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STUDY OF METHICILLIN RESISTANT COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM VARIOUS CLINICAL SPECIMENS

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ABSTRACT Coagulase Negative Staphylococcus being normal commensals of skin and mucus membrane are one of the leading cause of Hospital as well and Community acquired infections in humans. Present study considered 250 CONS isolates from clinical samples over a period of one year to study the trends of Methicillin resistance among them. Prevalence of MRCONS in our study is 73.2%. Predominantly affecting the productive (25.14%) and paediatric (24.59%) age groups and male population (57.92%). Maximum of MRCONS are isolated from pus samples and S. epidermidis is the most frequent isolate. Highest resistance is observed towards Nalidixic acid (98.91%), Cotrimoxazole (87.69%) and fluoroquinolones (78.69%) followed by Erythromycin (72.68%), Gentamicin (68.85%) and Clindamycin (63.39%) and lower resistance was observed towards Nitrofurantoin (7.16%), Linezolid (1.64%) and Teicoplanin (0.55%). None of the isolates were resistant to Vancomycin. The study stresses upon the significance of detection of methicillin resistance among CoNS on routine basis as MRCONS tend to be multidrug resistant and high prevalence of MRCONS in our set up drags the attention to implement effective infection control and antibiotic policies.

KEYWORDS : Cefoxitin, MRCoNS, Pus, Vancomycin

INTRODUCTION:

Coagulase-Negative Staphylococci are usually harmless commensals, have become important, commonly isolated pathogens in clinical microbiology laboratories around the world. [1] Widely used antibiotics including penicillins, cephalosporins, macrolides, aminoglycosides and tetracyclines, have proven to be ineffective against several prevalent species of CONS, thus augmenting the need for new and effective antimicrobials. [1, 2] Highly penicillin-resistant *S. epidermidis* isolates, responsible for fatal subacute bacterial endocarditis, were being reported as early as 1949.[3] This phenotype is caused by penicillinases, first described by Kirby in 1944.[4] In staphylococci the expression of an additional penicillin-binding protein (PBP2a) has considerably reduced binding affinities for -lactam antibiotics, in contrast to the intrinsic set of staphylococcal PBPs leading to complete -lactam resistance (to penicillins, most cephalosporins and carbapenems) with the only exception being recently introduced cephalosporins with MRSA activity, such as ceftobiprole and ceftaroline. [5]

Over the course of a 20-year study (1986 to 2005) in Zurich, Switzerland, the percentage of MRCoNS isolates recovered from burn patients increased from 11% to 55%. [4] The prevalence of oxacillin resistant *S. epidermidis* and *S. haemolyticus* isolates has now reached about 80% or more. CONS have historically been more resistant to antimicrobials than *S. aureus.* CONS form biofilms on device materials and devitalized tissue and turn out to be more resistant to penetration by antimicrobial agents. [6] Multiple antibiotic resistances are characteristics of hospital strains of CoNS that tend to carry plasmids and these resistance plasmids are transferable between different strains of CoNS and also between *Staphylococcus aureus*.[7]

Multi resistance in CONS as in *S. aureus* is carried out on a Staphylococcal chromosome cassette (SCC) which almost always includes the *mec*A gene for resistance to semi-synthetic penicillins (SCC*mec*). [8] Vancomycin resistance has also been emerged among the CoNS species and these resistant isolates were reported long before the advent of the first *S. aureus* isolates with reduced glycopeptide susceptibility, in 1997. [4]

Valid methicillin resistance determination is a necessity for CoNS since in several circumstances such as endocarditis and other invasive processes the use of -lactams should not be excluded *a priori*. Conversely, misidentification of MR-CoNS isolates as methicillin susceptible may lead to fatal treatment failure. [4, 9] According to the recent CLSI and EUCAST cefoxitin screening based on a disc diffusion assay (disc content of 30µg) is used as a surrogate marker for the determination of *mecA*-mediated methicillin resistance. [10,11]

In a view of changing trends of antibiotic resistance among MRCoNS isolates, this study was undertaken to find the current trends in our hospital set up using simple convention methods.

