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Research Article

ANTIBIOGRAM AND SUSCEPTIBILITY PATTERN OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* COLLECTED FROM VARIOUS CLINICAL SAMPLES IN BENGALURU

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial and community infections. A total of 671 clinical specimens were collected and subjected to standard microbiological screening methods for *S. aureus*. Subsequently, the antibiotic sensitivity test was performed for the confirmed MRSA isolates. Out of 323 (48.14%) strains of *S. aureus* isolated from clinical samples, 97 (30%) were found to be MRSA. The prevalence rate of MRSA was higher in clinical samples collected from males (58.8%) when compared to MRSA strains isolated from samples collected from females (41.2%). The highest number of MRSA isolates of 78.4% of MRSA strains were obtained from wound pus samples, 3.1% from urine, 4.1% from sputum, 5.2% from eye swab, and 1.0% from ear swab. The percentage of MRSA stains isolated among the age group 21-30 years is 3.5-fold higher than the strains isolated from the group aged between 61 and 70 years (6.19%). Almost all clinical MRSA strains (100%) were resistant to oxacillin, 90% resistant to ampicillin, 84.5% to penicillin-G, 76.2% erythromycin, 71% to cephalexin, and 40.2% to cotrimoxazole. However, here we reported 7.22% MRSA strains resistance to linezolid and 28.87% to vancomycin. The higher percentage of intermediate resistance was noted against antibiotics such as gentamycin and ciprofloxacin.

Keywords: *Staphylococcus aureus*, Methicillin resistance, Nosocomial infections, Vancomycin, Linezolid.

INTRODUCTION

Staphylococcus aureus, an opportunistic pathogen, commonly found in the nose, throat, intestine, vagina, and skin of human body [1]. It is a pathogen of greater concern because it has the ability to cause a wide variety of diseases ranging from self-limiting skin infection to life-threatening conditions [2]. Furthermore, it has an incredible ability to adapt fast to various antibiotic therapies.

Multi-drug resistance (MDR) is a major health concern in the treatment of staphylococcal infections; more specifically infections of methicillin-resistant *S. aureus* (MRSA) pose a serious challenge to hospital industry (Guidelines, 2008). The factors that are responsible for the pathogenicity of the *S. aureus* include enterotoxin, exfoliative toxin, and toxic syndrome toxin [3]. Recently, it has been reported that most of the MRSA strains are becoming resistant and are susceptible only to glycopeptides antibiotics such as vancomycin [4]. Another literature reported the prevalence of vancomycin-resistant *S. aureus* and vancomycin-intermediate *S. aureus*, therefore, development of resistance toward vancomycin warrants the search for a new class of antibiotics [4-6].

With respect to the Indian scenario, the prevalence of MRSA has increased from 12% (1992) to 80.83% in 1999 [7]. Today, MRSA strains are widespread not only in the hospitals of most countries but also in communities and are often resistant to several antibiotics [4,8]. The wide spread of MRSA in both hospitals and communities pose a difficult situation in terms of the control and prevention to both hospitals and the health care settings.

Prolonged hospital stay and arbitrary use of antibiotics will increase the emergence and spread of MRSA [9]. There are several reports focusing on MRSA isolates prevalence in different parts of India [10-12]. On the other hand, not many studies have been conducted addressing MRSA/methicillin-sensitive *S. aureus* (MSSA) prevalence in the southern India, especially in Bengaluru, which has relatively better health care facilities, offers easier access to antibiotics, and receives many patients from neighboring places of Bengaluru. Therefore, the present study estimates the percentage of MRSA strains and investigates their antibiotic resistance profiles in Bengaluru. Moreover, since Bengaluru, IT hub of India has become a preferred destination for various kinds of individuals and receives a significant number of students, and IT professionals throughout the year, this study is more important given the capacity of MRSA to spread.

