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COMPARISON OF COMMUNITY AND HOSPITAL-ACQUIRED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN THE HOSPITAL IN-PATIENT AND OUT-PATIENT SETTINGS OF NORTH KARNATAKA

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ABSTRACT

Background: *Over the years, the misuse of antibiotics has become a significant factor in the emergence of resistant bacteria. Methicillin-resistant Staphylococcus aureus (MRSA) is staphylococci that are resistant to Beta-lactam drugs. There are two types of MRSA infections: community-acquired MRSA (CA-MRSA) and hospital-acquired MRSA (HA-MRSA).* ***Objective:*** *This study was aimed to determine comparison of community-acquired and hospital-acquired methicillin-resistant Staphylococcus aureus isolated from clinical samples of in-patient and out-patient settings of North Karnataka.* ***Materials and methods:*** *The clinical samples were collected from anterior nares of the patients and their accompanists, pus of the wounds of patients attending out-patient department and various in-patient departments' different hospitals of North Karnataka. The samples were inoculated in nutrient broth for enrichment of S. aureus then streaked on Mannitol Salt Agar (MSA) and incubated at 37 °C for 24 h. For characterization of the S. aureus, conventional methods such as growth characteristics on selective medium, Gram's staining, and biochemical characteristics have been performed. Antibiotic sensitivity test and screening for MRSA were carried out for the isolated S. aureus using 12 different antibiotics.* ***Results:*** *A total of 593 samples were collected from which 265 strains of S. aureus were isolated. Among the 265 S. aureus isolates, 76 had been confirmed as MRSA. Clinical data as well as antimicrobial susceptibility data was analyzed and compared. In conclusion, our results suggest that the prevalence rate of HA and CA-MRSA are 17.35%% and 11.32% respectively.*

KEY WORDS

CA-MRSA, HA-MRSA, comparison, antibiotics, North Karnataka.

INTRODUCTION

Staphylococcus aureus is one of the most common human pathogens with ability to cause a wide range of infections. On an average 20-40% of the adults are carriers of *S. aureus* in the anterior nares [1]. The emergence of community-acquired (CA) and hospital acquired (HA) methicillin resistant *S. aureus* (MRSA) has led to increasing in cases of invasive infections [2, 3]. In 1965 first case of MRSA infection recorded in Sydney, Australia [4, 5] and in 1980 first case of a CA-MRSA infection in the United States was reported. Both HA-MRSA and CA-MRSA strains are transmitted by skin to skin contact although they have distinct clinical characteristics.

Once prevalent in health care setup for more than 40 years, MRSA has migrated to the community in recent years [6]. The prevalence of multidrug-resistant strains in Indian hospitals [hospital acquired MRSA (HA-MRSA)] ranges from 15 to 70% [7]. Community-acquired MRSA (CA-MRSA) has evolved as a novel emerging pathogen in patients who had no contact with health care setup [8]. Unlike HA-MRSA which typically are resistant to multiple antibiotics, CA-MRSA tend to be susceptible to other antibiotic classes and often are resistant to only β

lactam antibiotics [9]. They also differ from HA-MRSA in epidemiological association, drug resistance determinants, putative virulence factors, and genetic background [10].

Phenotypic and genotypic features can help distinguish between community and nosocomial MRSA strains; [11] absence of hospital-associated risk factors; [12] susceptibility to most antibiotics other than β-lactams; [13] presence of fourth type of *SCCmec* (the element that contains the methicillin resistance determinant), in contrast to the types I-III which are typical of nosocomial MRSA strains; [14] the presence of genes encoding for toxins such as pantone-valentine leukocidine (PVL) and the many staphylococcal enterotoxins in CA-MRSA [15-18]. The present study was aimed to understand the differences between community and hospital acquired MRSA and their profile in the in-patient and out-patient settings of North Karnataka. The present study helps to describe the comparative phenotypic differences of *S. aureus* and MRSA in community as well as in hospital.

MATERIALS AND METHODS

This prospective study was conducted at Department of Studies and Research in Microbiology, Gulbarga University, Kalaburagi, Karnataka, India, from March 2012 to March 2015. Overall a total of 593 samples were collected from the hospital in-patient and out-patient settings. Required samples were collected from the anterior nares, skin, and pus from the skin infections with the help of sterile cotton swabs. The clinical samples were collected from pus of the wounds of patients attending in-patient and out-patient department of various places of North Karnataka including Gulbarga, Raichur and Dharwad. All the details of the individual person were filled in a datasheet which contained brief information of name, age, sex, occupation, living status, sample number and history about health status.

