



PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF METHICILLIN RESISTANCE *STAPHYLOCOCCI* ISOLATED IN A TERTIARY CARE HOSPITAL OF RURAL GUJARAT, INDIA

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ABSTRACT

Antibiotic resistance, a global concern, is particularly emerging in developing nations, including India; where the burden of infectious disease is high and healthcare spending is low. Methicillin resistant *S. aureus* (MRSA) and Methicillin resistant coagulase negative *Staphylococci* (MRCoNS), are significant pathogens of concern causing both nosocomial and community acquired infections associated with increased morbidity and mortality. A prospective cross-sectional study was therefore conducted to determine the prevalence and antimicrobial sensitivity of Methicillin resistant Staphylococci isolated at Shree Krishna Hospital, Karamsad, Gujarat, India from June 2012 to August 2014. Identification and antimicrobial susceptibility testing (AST) were carried out by VITEK 2 Compact and the Methicillin resistance was confirmed by the modified Kirby Bauer Disc Diffusion method as per CLSI- 2012 guidelines. A total of 14,415 clinical specimens were processed, of which 707 Staphylococcal isolates were obtained. Among which, 362 (51.2%) were *S. aureus* and 345 (48.8%) were coagulase negative *Staphylococci* species. The prevalence of MRSA and MRCoNS were found to be 44.8% and 78.8% respectively. Methicillin resistant isolates showed high level of resistance to Penicillin followed by Erythromycin, Levofloxacin, Co-trimoxazole, Gentamicin and Clindamycin. Low level of resistance was seen against Vancomycin. Linezolid-resistant strain of Staphylococci was not found at all in the present study. Methicillin resistant strains showed higher antimicrobial resistance as compared with Methicillin sensitive strains. Thus, a continuous surveillance of Methicillin resistant Staphylococci (MRS) is essential for proper guidance of antimicrobial therapy and to minimize the irrational use of reserved antibiotics.

Key words: MRSA, MRCoNS, Staphylococcal isolates, multidrug resistance, risk factors

INTRODUCTION

Historically, *S. aureus* has been the major species as a leading cause of disease ranging from mild skin and soft tissue infections to life threatening illness and coagulase negative *Staphylococci* (CoNS) were encountered as a contaminant or rare pathogen. Despite this, they are associated with a myriad of disease like urinary tract disease, catheter related infections, shunt infections, pneumonia, endophthalmitis surgical wound infections, breast abscess, osteomyelitis, and native valve endocarditis.^[1]

Methicillin resistance is of special concern not only because of its resistance to methicillin but also because it is generally resistant to many others chemotherapeutic agents.^[2] In India, the prevalence

rate of MRSA and MRCoNS varies from 17.2% to 84% and 22.5% to 73.5% respectively.^[3-8] While in other countries MRSA varies from 1% to 54% and CoNS has shown significant increase in antibiotic resistance trend in a 13 year study of USA.^[7,9] They causes both nosocomial and community acquired infections resulting in increased morbidity and mortality.^[2]

Emergence of resistance against Vancomycin and Linezolid, the reserved drugs for the treatment of MRSA infections suggests the importance of antibiogram of MRS to minimize the irrational use of reserved antibiotics.^[10,11]

It is therefore decided to study the scenario of the Staphylococcal species distribution in different clinical specimens, to assess the prevalence and antimicrobial susceptibility pattern of the clinical

isolates in this area and their association with the co-morbid condition.

MATERIAL AND METHODS

Selection and description of participants:

This was a prospective cross-sectional study carried out after Institutional Human Research Ethical Committee, in a 550 bedded tertiary care hospital at Karamsad, located in rural Gujarat, India. The study included those patients from whom *Staphylococci* have been isolated from various clinical specimens submitted to Microbiology Laboratory for culture and sensitivity and excluded the specimens where *Staphylococci* isolates have been considered due to Laboratory or skin flora contamination as confirmed on the basis of clinical co-relation.