METHODS

Sample collection

The total of 671 clinical specimens, such as urine, pus, sputum, semen, ET secretion, and swabs from the vagina, umbilical, nasal, throat, ear, and eye, were collected for *S. aureus* screening during 2012-2015 from hospitals different parts of south India. All the samples were aseptically handles and processed.

Identification of *S. aureus*

The morphotypes were done for all the samples based on the Gram-staining method to determine the likely organism present. The colonies of Gram-positive cocci in clusters were further inoculated on to the blood agar plates (aerobic with 5% carbon-di-oxide), MacConkey agar, and Robertson cooked meat medium for further sub culturing and incubated at 37°C for 24 hrs.

Identification of MRSA

All the confirmed *S. aureus* stains were subsequently tested for methicillin resistance based on the Kirby-Bauer disk diffusion method using oxacillin disc contained from Hi-Media Laboratories Pvt. Ltd. The isolates were considered methicillin-resistant if the zone of inhibition was 10 mm or less.

Antibiotic susceptibility testing

Further, the antibiotic susceptibility pattern of MRSA strains were subjected to *in vitro* antimicrobial testing method on Muller-Hinton agar containing 2-3% NaCl, using 2-hrs-old nutrient broth culture and HIMEDIA make antibiotic discs as per the standard methods. The clearing zone around the discs were measured and used to define the

sensitivity or resistance to different antimicrobials. The antibiotics used were penicillin-G (10 units); ampicillin (10 µg); cloxacillin (30 µg); cephalexin (30 µg); cephotaxime (30 µg); erythromycin (15 µg); gentamycin (10 µg); amikacin (30 µg); ciprofloxacin (5 µg); ofloxacin (5 µg); norfloxacin (10 µg); cotrimoxazole (25 µg); vancomycin (30 µg); and linezolid (30 µg). Finally, the data were recorded and analyzed at the completion of the study as per recommendations of the CLSI. *S. aureus* ATCC 29213 was used as the reference strain for the standardization of antibiotic susceptibility testing.

RESULTS

In this present study, we reported the prevalence and antibiotic susceptibility patterns of various MRSA strains isolated from clinical specimens, such as urine, pus, sputum, semen, ET secretion, and swabs from vaginal, umbilical, nasal, throat, ear, and eye, umbilical were determined. The total number of 671 clinical specimens were subjected for *S. aureus* screening. Out of which 323 (48.14%) were *S. aureus* (Fig. 1).

Then, all the confirmed *S. aureus* isolates were subjected to methicillin resistance screening using the Kirby-Bauer disk diffusion method with oxacillin disc. Table 1 represents the percentage distribution of *S. aureus*, MSSA, and MRSA isolated from various clinical specimens. Out of 323 identified *S. aureus*, 226 (70%) and 97 (30%) isolates were MSSA and MSSA, respectively.

The percentage of MRSA isolates was significantly different between males and females. The prevalence rate of MRSA was higher in clinical samples collected from males (68.9%) when compared to MRSA strains isolated from females (39.13%). Out of 97 MRSA isolates, the highest number of MRSA isolates (78.4%) was obtained from wound pus samples, 3.1% from urine, 4.1% from sputum, 5.2% from eye swab, and 1.0% from ear swab (Table 1).

The distribution pattern of MRSA isolates was noticeably different among clinical samples collected from males and females (Table 2). In females, the maximum number of MRSA isolates (87.5%) was obtained from pus samples, followed by urine (7.5%), and swabs from nasal (7.5%) and eye (2.5%). On the other hand, in males, the highest number of MRSA isolates of were obtained from pus samples (76.0%), followed by 7.4% from eye swab, 5.6% from sputum, and 5.6% from semen. A considerable difference was observed in the distribution of MRSA isolated from different age groups (Fig. 2). The highest number of MRSA strains was isolated from the clinical samples collected between 21 and 30 years (22.88%) of age group, followed by 31-40 years (20.62%) and 51-60 years (15.46%).

