

Speciation and resistotyping of coagulase negative staphylococci from clinical samples in a tertiary care hospital

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Abstract

Background: Coagulase negative Staphylococci (CONS) are the indigenous flora of the human skin and mucous membrane. They have long been considered as non-pathogenic and were rarely reported to cause severe infections. However, as a result of the combination of increased use of Intravascular devices and an increase in the number of hospitalised immunocompromised patients, CONS have become the major cause of Nosocomial blood stream infections and they account for 9% of all nosocomial infections. These infections are difficult to treat because of the multiple drug resistant nature of the organisms. **Objective:** Isolation, identification and speciation of coagulase negative Staphylococci from various clinical specimens and study their antimicrobial susceptibility pattern. **Material and Methods:** Out of 1280 samples collected for this study, 100 strains of CONS were isolated from exudates, urine and blood of clinically diagnosed cases by standard identification methods. Antibiotic susceptibility testing of the isolated strain was carried out by Kirby-Bauer disc diffusion method. **Results:** The most common CONS species isolated were *S. epidermidis* 59 (59%), followed by *S. saprophyticus* 29 (29%), *S. haemolyticus* 9 (9%), *S. xylosum* 2 (2%) and *S. capitis* 1 (1%). Maximum number of *S. epidermidis* were isolated from exudates 36 (72%) and *S. saprophyticus* from urine 23 (74.19%). The antibiotic susceptibility testing showed, Vancomycin as the most effective drug followed by Ciprofloxacin. Methicillin resistant CONS were 38 (38%). **Conclusion:** The most common species isolated was *S. epidermidis*, which indicates it is a pathogen of significance and not merely a commensal. *S. saprophyticus* was the most common urinary isolate which indicates that this is a very common urinary pathogen. The antibiotic resistance pattern in CONS is a great threat to the clinicians and the widespread occurrence of methicillin resistance poses a great problem, which needs special attention and intensive study.

Key Words: Antibiotic susceptibility, clinical isolates, coagulase negative staphylococci, identification.

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INTRODUCTION

Coagulase negative Staphylococci (CONS), previously dismissed as contaminants are now emerging as important potential pathogens with the increase in number of severely debilitated patients and increased use of implants in hospitals. More than 30 species of CONS are recognised but only a few are commonly incriminated in human infections. Multidrug resistant strains are common.¹ Skin of patients and health care workers, medical equipment, clothing of personnel and environment surfaces can be sources of antibiotic resistant strains.² CONS are one of the main causal agents of bacteraemia in patients with indwelling medical

devices such as central and peripheral venous catheters, valvular prostheses, artificial heart valves, pace-makers and orthopaedic prostheses.³ Numerous species of CONS have been recognised recently. *Staphylococcus epidermidis* is the species most frequently isolated from infections.⁴ It has been implicated as the etiological agent in infections of wounds, urogenital tract, respiratory tract, meninges, conjunctiva and skin.⁵ *S.saprophyticus* is second to coliforms as the most common cause of the acute urethral syndrome in women.⁶ Due to the increasing clinical significance, accurate species identification of CONS is highly desirable to permit a more precise determination of host-pathogen relationship of CONS.⁷ The worldwide increase in antibiotic resistance is a concern for public health.. Proper use of antibiotics reduces unnecessary expenses, reduces development of resistance to useful and life saving antibiotics, minimize many side effects. Knowledge of the susceptibility/resistance pattern of CONS is a prerequisite for rational use of antibiotics. Scant data are available on CONS responsible for infections in developing countries.⁸ A study carried out at Kasturba Medical College Hospital, Manipal reported the prevalence of MRCONS as 13.84 % of the total isolates of CONS.⁹ Another survey in India recorded 62.7 % MRCONS among clinical isolates.¹⁰ Considering the importance of CONS as a causal agent of nosocomial infections, the present study was undertaken to analyse species distribution and antimicrobial susceptibility of CONS isolated from different clinical specimens.

