Section: Microbiology

Original Research Article

RETROSPECTIVE STUDY ON INCIDENCE OF METHICILLIN-RESISTANT COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM VARIOUS CLINICAL SAMPLES IN A RURAL MEDICAL COLLEGE HOSPITAL

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Received : 05/08/2025
Received in revised form : 22/09/2025
Accepted : 10/10/2025

Keywords:
Coagulase-negative Staphylococci, Clinical infections, Pathogenic, Drug resistance.

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DOI: 10.47009/jamp.2025.7.5.205

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2025; 7 (5); 1086-1090

ABSTRACT

Background: Skin of human beings is densely colonized with several species of Coagulase-Negative Staphylococci (CONS), and to a lesser extent with coagulase positive Staphylococcus aureus. Increasing evidence documented from various studies have been flagging the alarming increase in etiology of methicillin-resistant CONS causing simple to fulminant infections both in the immune-compromised patients as well as other patients. **Materials and Methods:** This study was conducted at Nandha Medical College and Hospital, Erode from July 2024 till June 2025, and clinical samples such as urine, pus, blood, high vaginal swabs, body fluids, tissue and sputum which corroborated with etiological cause of infections were included in the study. All the clinical samples were subjected to direct Gram staining, culture and sensitivity. Phenotypic identification for CONS was done using standard methods. Antibiotic susceptibility testing was by Kirby-Bauer's method using Cefoxitin as surrogate marker for methicillin-resistance and various other antibiotics were also tested. **Result:** This study yielded a total count of 213 CONS isolates out of which 91.54 % (n=194) were methicillin-resistant CONS. Among MRCONS isolates (n=194), highest resistance was observed for Erythromycin (75.7%), followed by Cotrimoxazole (35.8%), Clindamycin (32.6%), Ciprofloxacin (32.3%), Tetracycline (29.23%), Gentamicin (16.78%), Nitrofurantoin (9.74%) and Linezolid (8.7%). All MRCONS isolates were sensitive to Vancomycin.
Conclusion: Implications of our study outcome confirm the overwhelming increased isolation of MRCONS as etiology in causing pathogenic infections, and it is high time that clinicians and laboratory specialists acknowledge that CONS cannot be ignored merely as a contaminant or commensal in culture and sensitivity reports henceforth.

INTRODUCTION

Micrococcaceae comprises of Gram positive spherical cocci which include Staphylococci, Micrococci and Stomatococcus. Commonest pathogen causing clinical infections are Staphylococci which are non-motile and non-spore forming catalase positive bacteria. Staphylococci are ubiquitous in nature, inhabiting skin and mucous membranes of mammals and birds. Skin of human beings is densely colonized with several species of Coagulase-Negative Staphylococci (CONS), and to a lesser extent with coagulase positive Staphylococcus aureus. CONS include numerous species such as Staphylococcus epidermis, S haemolyticus, S hominis, S warneri, S capitis, S lugdunensis, S cohnii, S.simulans and S xylosus[1].

CONS form clusters of spherical cocci, which are Gram positive, and colonies on solid media are smaller grey or white in colour. Species identification can be made by various manual biochemical tests,[2] or automated systems. Identification scheme used to identify CoNS groups/species commonly encountered in clinical practice, includes detection of heat stable nuclease, ornithine decarboxylase test, pyrollidone, urease production, Voges- Proskauer test, O-Nitrophenyl -D galacto-pyaranoside alkaline phosphatase, polymyxin B susceptibility, novobiocin

ISSN (O): 2687-5365; ISSN (P): 2753-6556

sensitivity, nitrate reduction, fermentation of glucose, maltose, sucrose, mannitol, lactose and mannose.[3]

Over the years, various studies have reinforced the increasing pathogenicity of CONS in causing simple to complex clinical infections, and few CONS species have been documented to possess virulence factors similar to that of Staph aureus.[4]

Production of slime which is a complex glycoprotein is a major armour in colonizing foreign bodies such as indwelling prosthetic bodies and vascular catheters. Slime-producing bacteria become encased in an extracellular glycocalyx, rendering them resistant to antimicrobial agents.[5]

Bio-film by Staph epidermidis specifically binds to fibronectin, fibrinogen, collagen & vitronectin as well as to the plastic catheter material using a wide spectrum of adhesin molecules such as Aae & AltE (autolysin-adhesins), Ebp (Elastin-binding protein), Empb (Extracellular matrix binding protein), and Fbe (Fibrinogen binding protein).[6]

CONS are known to be the commonest pathogens in neonatal ICUs, sternotomy wound infections, peritonitis in patients on peritoneal dialysis and bacteremia in immune-suppressed adults.[7]