MATERIALS AND METHODS:

This cross sectional study is conducted from January 2015 to December 2015 in department of Microbiology, Karnataka Institute of Medical Sciences Hubballi. Various clinical specimens received were inoculated onto Chocolate agar, CoNS isolates were identified and speciated based on Standard conventional methods. [12] Methicillin resistance was detected by using cefoxitin (30µg) disc. Isolates showing less than 24 mm zone of inhibition around cefoxitin disc were considered as MRCONS and included in the study [10] Antimicrobial susceptibility test was performed by Kirby-Bauer's disc diffusion method with the following panel of antibiotics.

Amoxicillin/Clavulanic acid (20/10µg), Gentamicin (10µg), Ciprofloxacin (5µg), Clindamycin (2µg), Erythromycin (15µg), Trimethoprim/Sulphamethoxazole (1.25/23.75µg), Linezolid (30µg), Vancomycin (30µg), Teicoplanin (30µg), Nitrofurantoin (300µg) and Norfloxacin (10µg).

Standard reference strain of Staphylococcus aureus ATCC 25923 was included and parallel tests were carried out for quality control. Interpretation of the results was done using CLSI guidelines.[10]

RESULTS:

Among 250 CoNS species isolated over a year, 183 species showed resistance to cefoxitin, constituting 73.2% of MRCoNS. Among which 57.92% of MRCoNS are isolated from male patients and 42.08% from female patients. Highest occurrence of MRCoNS is seen in the age group of 21-30 years (25.14%) followed by neonates (15.85%). (Table-1)

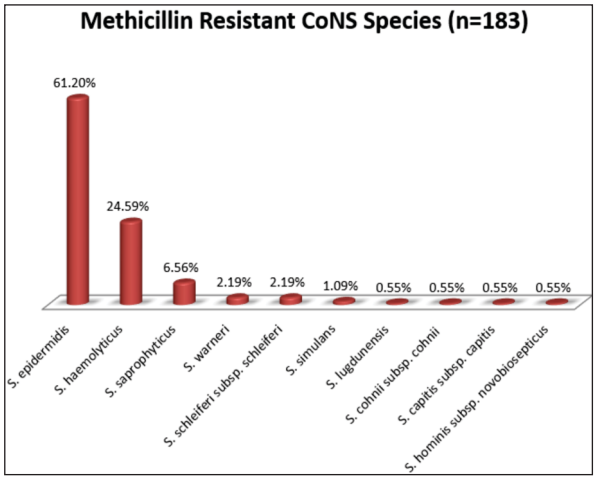
Table 01: Age wise distribution of MRCoNS species isolated

Age group	Number of CoNS species (n=183)		Percentage	
Day 1 – Day 30	29	45	15.85%	24.59%
1Month -1Year	04		02.19%	
2-10 year	12		06.56%	
11-20 year	14		07.65%	
21-30 year	46		25.14	
31-40 year	17		09.29%	
41-50 year	21		11.48%	
51-60 year	16		08.74%	

61-70 year	16	08.74%
71-80 year	07	03.82%
81-90 year	01	00.55%
Total	183	

Maximum numbers of the MRCoNS are isolated from the samples from various wards (57.38%), followed by ICUs (30.06%). Among 55 ICU isolates, 29 (15.85%) are obtained from NICU samples. 10.38% of total MRCoNS are isolated from OPD clinics. And 2 samples each received from Labour room and Cath lab (1.09%).

S. epidermidis accounted the maximum (61.20%) followed by *S. haemolyticus* (24.59%) among different species of CoNS (Graph -1). Majority of MRCoNS are isolated from pus sample constituting 39.34% followed by Blood samples (24.04%) (Table-2)



Graph – 1: Species wise Distribution of MRCoNS

Table 02: Sample wise Distribution of MRCoNS

Samples	MRCoNS (n=183)	
	Number	Percentage
Pus	72	39.34%
Blood	44	24.04%
Urine	20	10.92%
Ear discharge	13	7.10%
Sputum	12	6.56%
CSF	09	4.92%
Vaginal swab	04	2.19%
Cervical swab	03	1.64%
Devices	03	1.64%
Throat swab	01	0.55%
Ascitic fluid	01	0.55%
Pleural fluid	01	0.55%

Among underlying risk factors, 95.25% MRCoNS are isolated from patients who were on antibiotics, 89.62% among hospitalized patients; among which 30.06% were from ICUs and 29.51% of the patients had history of surgery, 43.72% were having in situ foreign bodies, common ones being I. V. catheters, Suture and Implants. 26.23% of them were associated with the underlying conditions like diabetes mellitus accounting for 16.39%. (Table-3)

