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RESEARCH ARTICLE

PREVALENCE OF COAGULASE NEGATIVE STAPHYLOCOCCAL SPECIES AND THEIR ANTIBIOTIC RESISTANCE PATTERN AT TERTIARY CARE RURAL HOSPITAL

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ABSTRACT

Background & objective: CONS species have been recognized as etiological agents in wide variety of infections especially in immunocompromised patients, patients with indwelling or implanted foreign bodies. Furthermore, there is increasing rates of antibiotic resistance which varies within species of CONS and limits therapeutic options .Hence the present study was undertaken to determine common CONS species isolated from clinically significant specimens and to evaluate antimicrobial susceptibility pattern of these species. **Methods:** This cross sectional study was carried out from September, 2016 to September, 2018in which 200 CONS isolates from clinically significant samples were identified upto species level by conventional phenotypic methods. Antibiotic Susceptibility profile was studied by Kirby Bauer Disk Diffusion method with special emphasis on methicillin & clindamycin resistance. **Result:** Total 9 species were isolated, the most common being S. epidermidis(40%) followed by S. haemolyticus (25%) , S. schleiferi (11%) and most commonly from blood ,pus,urine.Overall there was high rate of resistance to penicillin & erythromycin(60%)and all were sensitive to vancomycin & linezolid.66% isolates were methicillin resistant CONS of which 18.18% showed iMLSB phenotype. **Conclusion:** Species identification of CONS is important because of rising clinical importance and variation in antibiotic resistance pattern within species. Accuracy and promptness in the detection of species wise antibiotic resistance pattern will help in understanding definitive therapy for CONS.

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INTRODUCTION

Coagulase negative Staphylococci (CONS) are found as part of the normal flora of skin and mucous membrane of humans and animals. They are considered as non- pathogenic (Davenport, 1986 and Christensen, 1982). Healthy human skin or mucous membrane normally support from 10^1 to 10^6 colony forming units(CFU)/cm^2 of CONS, depending on the anatomical site. There are around more than 40 recognized species and subspecies of CONS, making them the most prominent microbes inhabiting on the normal skin and mucous membranes (John, 2009). Infrequently, CONS causes primary invasive disease but they are considered as contaminants. With the changes in underlying host populations and the changes in the practice of medicine, CONS has become formidable pathogens (John, 2009). Species identification of CONS is important because of rising clinical importance of CONS.CONS species have been recognized as etiological agents in wide variety of infections especially in immunocompromised patients, patients with indwelling or implanted foreign bodies. Furthermore, in CONS, there is increasing rates of antibiotic resistance which varies within species of CONS and limits therapeutic options (Becker, 2014). Another issue in CONS is higher incidence of methicillin resistance due to mecA gene (Lucía, 2003), encoding penicillin-binding protein (PBP2a) with altered properties responsible for antimicrobial resistance. Because of increasing antimicrobial resistance in CONS, few therapeutic options are available for treatment. The major barrier in the uses of clindamycin is development of inducible resistance in vivo and in vitro testing. This inducible resistance varies with geographical location from hospital to hospital and within bacterial species (Bansal, 2012). Hence the present study was undertaken to determine common CONS species isolated from clinically significant specimens and to evaluate antimicrobial susceptibility pattern of these species from tertiary care rural hospital.

MATERIALS AND METHODS

This cross sectional study was carried out in department of Microbiology of Jawaharlal Nehru Medical College and Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha which is a tertiary care hospital from September, 2016 to September, 2018 after obtaining approval from Institutional Ethics Committee. 200 CONS isolates from clinically significant samples like blood, pus, urine, body fluids received in department of Microbiology were processed. CONS isolates from sputum, stool, wound swabs, throat and vaginal swabs were excluded. The isolates were considered clinically significant when isolated in pure culture from infected sites or body fluids or if the same strain was isolated from repeat samples (Bansal, 2012 and Washington, 2006).