Opportunistic infections such as native and prosthetic heart valve endocarditis caused by CONS is well documented.[8]

Studies have shown that patients admitted in cardiology, orthopaedic wards or neonatal ICU acquire CONS endemic in these units, colonized in health-care personnel and clinical patients, and more likely drug-resistant strains contribute to hospital-acquired infections.[9]

Among CONS, Staph. saprophyticus causes both upper and lower UTI, being the second most common cause of UTI after Enterobacteriaceae, and is found in 3 % -9 % of acute uncomplicated cystitis. Clinical outcomes include pyelonephritis, sepsis, renal calculi and rarely endocarditis.[10,11]

Methicillin resistance mediated by mec gene is highly prevalent among Staph epidermidis isolates with rates of 75 % -over 90% constituting (Staphylococcal Cassette Chromosome) SCC mec types I, IIa, IIb, III, IV, IVa- IVd and several variants, SCC mec types I-V in Staph haemolyticus, IV E in Staph warneri, III in S hominis, I,IIa,IV,V in Staph capitis, III in S sciuri, S xylosus and S lentus.[12,13]

Vancomycin resistance has already emerged on a small scale among CONS.[14] The use of pre-operative antibiotics contributes in selecting the prevalent resistant CONS strains.[15]

MATERIALS AND METHODS

This study was conducted at Nandha Medical College and Hospital, Erode after obtaining Institutional Human Ethics Committee approval. Retrospective data regarding clinical samples received in the diagnostic microbiology laboratory for culture and sensitivity from July 2024 till June 2025 was included in the study. The clinical history of patients was recorded from the case records, and those samples which corroborated with etiological cause of infections only were included in the study.

Clinical samples included urine, pus, blood, high vaginal swabs, body fluids, tissue and sputum. All the clinical samples were subjected to direct Gram staining and inoculated on sterile sheep blood agar and McConkey agar. Body fluids were centrifuged and subjected to the same process of Gram staining and culture. Aerobic incubation was done at 37 deg C for 18-20 hours, and those samples which grew colonies were initially characterized by standard conventional phenotypic detection methods of Gram staining.[16]

Samples which grew Gram positive cocci were further characterized by catalase test using 3% hydrogen peroxide, coagulase test (both tube and slide), urease test using Christensen's urea medium, mannitol fermentation, bacitracin sensitivity (0.04 units) and acetoin production by Voges-Proskauer test for differentiating between Staphylococcus aureus and Staphylococcus lugdunensis, which were validated by ATCC Staph aureus 25923 strain.

Antibiotic susceptibility testing by Kirby-Bauer disc diffusion method was done using Mueller-Hinton agar and sterile antibiotic discs were placed. Cefoxitin was used as a surrogate marker for methicillin resistance. The other discs were Co-trimoxazole, Tetracycline, Linezolid, Ciprofloxacin, Gentamicin, Nitrofurantoin for urine samples, Erythromycin and Clindamycin. Vancomycin E-Strips were used for all the isolates. The plates were incubated at 37 deg C aerobically for 18-20 hours. Pure growth in all the plates were considered for measuring zone of inhibition around the discs. The results were interpreted using CLSI 2025 guidelines as either sensitive, intermediate or resistant as shown in the following table. [17]

S.no	Antibiotic	Disc content (µg)	Zone diameters in mm		
			Sensitive	Intermediate	Resistant
1	Cefoxitin (surrogate test for oxacillin)	30	>=25	-	<=24
2	Chloramphenicol	30	>=18	13-17	<=12
3	Ciprofloxacin	5	>=21	16-20	<=15
4	Clindamycin	2	>=21	15-20	<=14
5	Cotrimoxazole	1.25/23.75	>=16	11-15	<=10
6	Erythromycin	15	>=23	14-22	<=13
7	Gentamicin	10	>=15	13-14	<=12
8	Levofloxacin	5	>=19	16-18	>=15
9	Linezolid	30	>=26	23-25	<=22

ISSN (O): 2687-5365; ISSN (P): 2753-6556

10	Nitrofurantoin(only for urine)	300	>=17	15-16	<=14
11	Tetracycline	30	>=19	15-18	<=14
12	Vancomycin (MIC)		<=4µg/ml	8-16µg/ml	>=32µg/ml

RESULTS

This study yielded a total count of 213 isolates of CONS (Coagulase-negative staphylococci)/SOSA (Staphylococci other than Staph. aureus) from various clinical samples considered pathogenic received from patients in our laboratory between July 2024 till June 2025. Out of the total 213 CONS isolates, around 91.54 % (n=194) were methicillin-resistant CONS.