Table 03: Underlying risk factors among patients with MRCoNS infections (n=183)

Risk factors		No. of MRCoNS isolated (n=183)	Percentage
H/O of antibiotics		156	95.25%
H/O surgery		54	29.51%
ICU admission		55	30.06%
	NICU	29	15.85%
	PICU	7	3.83%
	SICU	7	3.83%
	OICU	7	3.83%
	MICU	5	2.73%
Foreign body insitu		80	43.72%
	I.V. Catheter	36	19.67%
	Suture	22	12.02%
	Implant	16	8.74%
	Urinary Catheter	1	0.55%
	CVP	3	1.64%
	Abdominal Drain	1	0.55%
	ICD drain	1	0.55%
Underlying condition		48	26.23%
	DM	30	16.39%
	Pregnancy	8	4.37%
	COPD	5	2.73%
	Cancer	3	1.64%
	HIV	1	0.55%
	CKD	2	1.09%
	Pulm. TB	1	0.55%
	Anemia	1	0.55%
	SAM	1	0.55%
	Liver cirrhosis	1	0.55%
Hospitalized patients HA-MRCONS		164	89.62%

Most of the MRCoNS isolates are multidrug resistant. Highest resistance is observed towards Nalidixic acid (98.91%), Cotrimoxazole (87.69%) and fluoroquinolones (78.69%) followed by Erythromycin (72.68%), Gentamicin (68.85%) and Clindamycin (63.39%) and lower resistance was observed towards Nitrofurantoin (7.16%), Linezolid (1.64%) and Teicoplanin (0.55%). None of the isolates were resistant to Vancomycin. Of the 183 MRCoNS isolates 19 (10.38%) are community acquired and 164 (89.62%) are hospital acquired. Among various antibiotics tested, there is no much statistically significant difference between the resistance patterns of CA-MRSA and HA-MRSA. (Table-4)

Table 04: Resistance pattern of HA-MRCoNS and CA- MRCoNS

ANTIBIOTICS	TOTAL		HA-MRCoNS		CA-MRCoNS	
	Number of Resistant MRCONS	Percentage	No	%	No.	%
Amoxicillin-clavulanic acid	183	100%	164	100 %	19	100 %
Gentamicin	126	68.85%	116	70.73%	10	52.63%
Ciprofloxacin	141	77.055	128	78.05%	13	68.42%
Clindamycin	116	63.39%	104	63.72%	12	63.16%
Erythromycin	133	72.68%	119	72.56%	14	73.68%
Cotrimoxazole	161	87.98%	144	87.79%	17	89.78%
Norfloxacin	144	78.69%	130	79.27%	14	73.68%
Nitrofurantoin	13	7.16%	12	07.32%	01	05.26%
Linezolid	03	1.64%	02	01.22%	01	05.26%
Teicoplanin	01	0.55%	01	00%	00	00%
Vancomycin	0	0%	00	00%	00	00%
Nalidixic Acid	181	98.91%	162	98.78%	19	100 %

DISCUSSION:

The susceptibility of CoNS to antimicrobial agents is extremely variable. Although community-acquired isolates are frequently susceptible to a wide variety of agents, strains isolated from hospitalized patients that have been exposed to antibiotic selection pressure in the health care environment have been noted to be resistant to an increasing number of antibiotics. The main aim of our study was to identify the prevalence of Methicillin resistance among clinically isolated CONS along with to study their Antimicrobial susceptibility pattern and to analyse their trends in relation with patient's demographic profile, clinical specimen, species and risk factors. Current study has studied antimicrobial resistance patterns of 183 MRCoNS in the tertiary care hospital using conventional methodology for the antimicrobial susceptibility test using routine anti-staphylococcal antibiotic panel and analysed the results by comparing with similar studies conducted all over the country and globe.

In the present study prevalence of MRCONS is 73.2%. Occurrence of methicillin resistance among CoNS ranges from 19.2% to 84.7% in various other studies. (Table-4) Study conducted by Gunti R et al [13] shows similar prevalence of MRCoNS (72%). However study done by Goudarzi M et al [14] revealed even more higher rate of MRCONS

(84.7%) and lower incidence of MRCoNS was seen in the studies conducted by Mila et al [15] (19.2%) and Mohan U et al [16] (20.8%).