Processing of sample: Direct microscopy which includes wet mount preparation and gram staining were carried out on appropriate samples. Samples were cultured on Nutrient agar , Blood agar and MacConkey agar and incubated over night at 37^0C .The organisms isolated were identified on the basis of colony characters, gram staining, catalase test and coagulase test (Washington, 2006 and Kloos, 1986). Genus Staphylococcus was differentiated from Micrococcus by the glucose oxidation and fermentation test, sensitivity to Furazolidone (100ug) and resistance to Bacitracin (0.04unit/disc) as described by koneman et al (Washington, 2006). According to koneman et al. (Washington, 2006), for species identification of CONS the following biochemical tests were used:

Ornithine decarboxylase test, Carbohydrate fermentation test (utilization of xylose, trehalose, maltose, fructose, lactose, mannose and mannitol were employed), Phosphatase test 'Nitrate reduction test, Urease production test PYR TEST (Pyrrolidonyl Arylamidase test) Voges–Proskauer (VP) test.
According to Koneman et al (Washington, 2006), for species identification of CONS, the following antibiotic discs were used: Polymyxin – B Sensitivity test, Novobiocin Susceptibility test.

Antibiotic Susceptibility Test: Antibiotic Susceptibility profile of 200 CONS strains isolated from different clinical samples was studied by Kirby Bauer Disk Diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2016) The following antibiotic discs were used Penicillin-G(10 µg) Erythromycin (15µg), Clindamycin (2µg), Cefoxitin (30µg) Linezolid (15µg), Tetracycline (30 µg), Vancomycin (30 µg) Rifampicin (5 µg), Chloramphenicol (30µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Nitrofurantoin (300 µg).

Methicillin resistance (Clinical and Laboratory Standards Institute, 2016**):** Methicillin resistance was detected according to CLSI guidelines by using cefoxitin (30 µg) disc[zone of inhibition ≤ 24 mm (resistant – mec A positive) and ≥ 25 mm (sensitive-mec A negative).] [photo 1]

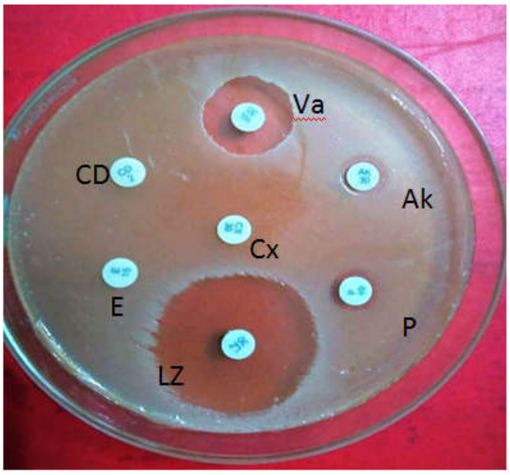


Photo 1. Methicillin resistant CONS (Cefoxitin-Resistant)

Clindamycin resistance (Clinical and Laboratory Standards Institute, 2016): If strains were erythromycin resistant (zone size ≤13mm), then such strains were subjected to detection of inducible clindamycin resistance by D test according to CLSI guidelines. In this test, erythromycin (15 µg disc) and clindamycin (2 µg disc) were placed at a distance of 15 mm edge to edge on a Muller Hinton agar plate already inoculated with test strain(turbidity adjusted to 0.5 McFarland standard) and incubated over night at 37□ C.CONS strains showing resistance to erythromycin (zone size ≤ 13mm) while being sensitive to clindamycin (zone size ≥ 21mm) and were giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having inducible MLS_B phenotype.[photo 2a] CONS strains showing resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥21mm) and giving circular zone of inhibition around clindamycin was labelled as MS Phenotype.[photo 2b]. CONS strains showing resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤ 14mm) with circular shape of zone of inhibition if any around clindamycin were labeled as having Constitutive MLS_B ($cMLS_B$) phenotype.[photo 2c]

OBSERVATION AND RESULTS

Total 200 CONS isolates from clinically significant samples were processed. Out of 200 CONS isolates, 91 (45.5%) isolates were from blood samples, 48 (24%) isolates from pus samples, 43(21.5%) isolates from urine samples, 9 (4.5%) isolates from catheter tip samples and 9 (4.5%) isolates from body fluids respectively (Figure 1).