Table 1: Comparison data of MRCONS isolation from clinical samples in various studies

	Name of study	Year of study	MR CONS incidence rate
1.	Bhathala et. al, [18]	2014-15	86.21 %
2.	Bhatt et. al, [19]	2011-14	32.7%
3.	Prapti Bora et al, [20]	2017	68.67%
4.	Asangi et al, [21]	2011	67.7%
5.	Abhishek Debnath et al, [22]	2016-18	70%
6.	Sharma et al, [23]	2008-9	52%
7.	Mehreen et al,[24]	2011-12	59.64%
8.	Goudarzi et al,[25]	2012-13	84.7%
9.	Khadri et al,[26]	2007	39.4%
10.	Haque et al,[27]	2007-8	56.25%
11	Laithangbam et al,[28]	2021-22	64.15%
12.	Saraswathi Jayanthi et al,[29]	2010	67%
13.	Sita Mahalakshmi et al,[30]	2015	68.18%
14	Namratha et al,[31]	2015	73.2%
15.	OUR STUDY	2025	91.54%

Sample-wise yield of MRCONS(n=194) was highest in urine (46.94%), followed by pus samples (19.71%), high vaginal swabs (18.3%), sputum (4.22%), blood culture (1.4%) and sterile body fluids (0.93%) FIG:1.

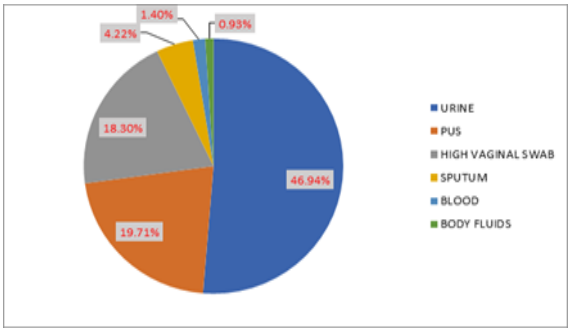

Figure 1: Distribution of MRCONS among clinical samples

Table 2: Resistance rate of CONS isolates

	MSCONS(n=18)	MRCONS(n=194)
Cotrimoxazole	22.22%	35.8%
Tetracycline	-	29.23%
Gentamicin	-	16.78%
Ciprofloxacin	5.5%	32.3%
Erythromycin	-	75.7%
Clindamycin	25%	32.6%
Nitrofurantoin	-	9.74%
Linezolid	-	8.7%

Among MRCONS isolates (n=194), highest resistance was observed for Erythromycin (75.7%), followed by Cotrimoxazole (35.8%), Clindamycin (32.6%), Ciprofloxacin (32.3%), Tetracycline (29.23%), Gentamicin (16.78%), Nitrofurantoin (9.74%) and Linezolid (8.7%). All MRCONS isolates were sensitive to Vancomycin. [Figure 2]

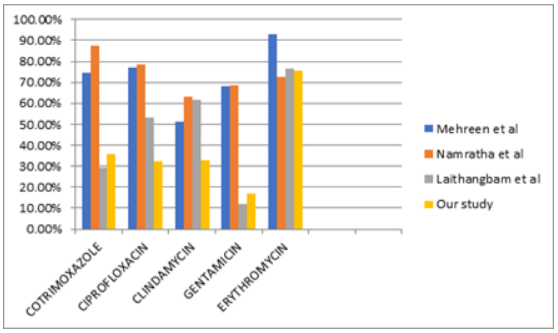

Figure 2: Comparison of Antibiotic Resistance % among other studies in Mrconsisolates

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 3: Age-Group wise distribution of MRCONS isolates

Age group	Number of MRCONS isolates
Less than 20 years	5
21-30	48
31-40	35
41-50	28
51-60	26
61-70yrs	27
71-80 yrs	13
81-90 yrs	12

DISCUSSION

Coagulase negative staphylococci (CoNS) are the commonest commensals encountered in the human species. Isolation of CONS was largely confined to being labeled as commensals or contaminants in clinical specimens received in the laboratory until few decades ago. Nevertheless increasing evidence documented from various studies have been flagging the alarming increase in etiology of CONS causing simple to fulminant infections both in the immune-compromised patients as well as other patients.

Largely prolonged hospitalization, cross-infections in health-care centres, use of prosthetic implants, poor infection-control practices, rampant use and misuse of antimicrobials has been contributing in the spread of pathogenic CONS bacteria.

Various species of CONS have been classified according to their biochemical characteristics such as Staphylococcus epidermidis, Staph. hemolyticus, S.schleiferi, S.lugdunensis, S,saprophyticus, S.xylosus, S.intermedius, S.warnieri and S.hominis. Historically, the acronym MR-CoNS (methicillin-resistant Coagulase-negative Staphylococci) is still commonly used, even though methicillin is currently not the agent of choice for testing or treatment.