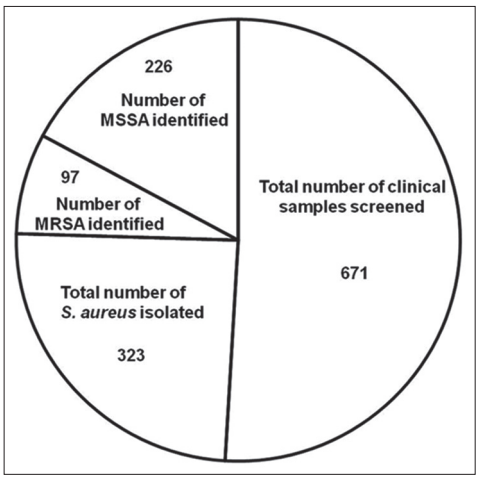

Fig. 1: Pie chart showing the prevalence rate of *Staphylococcus aureus* and methicillin-resistant *S. aureus* in clinical specimens

The drug resistance patterns of the isolated MRSA were found to be highly variable (Fig. 3). The antibiotics resistance patterns among the MRSA and MSSA isolates showed, 100% resistant to oxacillin, 90% resistant to ampicillin, 84.5% to penicillin-G, 76.2% erythromycin, 71% to cephalexin, and 40.2% to cotrimoxazole. However, here we reported 7.22% MRSA strains resistance to linezolid and 28.87% to vancomycin. The higher percentage of intermediate resistance was noted against antibiotics such as gentamycin and ciprofloxacin.

Table 1: Source and percentage distribution of isolated MSSA and MRSA present in the various clinical samples

Clinical sample	*S. aureus* Total=323 (%)	MSSA Total=226 (%)	MRSA Total=97 (%)
Urine	7.7	9.7	3.1
Pus	68.7	64.6	78.4
Sputum	8.0	9.7	4.1
Semen	1.2	0.4	3.1
ET secretion	2.2	2.7	1.0
Vaginal swab	0.6	0.6	0.0
Nasal swab	5.3	5.3	4.1
Umbilical swab	0.6	1.0	0.0
Throat swab	0.9	0.6	0.0
Ear swab	2.5	2.5	1.0
Eye swab	2.2	2.2	5.2

S. aureus: Staphylococcus aureus, MSSA: Methicillin-sensitive *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*

Table 2: Sex wise distribution of MSSA and MRSA isolated from various clinical samples

Clinical sample	MSSA total=226 (%)		MRSA total=97 (%)	
	Male Total=105	Female Total=99	Male Total=57	Female Total=40
Urine	3.8	18.2	0.0	7.5
Pus	78.1	64.6	76.0	87.5
Sputum	1.0	21.2	5.6	0.0
Semen	1.0	0.0	5.6	0.0
ET secretion	5.7	0.0	1.9	0.0
Vaginal swab	0.0	2.0	0.0	0.0
Nasal swab	5.7	7.1	1.9	7.5
Umbilical swab	0.0	2.0	0.0	0.0
Throat swab	2.9	0.0	0.0	0.0
Ear swab	3.9	3.0	1.9	0.0
Eye swab	1.9	0.0	7.4	2.5

MSSA: Methicillin-sensitive *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*