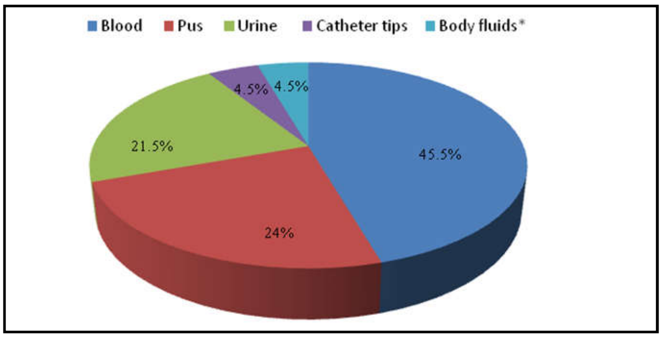


Figure 1. Sample wise distribution of CONS (n=200)

Out of 200 CONS isolates, 73(36.5%) isolates were from patients with catheter, 57(28.5%) isolates were from ICU patient, 54(27%) isolates were from patients on parenteral nutrition, 48(24%) isolates were from patients on mechanical ventilation, 46(23%) isolates were from new born and 36(18%) isolates were from very low birth weight babies (Figure 2).

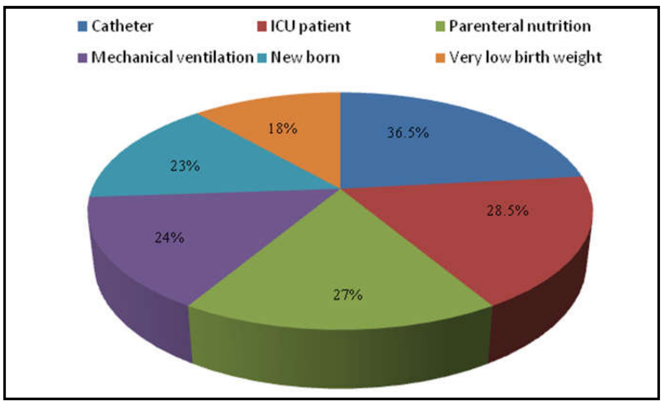


Figure 2. Risk factors for infection with CONS

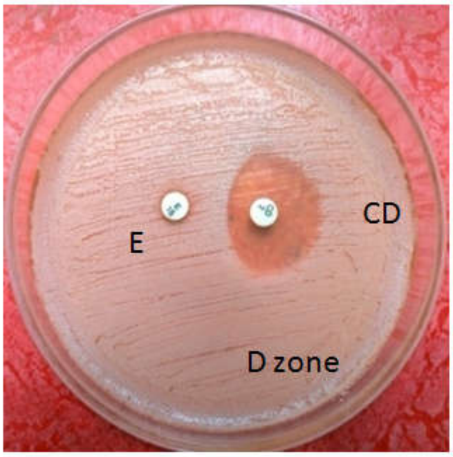
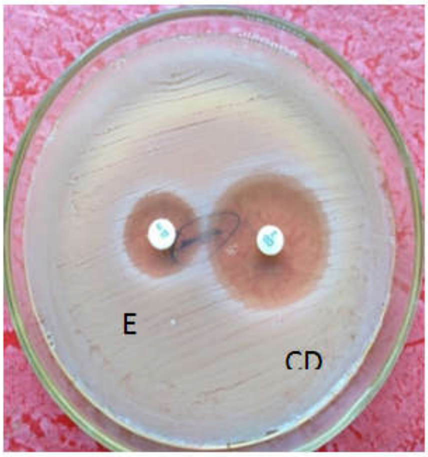
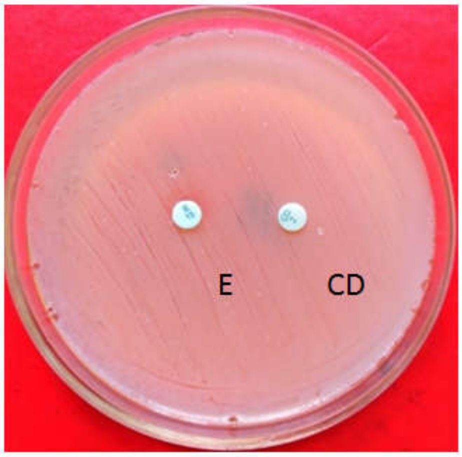