It is characterised by resistance to the anti-staphylococcal, penicillinase-stable penicillins, where mec is the genetic element responsible for resistance to methicillin and mecA gene encodes for a particular pencillin-binding protein PBP called PBP2A which has a low affinity for all β-lactam antibiotics.

In our present study conducted from July 2024 till June 2025, the total number of pathogenic CONS (Coagulase-negative staphylococci) from clinical samples was 213, out of which around 91.54% (n=194) were methicillin-resistant (MRCONS).

Increasing isolation of MRCONS from clinical samples which establishes the etiology in patients has been documented in previous similar studies where incidence of MRCONS was 86.21% as per Bhathala et al,[18] 84.7%, Goudarzi et al,[25] 73.2% Namratha et al,[31] 70% Abhishek Debnath et al,[22] 68.67 % Prapti Bora et al,[20] 68.18% Sita Mahalakshmi et al,[30] 67% Saraswathi Jayanthi et al,[29] 66.4% Asangi et al,[21] 64,15% Laithangbam et al,[28] and 59.64% as per Mehreen et al,[24] [Table 1].

Regarding gender distribution in yield of MRCONS (n=194) in our study, 61 isolates were from males and 133 from female patients. The clinical sample wise yield of MRCONS in our study was highest in urine (46.94%), followed by suppurative pus samples (19.71%), high vaginal swabs (18.3%), whereas sputum yield was 4.22%, blood culture 1.4% and sterile body fluids was the lowest at 0.93%. Comparatively Namratha et al,[31] have documented the highest yield of MRCONS from pus samples (39.34%),followed by blood culture (24.04%), urine (10.92%), sputum (6.56%), high vaginal swabs (3.83%) and the least from sterile body fluids at 1 %. Bhathala et al,[18] in their study have observed MRCONS highest from blood at 90% followed by 88% from sputum samples and 80% from urine samples in their study. In a similar study conducted by Bhatt et al,[19] blood culture showed highest recovery of MRCONS at 35%, following IVC tip at 28%, urine cultures - 27% and pus samples - 9 %.

The above comparative data analysis proves that isolation and recovery of Methicillin resistant Coagulase-negative Staphylococci from various clinical samples received validates the etiology and pathogenesis in causing infections in patients.

In our study, antibiotic susceptibility testing was done by Kirby-Bauer method and multiple commonly used antibiotics were tested. Comparing both CONS and MRCONS, resistance to antibiotics were higher in MRCONS isolates [Table 2].

Among MRCONS isolates(n=194), highest resistance was seen for Erythromycin (75.7%), followed by Co-trimoxazole at 35.8%, Clindamycin 32.6%, Ciprofloxacin 32.3%, Tetracycline 29.23%, Gentamycin-16.78%, Nitrofurantoin 9.74 % and the least for Linezolid at 8.7%. All the isolates in our study were sensitive to Vancomycin.

Comparing our data with previous similar studies and their outcomes, we observed that Mehreen et al,[24] have observed similar antibiotic resistance patterns with Erythromycin resistance at 93 % of MRCONS isolates, Ciprofloxacin -77%, Cotrimoxazole-74.8%, Gentamicin -68.3%, Clindamycin -51.06 5 followed by Tetracycline -44.6 %. (FIG:2). Laithangbam et al,[28] study on MRCONS recorded Erythromycin resistance was highest at 76.5%, following Clindamycin at 61.8%, Ciprofloxacin at 53 %, Tetracycline-44.1%, Cotrimoxazole at 29.4%, Gentamicin -11.8% whereas no Linezolid resistance was reported.

Namratha et al,[31] have documented highest resistance of MRCONS isolates to Cotrimoxazole at 87.69%, Ciprofloxacin -78.69%, Erythromycin – 72.68 %, Gentamicin - 68.85 %, Clindamycin - 63.39%. Khadri et al,[26] also have recorded similar

ISSN (O): 2687-5365; ISSN (P): 2753-6556

outcomes with regards to antibiotic resistance patterns in MRCONS isolates in their study.

CONCLUSION

Implications of our study outcome herewith confirm the overwhelming increase in isolation of MRCONS as etiology in causing pathogenic infections, and it is high time that clinicians and laboratory specialists acknowledge that CONS cannot be ignored merely as a contaminant or commensal in culture and sensitivity reports henceforth. Multi-drug resistance antibiograms are becoming a reality along with increasing incidence of methicillin-resistant strains, thereby posing an immense challenge in health-care delivery systems and disease management.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556