I. Collection of samples

Required samples were collected with the help of sterile cotton swabs. Before taking samples, the swabs were dipped in sterile saline in propylene tubes (Hi-Media Pvt. Ltd; Mumbai). The collected swabs were immediately kept into the sterile propylene tubes provided with the swabs and put in the transport box. These swabs were transported to laboratory for processing within 6 hours.

II. Isolation of *S. aureus* from the collected specimens

In the laboratory, these samples were enriched by incubating for 6-12 hrs in the nutrient broth [19] then it was inoculated on to the mannitol salt agar (MSA) medium which serves as the selective medium for isolation of *S. aureus.*

III. Phenotypic Identification

For characterization of the *S. aureus,* conventional methods like growth characteristics on mannitol salt agar, Baird Parker agar, Grams staining and biochemical characteristics such as coagulase test and catalase test have been performed. Antibiogram was carried out using 12 different antibiotics including methicillin, vancomycin and for phenotypic characterization of MRSA. Antibiotic sensitivity tests had been performed as per CLSI guidelines [20].

IV. Antibiotic Sensitivity Test

Antibiotic Sensitivity test was done for each *S. aureus* isolate by the Kirby-Bauer disc diffusion method against ciprofloxacin (5µg), erythromycin (15µg), cloxacillin (30µg), vancomycin (30µg), ceftizoxime (30µg), ampicillin (10µg), penicillin (10µg), methicillin (5µg), amikacin(30µg), cefoxitin(30µg), oxacillin(1µg), and gentamycin(10µg) [21, 22]. The *S. aureus* confirmed isolates were inoculated to 2-3 ml of nutrient broth. The tubes were allowed for incubation for about 6 hours then turbidity was adjusted to 0.5 MacFarland's standard. That standard inoculum of each testing *S. aureus* was used for formation of uniform lawn on Mueller Hinton agar plates with the help of sterile cotton swabs. The plates were allowed to dry for 5 minutes. After that the antibiotic discs were kept in such a way that space between two dices was 12-15 mm on the plates with the help of sterile forceps. Then the plates were incubated for about 18-24 hours in inverted position in an incubator at 37^0 C. After the incubation, individual antibiotic sensitivity zone was measured with the help of zone measuring scale. *S. aureus* strain ATCC 43300 and *S. aureus* strain MTCC 96 were used as the Methicillin-resistant and Methicillin-sensitive control test standard organisms respectively [21, 22]. Then the tested all *S. aureus* isolates were preserved at -10^0C in tubes containing nutrient broth and 25% glycerol for further study.

RESULTS

A total of 265 *S. aureus* were isolated from both out-patient and in-patient departments from which 76 MRSA were isolated with a prevalence of 28.68%. The MRSA isolates were classified into HAMRSA and CAMRSA, based on the clinical history. 46 MRSA isolates were grouped into HAMRSA and 30 were grouped into CAMRSA, with a prevalence of 17.36%% and 11.32% respectively. Prevalence of MRSA is shown in **Table 1.**

Table 1: Prevalence of MRSA

Type	Number (n = 265)	Prevalence (%)
MSSA	189	71.32%
MRSA	76	28.68%
HAMRSA	46	17.36%
CAMRSA	30	11.32%

Overall 282 samples were collected from the hospital out-patient settings of various places of North Karnataka. Among them *S. aureus* incidence rate was found to be 42.55% (120) and incidence rate of MRSA was found to be 10.63% (30) as shown in **Table 2.**

Table 2: Incidence of *S. aureus* and MRSA in the hospital out-patient setting (CA-MRSA)

Sources	Hospital	No. of Samples tested			Incidence of *S. aureus*		Carriage rate of MRSA	
		Total	Male	Female	Male	Female	Male	Female
OPD	Govt.	148	97	51	42/97 (43.29%)	23/51 (45.09%)	13/97 (13.40%)	08/51 (15.68%)
	KBN	67	49	18	21/49 (42.85%)	06/18 (33.33%)	02/49 (4.08%)	00/18 (00.00%)
	Navodaya Raichur	26	15	11	7/15 (46.66%)	4/11 (36.36%)	2/15 (13.33%)	1/11 (9.09%)
	RIMS Raichur	21	14	07	06/14 (42.85%)	03/07 (42.85%))	1/14 (7.14%)	1/7 (14.28%)
	SDM Dharwad	20	14	06	06/14 (42.85%)	02/06 (33.33%)	02/14 (14.28%	0/6 (0.0%)
Total OPD		282	189	93	82/189 (43.38%)	38/93 (40.86%)	20/189 (10.58%)	10/93 (10.75%)
Overall OPD		282			120/282 (42.55%)		30/282 (10.63%)	

Overall 311 samples were collected from the hospital in-patient settings of various places of North Karnataka. Among them *S. aureus* incidence rate was found to be 46.62 % (145) and MRSA incidence rate was found to be 14.79% (46) as shown in **Table 3.**