Photo 1. Methicillin resistant CONS (Cefoxitin-Resistant)



2b. MS phenotype



2c. Constitutive Clindamycin resistance CD-resistant

Out of 200 CONS isolates from different clinical samples, predominant isolated species were S. epidermidis 80 (40%), S.haemolyticus 50 (25%), S. schleiferi 22 (11%) and S.lugdunensis 20 (10%). Least commonly isolated CONS species were S.saprophyticus 11 (5.5%), S.xylosus7 (3.5%), S.intermedius 6 (3%), S.warneri 3 (1.5%) and S. hominis 1 (0.5%) (Figure 3). Table 1 shows samplewise & specieswise distribution of CONS from clinically significant samples. From table 1, most common species isolated from all clinically significant samples was S.epidermidis. In present study, among 9 S. saprophyticus from urine samples, 8 (88.89%) isolates were from female patients. Analysis of each individual isolated species of CONS showed variable distribution of all species among different sources of clinical specimens and different hospital wards. S. epidermidis, the most common species of CONS was isolated from all types of specimens and was isolated from all hospital wards (Table 1,2).

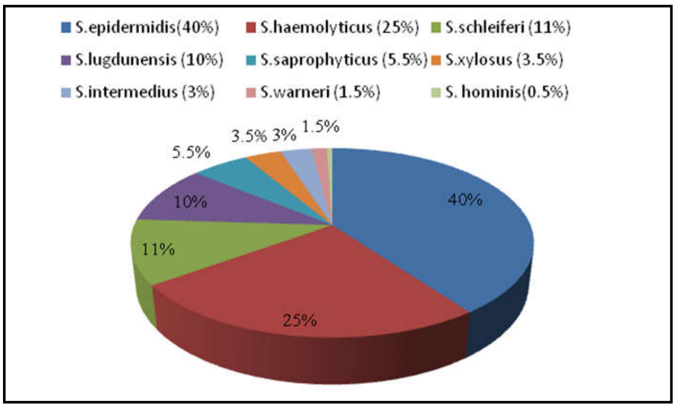


Figure 3. Species distribution of CONS isolates (n=200)

It was observed that various species of CONS showed variation in antibiotic resistance patterns. But overall there was high rate of resistance to penicillin, erythromycin, tetracycline, chloramphenicol and ciprofloxacin. All the 200 CONS isolates showed high sensitivity to vancomycin, linezolid and rifampicin [Table 3]. Out of 200 CONS isolates, 120(60%) showed erythromycin resistance, out of which, 30 (25%) isolates showed $iMLS_B$, 58(48.33%) isolates showed Constitutive MLSB and 32(26.67%) isolates showed MS Phenotype.

Fig.4 shows correlation of MRCONS and MSCONS with MLS_B phenotypes**.** Out of 200 CONS isolates, 132 (66%) isolates were MRCONS and 68 (34%) isolates were MSCONS. Out of 132 MRCONS isolates, 24(18.18%) isolates showed $iMLS_B$phenotype, 51(38.63%) isolates showed constitutive MLSB phenotype and 14(10.60%) isolates were having MS Phenotype. Out of 68 MSCONS isolates, 6(8.82%) isolates showed $iMLS_B$ phenotype, 7(10.29%) isolates showed constitutive MLSB phenotype and 18(26.47%) isolates showed MS Phenotype. It was observed that $iMLS_B$ phenotype was more among MRCONS as compared to MSCONS.