Technical Information:

All the clinical specimens were processed according to Standard Operating Procedure and the antimicrobial profile were determined as per CLSI (Clinical Laboratory Standard Institute) 2012 guidelines. Blood and body fluids were collected in BacT/Alert bottle and loaded in BacT/Alert® system (Biomerieux, France). Further processing was done on detection of growth in the BacT/Alert® system. All the clinical specimens and positive detected samples from BacT/Alert® were primarily inoculated on their respective culture plates and incubated at 37^o C overnight aerobically. Following Gram stain, catalase and coagulase test, further identification and AST pattern were carried out via Vitek 2 Compact® system (Biomerieux, France) and the confirmation of methicillin resistance was interpreted by Kirby Bauer Disc Diffusion method using Cefoxitin (30µg) disc. Recommended quality control procedures were regularly carried out. As per CLSI 2012 guideline, the used antibiotic panel was Penicillin (10µg), Erythromycin (15µg), Clindamycin (2µg), Co-

trimoxazole (1.25/3.75µg), Tetracycline (30µg), Levofloxacin (5µg), Gentamicin (10µg), Vancomycin (30µg) and Linezolid (30µg). For urinary pathogens, Norfloxacin (10µg), Nitrofurantoin (300µg) were used additionally. The data was recorded and analyzed using Microsoft Excel 2010. Results are presented in frequency and percentage.

RESULTS

A total of 14,415 clinical specimens were processed during June 2012 to August 2014, of which 707 Staphylococcal strains were isolated. Demographic profile of patients from whom 707 Staphylococci were isolated, showed 456 (64.5%) were male and 251 (35.5%) were female; majority of the Staphylococcal isolates (28.4%) were isolated from the age group of 50-64 years. Maximum MRSA were isolated in the age group of 35-49 and 50-64 years while MRCoNS were isolated in the age group of ≤ 1 year.

Among these 707 strains, 362 (51.2%) were *S. aureus* and 345 (48.8%) were CoNS. Overall prevalence rate of Methicillin resistant strain is 61.4% and Methicillin sensitive strain is 38.6%. Methicillin resistance is higher in CoNS i.e 78.8% as compared to *S. aureus* i.e. 44.8%. MRSA showed high frequency in Pus (69.1%) while MRCoNS showed in Blood (57%).

The most common isolates among CoNS were *S. hemolyticus* (18.7%); followed by *S. epidermidis* (13.3%), *S. hominis* (8.5%). The frequency of the staphylococcal species is shown in table 1. Although the *S. aureus* predominates in the present study, the Methicillin resistant strains found to be higher in *S. hemolyticus*. *S. aureus* predominates in pus, indwelling respiratory device and respiratory secretions while *S. hemolyticus* predominates in blood, body fluid and CVP-tip (as shown in table 2).

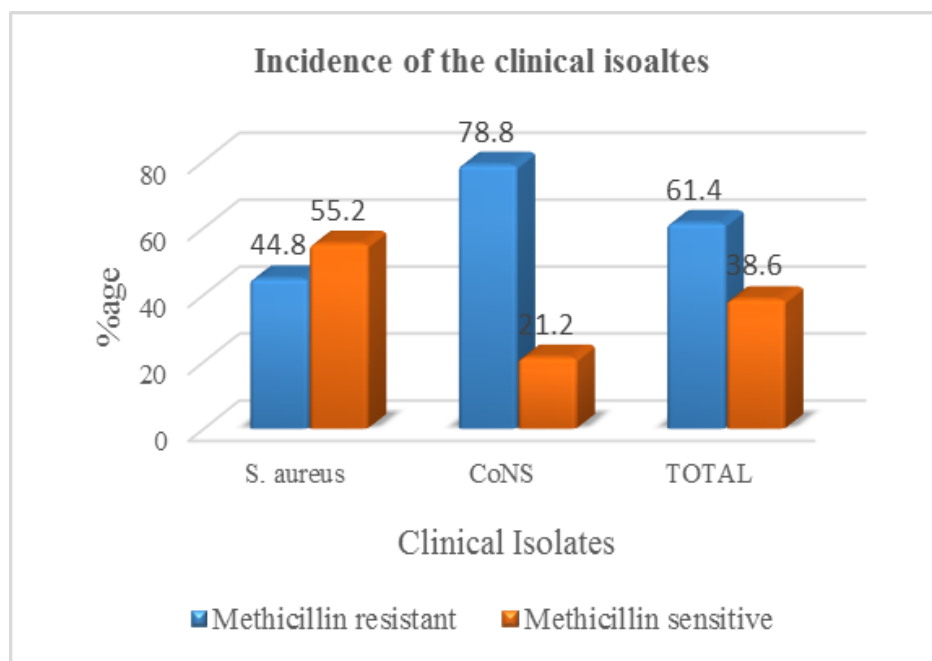


Figure 1: Prevalence of the clinical isolates

Table 1: Frequency of the Staphylococcal species isolates with their Methicillin sensitivity pattern