MATERIALS AND METHODS

The present study was performed after obtaining the Institutional Ethical Committee clearance. A total of 100 strains of CONS were isolated and studied over a period of 18 months, from November 2012 to June 2014. The Samples were collected from patients clinically diagnosed with urinary tract infections, respiratory tract infections, septicemia, peritonitis, cervicitis, conjunctivitis, pyogenic infections namely post-operative wound infections, burn wound infection, pyoderma, osteomyelitis, chronic suppurative otitis media, corneal ulcer, and diabetic ulcer. Only isolates grown repeatedly and in pure culture were included in the study. Specific identification of CONS was done based on Colony morphology, Gram stain, Catalase test and Coagulase test (Slide and Tube Coagulase). Bacitracin susceptibility was performed to exclude *Micrococci* and *Stomatococcus* species.^{11,12} The following tests are carried out for further speciation: Novobiocin sensitivity test (5 µg), Urease test, Phosphatase test and fermentation of Glucose, Sucrose, Mannitol, Maltose and Xylose.¹¹ Antibiotic susceptibility testing of the isolated strain was carried out by Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.^{13,14} A panel of 10 Antibiotics were tested namely, Ampicillin (Amp), Amoxy-clav (Amc), Erythromycin (E), Vancomycin (Va), Gentamicin (G), Tetracycline (Tet), Ciprofloxacin (Cip), Cephalexin (Cn), Cefotaxime (Ctx) and Co-trimoxazole(Cot) were used. Cefoxitin (30µg) was used for detection of Methicillin resistant CONS (MRCONS).^{13,14}

RESULTS

Table 1: Age and sex wise distribution of CONS

Age group	Male		Female		Total	
	No.	%	No.	%	No.	%
< 1 yr	1	2.63	2	3.22	3	3
1 - 14 yr	3	7.90	4	6.46	7	7
15 - 45 yr	24	63.15	48	77.42	72	72
46 - 60 yr	2	5.26	6	9.68	8	8
> 60 yr	8	21.06	2	3.22	10	10
Total	38	38	62	62	100	100

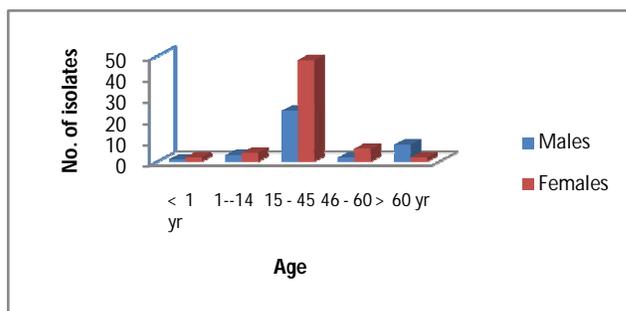


Figure 1: Showing Age and sex wise distribution of CONS

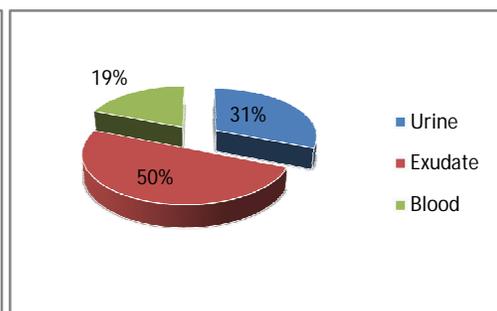


Figure 2: Showing distribution of CONS by specimen wise

Above table shows that, out of 100 CONS isolated, maximum isolates were from female patients 62 (62 %), than male patients 38 (38 %). Most common age group affected was 15-45 years 72 (72 %), followed by > 60 years 10 (10 %), 46-60 years 8 (8 %), 1-14 years 7 (7 %) and < 1 year 3 (3 %). Highest percentage among females and males were in the age group of 15-45 years, 48 (77.42 %) and 24 (63.15 %) respectively.

Table 2: Distribution of CONS specimen wise

Specimens	Exudates					Urine	Blood
	Pus	Ear swab	Vaginal swab	Pleural fluid	Catheter tip		
Number	40	3	3	2	2	31	19
Percentage	Total = 50 50.00						

Above table shows that, out of 100 CONS isolated, majority were from exudates 50 (50%), followed by urine 31 (31%), blood 19 (19%). In the exudates, 40 were isolated from pus, 3 from ear swab, 3 from vaginal swab, 2 from pleural fluid and 2 from catheter tip.

Table 3: Different species of CONS from various clinical specimens

Species of CONS	Specimen						Total	
	Urine		Exudate		Blood			
	No.	%	No.	%	No.	%	No.	%
S.epidermidis	5	16.12	36	72	18	94.73	59	59
S.saprophyticus	23	74.19	6	12	0	0	29	29
S.haemolyticus	1	3.22	7	14	1	5.27	9	9
S.xylosus	1	3.22	1	2	0	0	2	2
S.capitis	1	3.22	-	-	-	-	1	1
Total	31	100	50	100	19	100	100	100