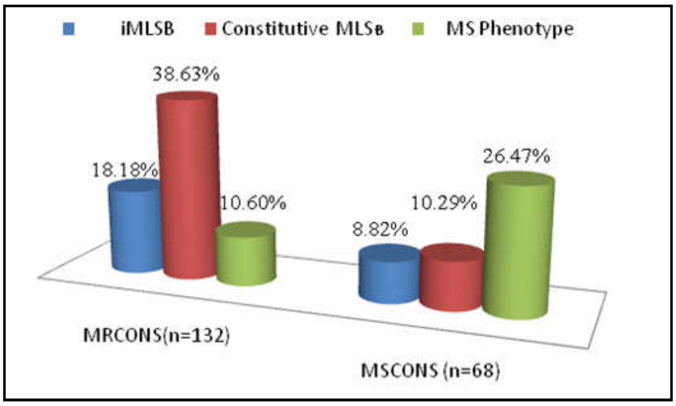


Fig. 4. Correlation of MRCONS and MSCONS with MLSB phenotypes

DISCUSSION

CONS are widespread in nature. Recently, CONS are emerging as opportunistic and nosocomial pathogens. Now a days in hospitalized patients, there is a significant rise of infections caused by the drug resistant strains of CONS. In present study, 14.5% CONS strains were isolated from neonates. This study correlates with the study done by Ibrahim Ali Al Tayyar *et al.* (Ibrahim Ali Al Tayyar, 2015) where11.7% CONS strains were isolated from neonates. Isolation of CONS from different samples correlates with the study done by Sadhvi Parashar *et al.* (2014). Risk factors for CONS infections includes medical conditions such as immune suppression, malignancy, neutropenia, premature birth, dependence of renal dialysis, long term hospitalization,

Table 1. Sample wise and species wise distribution of CONS species from clinically significant samples (n=200)

Species	No of CONS isolates (n=200)	Blood	Pus	Urine	Catheter tips	Body fluids
S.epidermidis	80	33(41.25%)	21(26.25%)	18(22.5%)	4(5%)	4(5%)
S.haemolyticus	50	24(48%)	15(30%)	10(20%)	0	1(2%)
S. schleiferi	22	12(54.54%)	4(18.18%)	2(9.09%)	2(9.09%)	2(9.09%)
S.lugdunensis	20	11(55%)	2(10%)	4(20%)	3(15%)	0
S.saprophyticus	11	2(18.18%)		9(81.81%)		
S.xylosus	7	4(57.14%)	3(42.85%)			
S.intermedius	6	3(50%)	3(50%)			
S.warneri	3	1(33.33%)				2(66.66%)
S. hominis	1	1(100%)				
Total	200	91	48	43	9	9

Table 2. ICU and ward wise distribution of CONS species

	No of CONS isolates (n=200)	S.epidermidis (n=80)	S.haemolyticus (n=50)	S.schleiferi (n=22)	S.lugdunensis (n=20)	S.saprophyticus (n=11)	S.xylosus (n=7)	S.intermedius (n=6)	S.warneri (n=3)	S. hominis (n=1)
ICU										
NICU	29	18(22.5%)	11(22%)							
MICU	19	12(15%)	7(14%)							
PICU	9	6(7.5%)	3(6%)							
Wards										
Surgery	36	7(8.75%)	6(12%)	9(40.9%)	7(35%)	4(36.36%)	1(14.28%)	1(16.66%)	1(33.33%)	
Pediatrics	33	18(22.5%)	8(16%)	3(13.63%)		1(9.09%)	1(14.28%)		1(33.33%)	1(100%)
Orthopaedics	28	5(6.25%)	5(10%)	5(22.72%)	7(35%)	1(9.09%)	1(14.28%)	3(50%)	1(33.33%)	
Medicine	24	7(8.75%)	4(8%)	1(4.54%)	2(10%)	5(45.45%)	3(42.85%)	2(33.33%)		
Dermatology	7	2(2.5%)	1(2%)	1(4.54%)	2(10%)		1(14.28%)			
Obs/gyane	6	1(1.25%)	1(2%)	3(13.63%)	1(5%)					
Urology	4	1(1.25%)	2(4%)		1(5%)					
Respiratory Medicine	3	1(1.25%)	2(4%)							
Neurosurgery	1	1(1.25%)								
ENT	1	1(1.25%)								
Total	200	80	50	22	20	11	7	6	3	1