<i>Staphylococcal spp.</i>	Methicillin resistance (%)	Methicillin sensitive (%)	TOTAL (%)
<i>S. aureus</i>	162 (44.8)	200 (55.2)	362 (51.2)
<i>S. hemolyticus</i>	128 (97)	4 (3)	132 (18.7)
<i>S. epidermidis</i>	73 (77.7)	21 (22.3)	94 (13.3)
<i>S. hominis</i>	44 (73.3)	16 (26.7)	60 (8.5)
<i>S. intermedius</i>	2 (33.3)	4 (66.7)	6 (0.8)
<i>S. saprophyticus</i>	4 (57.1)	3 (42.9)	7 (1)
<i>S. lugdunensis</i>	0 (0)	4 (100)	4 (0.6)
<i>S. warneri</i>	4 (66.7)	2 (33.3)	6 (0.8)
<i>S. gallinarum</i>	0 (0)	4 (100)	4 (0.6)
<i>S. xylosus</i>	2 (50)	2 (50)	4 (0.6)
<i>S. capitis</i>	1 (50)	1 (50)	2 (0.3)
<i>S. cohnii</i>	2 (100)	0 (0)	2 (0.3)
<i>S. auricularis</i>	0 (0)	1 (100)	1 (0.1)
<i>S. arlettae</i>	1 (50)	1 (50)	2 (0.3)
<i>S. caprae</i>	1 (100)	0 (0)	1 (0.1)
<i>S. lentus</i>	0 (0)	2 (100)	2 (0.3)
<i>S. sciuri</i>	0 (0)	2 (100)	2 (0.3)
<i>S. simulans</i>	0 (0)	2 (100)	2 (0.3)
<i>Other CONS</i>	10 (71.4)	4 (28.6)	14 (2)
TOTAL	434 (100)	273 (100)	707 (100)

Table 2: Distribution of clinical specimen amongst different *Staphylococci* spp.(n=707)

<i>Staphylococcal spp.</i>	Pus	Blood culture	IRD	Resp. secre.	Urine	CVP-tip	Body fluids	Other
<i>S. aureus</i>	265 (77.5)	40 (17.6)	21 (70.0)	14 (48.3)	6 (24)	1 (7.1)	9 (33.3)	6 (46.2)
<i>S. hemolyticus</i>	15 (4.4)	76 (33.5)	5 (16.7)	10 (34.5)	6 (24)	6 (42.9)	11 (40.7)	3 (23.1)
<i>S. epidermidis</i>	31 (9.1)	50 (22.0)	3 (10.0)	2 (6.9)	-	5 (35.7)	-	3 (23.1)
<i>S. hominis</i>	7 (2.0)	46 (20.3)	-	-	2 (8)	2 (14.3)	3 (11.1)	-
<i>S. arlette</i>	2 (0.6)	-	-	-	-	-	-	-
<i>S. auricularis</i>	-	-	1 (3.3)	-	-	-	-	-
<i>S. capitis</i>	-	1 (0.4)	-	-	-	-	1 (3.7)	-
<i>S. caprae</i>	-	-	-	-	-	-	1 (3.7)	-
<i>S. cohnii</i>	-	1 (0.4)	-	-	-	-	1 (3.7)	-
<i>S. gallinarum</i>	2 (0.6)	2 (0.9)	-	-	-	-	-	-
<i>S. intermedius</i>	6 (1.8)	-	-	-	-	-	-	-
<i>S. lentus</i>	-	-	-	-	2 (8)	-	-	-
<i>S. lugdunensis</i>	3 (0.9)	-	-	-	-	-	1 (3.7)	-
<i>S. saprophyticus</i>	-	2 (0.9)	-	-	5 (20)	-	-	-
<i>S. sciuri</i>	2 (0.6)	-	-	-	-	-	-	-
<i>S. simulans</i>	2 (0.6)	-	-	-	-	-	-	-
<i>S. warneri</i>	-	4 (1.8)	-	-	2 (8)	-	-	-
<i>S. xylosus</i>	4 (1.2)	-	-	-	-	-	-	-
Other CONS	3 (0.9)	5 (2.2)	-	3 (10.3)	2 (8)	-	-	1 (7.7)
TOTAL	342	227	30	29	25	14	27	13

