V, Karmarkar A, Rajput A, Bhardwaj R, Kagal A. Species wise distribution of coagulase negative staphylococci from various clinical specimens by standard bacteriological techniques. Indian J Basic Applied Med Res 2014;3(2):560-64.

31. Murugesan S, Perumal N, Mahalingam SP, Dilliappan S, Krishnan P. Analysis of antibiotic resistance genes and its associated SCCmec types among nasal carriage of methicillin resistant coagulase negative staphylococci from community settings, Chennai, Southern India. J Clin Dignostic Res 2015;9(8):1-5.

32. Sardar SA, Singh M, Basireddy S, Ali S, Kabra V. Coagulase negative staphylococci among clinical isolates in a tertiary care centre. Int J Pharma Bio Sci 2015; 6(1): 229-36.

33. Marsik FJ, Brake Sylvia. Species identification and susceptibility to 17 antibiotics of coagulase negative staphylococci isolated from clinical specimens. J Clin Microbiol 1982;15:640-45.

34. Koksal F YH, Samasti M. Antibiotic resistance patterns of coagulase negative staphylococcus strains isolated from blood cultures of septicemic patients in Turkey. Microbiol Res 2007;164(4):404-10.

35. Mohan U, Jindal N, Aggarwal P. Species distribution and antibiotic sensitivity pattern of coagulase negative Staphylococcus isolated from various clinical specimens. Indian J Med Microbiol 2002;20(1):45-6.

36. Akinkummi EO, Lamikanra A. Species distribution and antibiotic resistance in coagulase negative staphylococci colonizing the gastrointestinal tract of children in Nigeria. Trop J Pharm Res 2010; 9(1): 35-43.

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