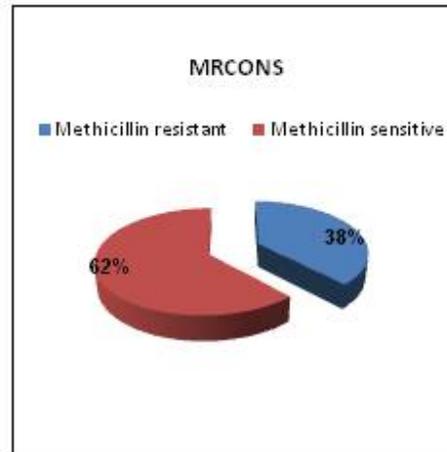
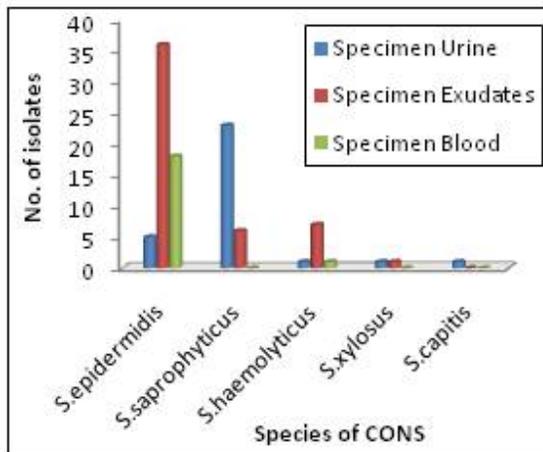


Figure 3: Showing different species of CONS from various clinical specimens **Figure 4:** Showing resistance to Methicillin

Above table shows that, majority of CONS species isolated were S. epidermidis 59 (59%), followed by S. saprophyticus 29 (29%), S. haemolyticus 9 (9%), S. xylosus 2 (2%) and S. capitis 1 (1%). Out of 50 samples of exudates, 36 (72%) isolates were S.epidermidis, 7 (14%) S. haemolyticus, 6 (12%) S. saprophyticus and 1 (2%) S. xylosus. Out of 31 samples of urine, 23 (74.19%) were S. saprophyticus, 5 (16.12%) S. epidermidis, S. haemolyticus, S. xylosus and S. capitis 1 (3.22%) each. Out of 19 samples of blood, 18 (94.73%) were S.epidermidis and 1 (5.27%) was S.haemolyticus.

Table 4: Resistance to Methicillin

Total number of CONS	Methicillin resistant CONS		Methicillin sensitive CONS	
	No.	%	No.	%
100	38	38	62	62

Above table shows that, out of 100 CONS isolated, 38 (38 %) were Methicillin resistant and 62 (62 %) were Methicillin sensitive.

Table 5: Antibiogram of CONS

Antibiotic	Resistant		Sensitive	
	No.	%	No.	%
Ampicillin	62	62	38	38
Amoxy-clav	24	24	76	76
Erythromycin	54	54	46	46
Vancomycin	0	0	100	100
Gentamicin	20	20	80	80
Tetracycline	32	32	68	68
Ciprofloxacin	8	8	92	92
Cephalexin	53	53	47	47
Cefotaxime	21	21	79	79
Co-trimoxazole	42	42	58	58

Above table shows that, all the strains of CONS were sensitive to vancomycin. However, they were resistant to Ampicillin 62%, Erythromycin 54%, Cephalexin 53%, Co-trimoxazole 42 % and showed low resistance to Ciprofloxacin 8%.