Where IRD (Indwelling respiratory devices) = ET/TT/TS, Respiratory secretions= Sputum and BAL, Body fluid= CSF/Ascitic/ Pleural/ Peritoneal and Synovial fluid, Other=Tissue/Genital and Eye swab [Note: Figures in parentheses indicates percentage]

Table 3: Prevalence of antibiotic resistance in clinical isolates (n=707)

Clinical Isolates	% of resistance to different antibiotics								
	P	ER	CD	COT	TE	LE	GEN	VA	LZ
MRSA	99.4	71.6	42.6	63	9.9	72.8	45.1	0.6	0
MRCoNS	98.5	82.4	54.8	61.4	32.7	63.2	52.6	0.4	0
MSSA	82	47	14.5	61	7.5	55	6.5	0	0
MSCoNS	47.9	24.7	20.5	31.5	4.1	8.2	4.1	0	0

Where P=Penicillin, ER=Erythromycin, CD=Clindamycin, COT=Co-trimoxazole, TE=Tetracyclin, LE=Levofloxacin, VA=Vancomycin, LZ=Linezolid

Table 4: Risk factors association with Methicillin Resistant infection (n=658)

Risk factors	Methicillin resistant (n=419)	Methicillin sensitive (n=239)	p-value	odd ratio (C.I.)	χ^2 cal value
History of previous hospitalization in last 1 year	168	47	0.000001*	2.73 (1.88-3.97)	28.87
Indwelling devices	51	15	0.0000003*	2.58 (1.78-3.75)	25.88
Surgery in last 30 days	30	5	0.000014*	2.75 (1.72-4.40)	18.83
Diabetes mellitus	76	34	0.02*	0.66 (0.47-0.94)	5.07
Hypertension	44	22	0.8	0.9 (0.66-1.42)	0.018
Neoplastic condition	23	6	0.07	1.78 (0.93-3.41)	3.12
Steroid use	5	3	0.9	1.01 (0.51-2.0)	0.003
Liver dysfunction	21	4	0.0002*	2.89 (1.61-5.18)	13.67
Renal dysfunction	45	14	0.014*	1.73 (1.10-2.70)	5.94
Duration of hospitalization >2 days (n=586)	131	22	0.000000*	4.56 (2.79-7.45)	41.46

Note: * indicates significance at 5%

The sensitivity pattern of the clinical isolates is shown in table 3. Methicillin resistant strains showed high level of resistance to Penicillin followed by Erythromycin, Levofloxacin, Co-trimoxazole, Gentamicin and Clindamycin. Low level of resistance has been seen for Vancomycin and no resistance seen against Linezolid. Table 4 shows significant correlation between Methicillin resistant *Staphylococci* infection and clinical risk factors such as history of previous hospitalization in last 1 year, indwelling devices, surgery in last 30 days, diabetes mellitus, liver dysfunction, renal dysfunction & more than 2 days of hospitalization.

DISCUSSION

Antibiotic resistance, a global concern, is particularly emerging in developing nations, including India, where the burden of infectious disease is high and healthcare spending is low.^[12] There were earlier harbingers about the MRSA of concern but during 1970s it became clear that methicillin resistance was more prevalent in CoNS (MRCoNS) than in *S. aureus* (MRSA), an observation that continues to be true today.^[13]

Indian Network of Surveillance of Antimicrobial Resistance (INSAR) group^[6], a multi hospital based study with support from World Health Organization, held in 2008-2009 in different parts of India to monitor the magnitude of antibiotic resistance

problem in India, showed diverse prevalence of MRSA in different region lowest 21% from Hyderabad to 84% from Imphal.

An overall prevalence rate of MRSA reported by INSAR group is 42% in 2008 and 40% in 2009. Comparatively, a higher MRSA prevalence in some parts of the country ranging from 46% to 54.2%, as well as, a lower prevalence rate of MRSA from 17.2% to 39.6% in other have also been reported.^[2,5,10,14-16]

Available literature from this region indicates the prevalence of MRSA ranging from as low as 16.27% to as high as 57%.^[6,17] Present study shows 44.8% prevalence rate of MRSA in a tertiary care hospital located in rural Gujarat. Types of patients included and sample size are important among many other factors responsible for a vast geographical variation in MRSA prevalence.