Table 3. Species wise antibiotic resistance pattern of CONS (n=200)

Species	CONS	P	CX	E	TE	AK	VA	RIF	LZ	C	CIP	NIT(n=43)
S.epidermidis	80	80 (100%)	55 (68.75%)	49 (61.25%)	48 (60%)	38 (47.5%)	0	34 (42.5%)	0	42 (52.5%)	39 (48.75%)	11(61.11%) (n=18)
S.haemolyticus	50	49 (98%)	34 (68%)	31 (62%)	29 (58%)	23 (46%)	0	19 (38%)	0	24 (48%)	22 (44%)	7(63.63%) (n=11)
S.schleiferi	22	22 (100%)	13 (59.09%)	12 (54.54%)	13 (59.09%)	8 (36.36%)	0	7 (31.81%)	0	10 (45.45%)	12 (54.54%)	1(50%) (n=2)
S.lugdunensis	20	19 (95%)	11(55%)	13 (65.15%)	8 (40%)	8 (40%)	0	6 (30%)	0	8 (40%)	11 (55%)	1(33.33%) (n=3)
S.saprophyticus	11	11 (100%)	8 (72.72%)	8 (72.72%)	7 (63.63%)	4 (36.36%)	0	3 (27.27%)	0	5 (45.45%)	6 (54.54%)	7(77.77%) (n=9)
S.xylosus	7	7 (100%)	4 (57.14%)	3 (42.85%)	4 (57.14%)	1 (14.28%)	0	1 (14.28%)	0	2 (28.57%)	3 (42.85%)	
S.intermedius	6	6 (100%)	4 (66.67%)	3 (50%)	3 (50%)	1 (16.67%)	0	1 (16.67%)	0	3 (50%)	3 (50%)	
S.warneri	3	3 (100%)	2 (66.66%)	1 (33.33%)	2 (66.67%)	1 (33.33%)	0	1 (33.33%)	0	1 (33.33%)	2 (66.67%)	
S. hominis	1	1 (100%)	1(100%)	0	1 (100%)	0	0	1 (100%)	0	0	1(100%)	
Total	200	198 (99%)	132 (66%)	120 (60%)	115 (57.5%)	84 (42%)	0	73 (36.5%)	0	95 (47.5%)	99 (49.5%)	27(62.79%)

- Nitrofurantoin was only used for urine samples.
- Abbreviations: P- Penicillin-G,CX-Cefoxitin,E-Erythromycin,TE-Tetracycline,AK-Amikacin,VA-Vancomycin,RIF-Rifampicin,LZ-Linezolid,C- Chloramphenicol, CIP- Ciprofloxacin, NIT- Nitrofurantoin.

cardiothoracic surgery. In present study, we also found risk factors such as ICU patient, mechanical ventilation, parenteral nutrition, catheter, very low birth weight babies. In this study, predominant isolated species were S. epidermidis 80 (40%), S.haemolyticus 50 (25%), S. schleiferi 22 (11%) followed by S.lugdunensis 20 (10%) and S.saprophyticus 11 (5.5%). This finding correlates with the study done by Badampudi *et al.* (2016), where predominant isolated species were S. epidermidis (40%), S. haemolyticus (26%), S. schleiferi (13%). S. saprophyticus (15 %). In present study, 41.25% S.epidermidis were isolated from blood samples, 26.25% S.epidermidis from pus samples, 22.5% S.epidermidis from urine samples and 5% S.epidermidis from body fluids (Table 1). Similar observation was seen in study done by Sadhvi Parashar et al. 2014 where 49.6% S.epidermidis were isolated from blood samples, 18.89% from urine samples. In present study, among 9 S. saprophyticus from urine samples 8 (88.89%) isolates were from female patients. Similar finding was observed in study done by Sheikh et al. 2012 where 90.9% of S. saprophyticus isolates were from urine samples from female patients.