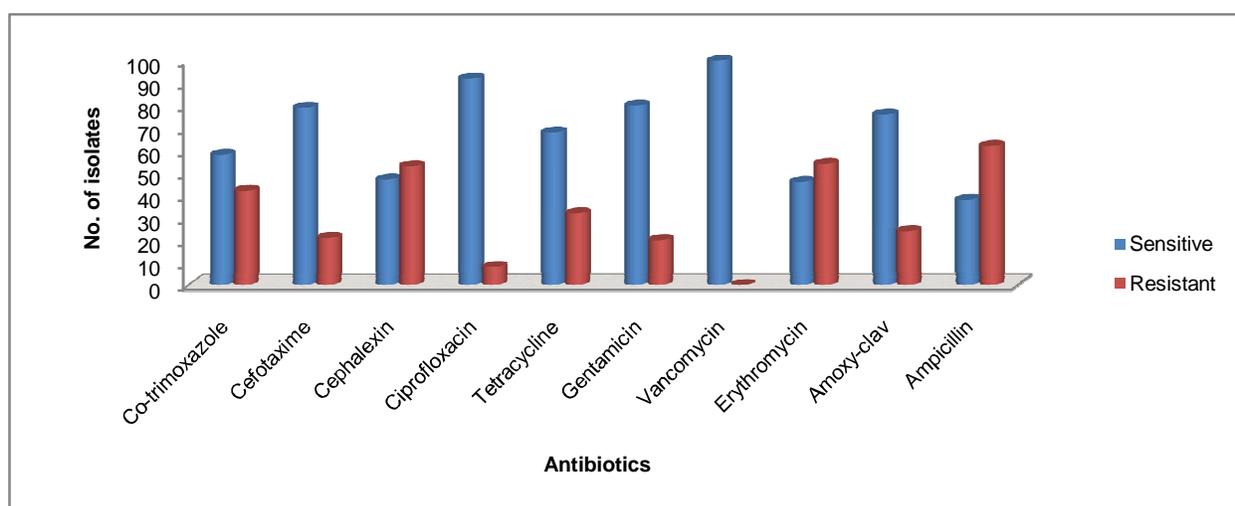


Figure 5: Showing antibiogram of CONS

DISCUSSION

CONS have been the major cause of nosocomial infections.¹⁵ Hospitals and communities have been struggling with increasing number of multi drug resistant MRCONS. Treatment is especially difficult due to biofilm formation and frequent antibiotic resistance. Distinguishing, clinically significant pathogenic strains from contaminant strains is one of the major challenges facing clinical microbiologists. Because many isolates are multiple antibiotic resistant, their infections are difficult to treat and can even be fatal. Taking into consideration the increased frequency of isolation of CONS from clinical specimens, they must now be individually evaluated as potentially true pathogens.⁴ In the present study, out of 100 CONS isolated, majority were from females (62 %) which correlated with the study of Namrata Kumari *et al.* (2001), who reported majority of CONS isolates from females (54.10 %).¹⁶ Majority of the CONS species in the present study were isolated from 15 - 45 years age group from both females 48 (77.42 %) and males 24 (63.15 %). (Refer Table No.1) These findings

are similar to a study done in Iran (2012) in which it was reported that majority of CONS isolates belong to 30 - 44 years age group in both the sexes.¹⁷ While some studies reported majority of isolates in males who were 45 years or older.^{16,18} In the present study, the predominance of CONS isolates from females in the 15 – 45 years age group is probably due to a high no. of UTI cases (31 %) during this period. It is well documented that UTI's are more common in females belonging to the reproductive age group.¹⁶ In the present study, majority of the isolates were from pyogenic skin lesions such as wound swab and abscess. Out of 100 CONS isolated majority were from exudates 50 (50 %) followed by urine 31 (31%), blood 19 (19 %) which correlates with another study conducted in south India by Surekha Y Asangi *et al* (2011).¹⁸ However, Mohan U *et al.* (2002), Sheikh and Mehdejad (2012) have reported majority of isolates from urine.^{1,17} In the present study, majority of CONS species isolated were *S. epidermidis* (59%), followed by *S. saprophyticus* (29%), *S. haemolyticus* (9%), *S. xylosus* (2%) and *S. capitis* (1%). These findings correlate with another study

conducted in south India (2011) by Surekha Y Asangi *et al* were *S.epidermidis* was the most frequent isolate 43 (44.8%), followed by *S.saprophyticus* 26 (27.1%) and *S. haemolyticus* 19 (19.7%).¹⁸ However other studies have isolated *S.haemolyticus* (18 %) as the second most common species.¹⁹ The incidence of methicillin resistance was 38 % in this study. This is in correlation with a study done in Government Medical College and hospital, Anantapur (39.4%) in 2010.²⁰ Many others have reported a much higher incidence of MRCONS.^{21,22} The relatively increased rate of MRCONS isolates in our study might be due to the fact that our specimens were taken from hospitalized patients where antibiotic policy to treat patients is lacking. Inadequate practice of antibiotic policy, indiscriminate use of antibiotics becomes common. In addition, environmental factors and hygienic conditions of the hospital were not adequate. Overcrowding of patients and attendants favour the spread of infectious agents. In such a scenario, hospital acquired infection in different wards should be quite high. As a result patients become infected during hospital stay with multi drug resistant pathogens. None of the isolates showed resistance to Vancomycin in our study. However, others have noted a reduced susceptibility to vancomycin.

CONCLUSION

Although the pathogenic role of CONS is now well established, the clinical significance of the various species is still being defined. We should not disregard any of the organisms until their clinical significance is resolved. In the hospital microbiology laboratory, non-aureus isolates are simply reported as CONS without speciation. Because there is increasing pathogenicity of these organisms, CONS should be identified to the species level by simple, reliable and preferably inexpensive method. The antibiotic resistance pattern in CONS is a great threat to the clinicians and the widespread occurrence of methicillin resistance poses a great problem, which needs special attention and intensive study. Future analysis of species-specific antibiotic resistance patterns could resolve the question of whether certain species by their very nature are more antibiotic resistant.

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