On the other hand MRCoNS, the opportunist pathogen causing wide spectrum of disease has shown high level prevalence. MRCoNS reported by SENTRY in Canada, Unites States, Latin America, Europe, West pacific ranges from 70-80%.^[18] A study from Iran has reported 94% MRCoNS.^[19] In our study, the prevalence rate of MRCoNS found to be 78.84% which is higher than other studies from India, which are ranging from 22.5% to 73.5%.^[2-4,7,8,20-22] This increasing and isolation rate of MRCoNS is alarming because of its self- involvement in the diseased

condition and possibility of transferring the *mecA* gene to *S. aureus*. Therefore continuous monitoring, strict antibiotic policies and resistance surveillance program is mandatory, which will contribute in implementation of infection control measures.

S Kumar et al has reported pus to be the maximum source of isolation of all clinical isolates including MRCoNS and MSCoNS.^[5] And Khadri and Alzohairy^[2] has shown the maximum MRSA amongst wound swab and pus while MRCoNS amongst urine followed by pus. The present study has shown frequent isolation of MRSA from pus while MRCoNS from blood.

In the present study, nineteen different Staphylococcal species from various clinical specimens were obtained as a clinical pathogen. Majority of the Staphylococcal species found in this study is *S. aureus* while in case of CoNS, *S. hemolyticus* predominated followed by *S. epidermidis* and *S. hominis*. Other species were less frequent ($\leq 1\%$) in occurrence. Many studies have reported *S. epidermidis* to be the most leading species among CoNS, which varies from this study.^[7,8,20-22]

Singh M et al and J Nagasudha Rani have reported nine and six different CoNS species respectively. In both the studies *S. epidermidis* predominates followed by *S. saprophyticus*, *S. hemolyticus* and *S. hominis*. The isolation rate of *S. epidermidis* and *S. hemolyticus* was frequent from blood while *S. saprophyticus* and *S. hominis* were frequently isolated from urine.^[18,19] In the present study, major CoNS like *S. hemolyticus*, *S. epidermidis*, *S. hominis* has shown its majority in blood and *S. saprophyticus* were frequent in urine which favors these studies. Blood to be the commonest source of CoNS has been reported in few studies which correlates our study.^[7,8,21] But few other studies are also there which has shown pus, wound swab and urine to be the commonest source of CoNS.^[2,19,22]

The majority of *S. aureus* were obtained from pus followed by blood, indwelling respiratory devices, respiratory secretions. Similar finding has been reported by INSAR group and Tiwari et al.^[6,10] Its maximum occurrence in pus, as seen in other studies too, signifies its association with abscess formation.

The urinary pathogen reported by M. Singh et al^[21] are *S. saprophyticus*, *S. hominis*, *S. lugdunensis*, *S. cohnii*, *S. capitis*, *S. hemolyticus* and *S. epidermidis*. Many studies has reported *S. saprophyticus* as the

leading urinary pathogen.^[1,18,21] Sharma et al has reported *S. hemolyticus* as predominant urinary isolate.^[22] In the present study the urinary isolates were *S. aureus*, *S. hemolyticus*, *S. saprophyticus*, *S. hominis*, *S. lentus*, and *S. warneri*. And *S. lentus* and *S. hemolyticus* has shown its majority in urine.

In view of antibiotic sensitivity pattern of the clinical isolate, INSAR group has reported a significant difference of erythromycin, clindamycin, gentamicin, co-trimoxazole and ciprofloxacin susceptibility amongst MRSA and MSSA.^[6] Sharma et al listed a significant difference for ciprofloxacin, gentamicin and amikacin susceptibility amongst MRCoNS and MSCoNS.^[21] Khadri and Alzodairy^[2] have also stated the resistance rate to different antibiotics among methicillin resistant were higher compared to that of methicillin sensitive which correlates the present study.

Eshwara VK et al^[23] has reported that previous hospitalization in last 1 year is a significant health care associated risk factor for acquiring CA-MRSA infection and other co-morbid conditions like diabetes, hypertension, neoplastic conditions, liver and renal dysfunction were non-significant. But the present study has shown a significant co-relation between Methicillin resistant *Staphylococci* infection and clinical risk factors such as history of previous hospitalization in last 1 year, indwelling devices, surgery in last 30 days, diabetes mellitus, liver dysfunction, renal dysfunction & hospitalization of more than 2 days.

CONCLUSION

MRSA and MRCoNS are still important pathogens among hospital as well as community associated infections and these strains have also been carrying high level of resistance to other antimicrobials too. It is therefore suggested that a continuous surveillance of Methicillin resistant *Staphylococci* is essential for proper guidance of antimicrobial therapy and to minimize the irrational use of reserved antibiotics.

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