In present study, ICU and ward wise distribution of CONS species showed that S. epidermidis and S.saprophyticus were the predominant species isolated from ICUs and pediatric ward which correlates with a study done by Goudarzi M et al (2014). In present study, CONS isolates showed high sensitivity to vancomycin, linezolid, rifampicin. These antibiotics may play important role in the treatment and prevention of nosocomial infections caused by CONS. However CONS species showed resistance to penicillin, erythromycin, tetracycline, chloramphenicol and ciprofloxacin. While bacteria continue to acquire resistance to antibiotics, selection of appropriate antibiotic is of paramount importance. When we compared other studies with present study, it can be inferred that antibiotic resistance among species varies with geographical location and resistance is increasing in CONS species. In present study, among 200 CONS isolates, 120 (60%) showed resistance to erythromycin, out of these, percentage of inducible ($iMLS_B$)and constitutive clindamycin resistance (constitutive MLSB) and MS phenotype were found to be 25%, 48.33% and 26.67 % respectively.

These findings correlates with a study done by Bansal et al. 2012 where 18%, 26% and 22% of CONS isolates were $iMLS_B$, constitutive MLSB and MS phenotype respectively. In present study, among 132 MRCONS isolates, 18.18%, 38.63% and 10.60% isolates showed $iMLS_B$, constitutive MLSB resistance and the MS phenotype respectively (Fig. 4.). These findings correlates with a study done by Bansal et al. 2012 where 25.8%, 51.7% and 12.4% of MRCONS isolates showed iMLSB, constitutive MLSʙ and MS phenotype respectively. In the present study, among 68 MSCONS isolates, 8.82%, 10.29% and 26.47% isolates were $iMLS_B$, constitutive MLSB and the MS phenotype respectively (Fig. 4.). These findings correlates with a study done by Bansal et al. (2012), where 13.7%, 11.8% and 27.3% of MSCONS isolates were $iMLS_B$, constitutive MLSB and MS phenotype respectively. In different studies, variations in the prevalence of $iMLS_B$, constitutive MLSB and MS phenotype is seen which could be explained by differences in antibiotic susceptibility pattern.

Inducible clindamycin resistance was significantly higher in MRCONS isolates as compared to MSCONS. This finding correlates with a study done by Bansal et al. (Bansal, 2012) where he also found higher incidence of inducible clindamycin resistance in MRCONS.

Limitations: As study duration is of 2 years and sample size was 200 CONS species, studies involving large sample size and more duration to know about prevalence and changing pattern of antibiogram of CONS will be more appropriate.
In this study, species identification was done by using conventional phenotypic methods. But species identification by automated methods and molecular studies will be appropriate if facilities are available.

Conclusion: From this study, it can be inferred that CONS have been increasingly gaining importance as an important pathogen and causing nosocomial and device associated infections in seriously ill and immunocompromised patients.
Present study showed significant antibiotic resistance among CONS specially MRCONS resistant to widely used antimicrobial agents. Hence, it is necessary to have regular surveillance which will be useful for selecting an appropriate antibiotic, to know the changing trends of the antibiotic susceptibility pattern and for limiting the use of powerful antibiotics like vancomycin and linezolid and save it for treatment of resistant and life threatening staphylococcal infections. Accuracy and promptness in the detection of species wise antibiotic resistance pattern will help in understanding definitive therapy for CONS.

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Conflict of interest: None declared.

Key points

- Sample wise and species wise distribution showed that various species of CONS have been isolated from clinically significant samples, S. epidermidis being predominant species.
- S. epidermidis & S. saprophyticus were predominant species isolated from ICU & paediatric wards causing nosocomial and device associated infections in seriously ill and immunocompromised patients
- Species wise antibiotic resistance pattern of CONS showed higher resistance to multiple antibiotics like penicillin-G(100%), cefoxitin (66%), erythromycin (60%), tetracycline (57.5%), ciprofloxacin (49.5%), Chloramphenicol (47.5%) and amikacin (42%). All 200 CONS isolates were sensitive to vancomycin and linezolid
- In the present study, 66% isolates were MRCONS and 34% isolates were MSCONS. Among MRCONS isolates, 18.18% and among MSCONS isolates, 8.82% isolates showed inducible clindamycin resistance. So, higher rate of inducible clindamycin resistance was seen among MRCONS.

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