Table 3: Incidence of *S. aureus* and MRSA in the hospital in-patient setting (HA-MRSA)

Sources	Hospital	No. of samples tested			Incidence of *S. aureus*		Carriage rate of MRSA	
		Total	Male	Female	Male	Female	Male	Female
IPD	Govt.	155	92	63	42/92 (45.65%)	29/63 (46.03%)	21/92 (22.82%)	07/63 (11.11%)
	KBN	74	42	32	20/42 (47.61%)	18/32 (56.25%)	06/42 (14.28%)	04/32 (12.50%)
	Navodaya Raichur	32	22	10	09/22 (40.90%)	04/10 (40.00%)	01/22 (4.54%)	01/10 (10.00%)
	RIMS Raichur	28	19	09	09/19 (47.36%)	03/09 (33.33%)	02/19 (10.52%)	01/09 (11.11%)
	SDM Dharwad	22	15	07	08/15 (53.33%)	03/07 (42.85%)	02/15 (13.33%)	01/07 (14.28)
Total IPD		311	190	121	88/190 (46.31%)	57/121 (47.10%)	32/190 (16.84%)	14/121 (11.57%)
Overall IPD		311			145/311 (46.62%)		46/311 (14.79%)	

Total of 12 antibiotics were used to study antibiotic susceptibility of isolated *S. aureus.* Among the 12 antibiotics used maximum of 93.33% isolates were resistant to Penicillin and minimum of 2.50% resistant to Vancomycin as shown in the **Table 4.**

Table 4: Hospital wise antibiotic % resistance in OPD (CA-MRSA)

HOSPITAL	TOTAL S.A.	ANTIBIOTICS RESISTANT											
		G	E	P	VA	M	CX	A	CIP	AK	CZX	COX	OX
GOVT	65	04	10	61	02	21	21	46	07	04	16	42	21
KBN	27	02	03	25	0	02	02	17	03	0	04	11	02
NIMS RCR	11	0	02	10	0	03	03	08	01	00	03	06	03
RIMS RCR	09	00	01	08	01	02	02	08	00	00	02	06	02
SDM DWD	08	00	01	08	00	02	02	06	02	01	03	03	02
TOTAL	120	06	17	112	03	30	30	85	13	05	28	68	30
% RESISTANCE		**5.00**	**14.16**	**93.33**	**2.50**	**25.00**	**25.00**	**70.83**	**10.83**	**4.16**	**23.33**	**56.66**	**25.00**

Total of 12 antibiotics were used to study antibiotic susceptibility of *S. aureus.* Among the 12 antibiotics used maximum of 93.79% resistance was shown to Penicillin and minimum of 4.14% resistance was shown Vancomycin as shown in the **Table 5.**

Table 5: Hospital wise antibiotic % resistance in IPD (HA-MRSA)

HOSPITAL	TOTAL S.A.	ANTIBIOTICS RESISTANT											
		G	E	P	VA	M	CX	A	CIP	AK	CZX	COX	OX
GOVT	71	22	35	68	04	28	29	45	29	19	24	46	29
KBN	38	21	18	34	02	10	10	21	21	06	11	22	10
NIMS RCR	13	03	05	12	00	02	02	07	02	02	04	09	02
RIMS RCR	12	03	03	11	00	03	03	07	02	01	03	07	03
SDM DWD	11	02	04	11	00	03	03	04	02	01	04	04	03
TOTAL	145	51	65	136	06	46	47	84	56	29	46	88	47
% RESISTANCE		**35.17**	**44.22**	**93.79**	**4.14**	**31.72**	**32.41**	**57.93**	**38.62**	**20.00**	**31.72**	**60.69**	**32.41**

Table 6: Overall % Resistance of antibiotics in OPD and IPD

ANTIBIOTIC	% RESISTANCE IN OPD (CA-MRSA)	% RESISTANCE IN IPD (HA-MRSA)
Gentamicin(G)	05.00	35.17
Erythromycin(E)	14.16	44.22
Penicillin(P)	93.33	93.79
Vancomycin (VA)	02.50	04.14
Mehticillin(M)	25.00	32.41
Cefoxitin (CX)	25.00	32.41
Ampicillin(A)	70.83	57.93
Ciprofloxacin (CIP)	10.83	38.62
Amikacin (AK)	04.16	20.00
Ceftizoxime (CZX)	23.33	31.72
Cloxacillin(COX)	56.66	60.69
Oxacillin (OX)	25.00	32.41