Table 05: Comparison of prevalence of MRCoNS:

Study	Year of study	% of MRCoNS.
Present study	2015	73.2%
Gunti et al [13]	**2015**	**72%**
Goudarzi et al [14]	2012-13	84.7%
Mila V S et al [15]	--	19.2%
Kumar S et al [17]	--	23.2%
Ahmed R et al [18]	2015-2016	52.83%
Ahirwar S S et al [20]	--	20.6%
Sardar et al [21]	2015	52%
Karigoudar R et al [22]	2016	40%
S. Mohan et al [23]	2017	33%
Singh S et al [24]	--	51.6%
Gilani et al [27]	2011-12	59.64%
Mir B A et al [28]	2011-2012	40%
Shah M U et al [31]	2008-11	40.01%
Mane P et al [32]	2009-12	68.93%
C. Roopa et al [33]	2013	33%
Usha M G et al [34]	--	56%
Asangi et al [35]	--	67.7%
Goliya S et al [36]	2013-14	66.4%

Male preponderance is seen in the present study similar to the study conducted by Kumar S et al [17] but contrasting results are seen in the studies conducted by Ahmed R et al [18] and Jogender J et al [19] where maximum of MRCONS are isolated from female patients. All the age groups from 0 to 90 years are affected by MRCONS infections among all most affected belongs to productive age group of 21 to 30 years (25.14%) and paediatric age group of 0 to 10 years (24.59%) followed by 41 to 50 years (11.48%). Similar observation is noticed in the study conducted by Ahmed R et al. [18] Similarly productive age group (33.33%) remains most affected in study done by Jogendar J et al [19] but 0 to 10 years belongs to least affected age group (5.55%) in their study.

Among 183 CONS maximum of methicillin resistance was observed in *S. epidermidis* (61.20%) followed by *S. haemolyticus* (24.59%) and then other species. Similar trend is seen in most of the studies. [15, 20-22] contrastingly methicillin resistance was more frequent among *S. haemolyticus* in few other studies [23-25]

Among various clinical samples Pus yielded maximum of isolates (39.34%) which is similar to the study conducted by S Mohan et al [23]. (44.04%) and Hajera M et al [26] (40%). 24.04% of MRCONS are isolated from blood stream infections in the present study which is comparable to study conducted by Hajera M et al [26] (20%) whereas in studies done by Ahmed R et al [19] , and Gilani M et al [27] maximum of MRCONS are isolated from Blood samples. Urine is 3rd frequent sample to yield MRCONS in the present study (10.92%) similar to the study conducted by Hajera M et al [26] (13.33%) however in the study conducted by Karigoudar et al [22] 52% of MRCONS are isolated from urine specimen alone. Most of the MRCoNS isolates are multidrug resistant. Similar trends are observed in most of the studies. [18, 19, 26-29] Comparatively higher resistance was noticed in the studies conducted by Karigoudar et al. [22] However studies conducted by Singh et al [24] and Kumar S et al [17] show Gentamicin and Clindamycin to be most effective antibiotic against MRCONS. None among 183 MRCONS showed resistance towards Vancomycin in the present study. This is in good correlation with other studies. [18,24,28]

Present study isolates 73.2% of MRCoNS which is substantially very high. The reasons for increased incidence of MRCoNS may be due to indiscriminate use of the antibiotics, poor hospital infection control practices, weak antibiotic policies, irrational antibiotic therapy, such as no control over the prescription pattern, usage of high level and broad spectrum antibiotics in the presumptive therapy. Chronic infections being treated with antibiotics alone without considering other treatment modalities to eliminate the focus of infection, i.e. removal of foreign bodies etc.

CONCLUSION:

The present study reveals that MRCONS tend to be multi drug resistant towards most of the available antibiotics in our setting. The lower level of resistance against Nitrofurantoin, Linezolid, Teicoplanin and no resistance against Vancomycin turns them to be the most effective antibiotic in the treatment of infections caused by MRCONS. Such high occurrence of MRCoNS in clinical set up indicates the need for compulsory antibiotic susceptibility testing of clinical isolates and detection of methicillin resistance among them along with implementation of effective antibiotic and infection control policies in the hospital.

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