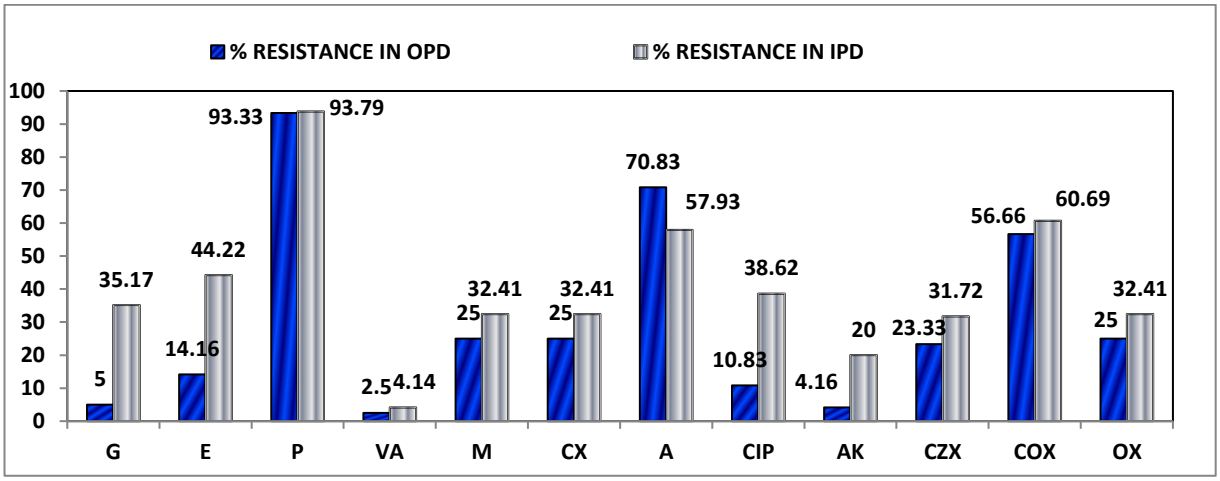

FIG 1: Graphical representation of overall % resistance of antibiotics in OPD and IPD

DISCUSSION

In developing countries like ours, in spite of the strict aseptic precautions which are being followed, MRSA causes important nosocomial infections. The improper usage of antibiotics can be identified as the root cause of the present condition. Since drug resistance is common among *Staphylococcus aureus* appropriate antibiotics should be chosen, based on the susceptibility pattern. Methicillin-Resistant *S. aureus* (MRSA) is a significant pathogen that has emerged over the past four decades, which causes both nosocomial and community-acquired infections. A rapid and an accurate detection of the methicillin resistance in *S. aureus* is important for the use of the appropriate antimicrobial therapy and for the control of the spread of the MRSA strains, because MRSA is an important nosocomial pathogen which causes significant morbidity and mortality. [23] Serious infections due to methicillin resistant *S. aureus* such as bacteremia, osteomyelitis, and sepsis are more prevalent in the hospital settings, but more importantly many cases of MRSA infections can be seen among previously healthy individuals with no exposure to health care setting, hence, these communities associated MRSA have become more important in daily practice. [24, 25, 26]

In our study prevalence of MRSA was found to be 28.68%. The similar prevalence rate of 27% was reported by Vysakh *et al;* [23]. Among total MRSA, 11.32% and 17.35% MRSA were isolated from hospital OPD (CA-MRSA) and hospital IPD (HA-MRSA) respectively from North Karnataka. CA-MRSA prevalence was same as that of the study carried out by Abdel-Maksoud *et al;* they reported the prevalence rate of 11.50% [27]. Vysakh *et al*; reported 20% of HA-MRSA prevalence which is slightly higher than that of our result [23]. All the CA-MRSA were isolated from the patients visiting for dressing at the out-patient settings of the hospital who did not have recent history of hospitalization and fulfilled the criteria for CA-MRSA. HA-MRSA were isolated from patients attending the various in-patient settings of the hospital and had recent history of hospitalization and fulfilled criteria for HA-MRSA.

The present study revealed that more than 90% of the CA-MRSA strains were susceptible to gentamycin, vancomycin, ciprofloxacin, and amikacin, suggesting that these drugs could provide a better option to treat CA-MRSA infections. High rate of resistance was observed to all antibiotics tested. by HA-MRSA compared to CA-MRSA. It is interesting to note that the resistance pattern of Ampicillin was more in CA-MRSA than HA-MRSA where remaining all antibiotics tested were shown higher degree of resistance to HA-MRSA.

CONCLUSION

Prevalence of MRSA is alarmingly high and causes a great worry to hospital settings in causing infections. Therefore, routine monitoring of MRSA infections along with their antimicrobial susceptibility pattern can assist in formulating a suitable antibiotic policy which may be helpful in reducing the burden and spread of MRSA infections in hospitals across North Karnataka.

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