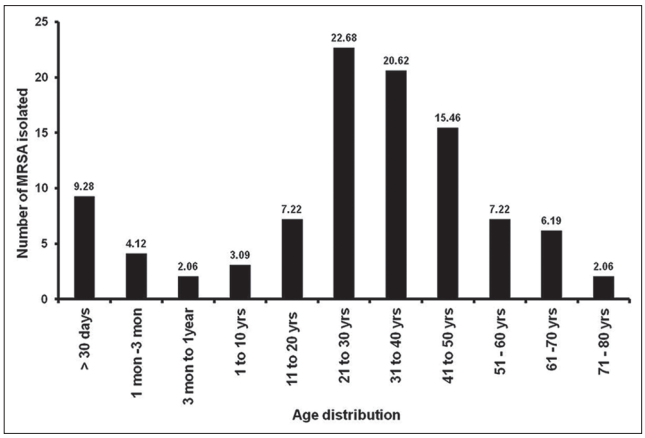

Fig. 2: Age wise distribution of methicillin-resistant *Staphylococcus aureus*

If bacteria are resistant to one or more classes of antimicrobial agents, then Centre for Disease Control classified as MDRs (Siegel JD *et al*., 2006) [13,4]. In the present study, 10 and 9 different multi-drug patterns were observed among MRSA samples collected from males and females' clinical samples, respectively (Fig. 4).

24.1% and 22.2% of MRSA isolated from males were resistant to at least 10 and 9 antibiotics, respectively. On the other hand MRSA, strains from female's samples showed 27.5% and 20% resistance to at least 7 and 8 antibiotics respectively. The percentage of antibiotic resistance among MRSA isolates from various clinical isolates was completely different. The percentage of antibiotics resistance of the MRSA isolated from different sources is shown in Table 3.

The MRSA strains isolated from pus samples showed resistance to a maximum number of antibiotics tested. The MRSA isolated from pus (4.1%), sputum (1.0%), and ET secretion (2.1%) showed resistance to the antibiotic linezolid. Furthermore, a significant number of vancomycin-resistant strains was observed from all the collected clinical samples.

DISCUSSION

Among the Gram-positive pathogens, *S. aureus*, especially MRSA has become a major nosocomial pathogen causing skin and soft tissue infections in the both community and hospitalized patients [2]. Multiple studies have been carried out on the growing concern over MDR across the world [14,15,12,10,11]. During the past 20 years, the increased

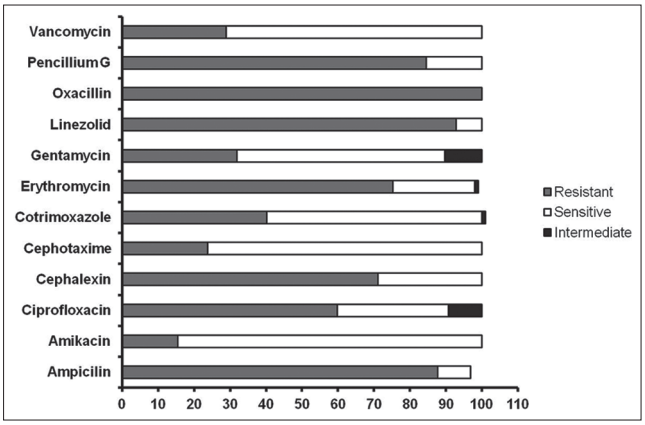

Fig. 3: Drug resistance patterns of methicillin-resistant *Staphylococcus aureus* isolated from clinical specimens

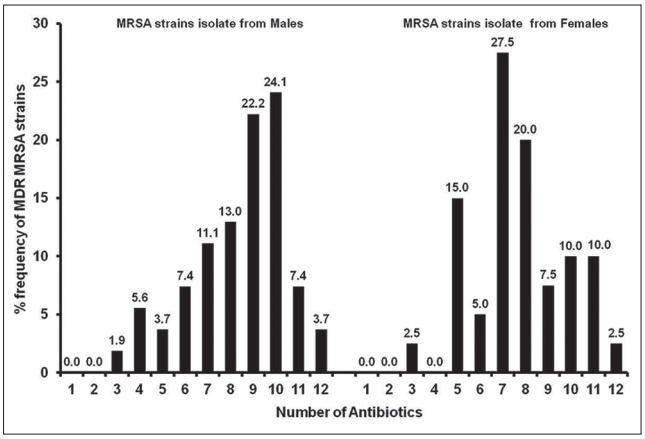

Fig. 4: Frequency of multi-drug resistance patterns of methicillin-resistant *Staphylococcus aureus* isolated from samples collected from males and females. Numbers denote the antibiotics that strains were resistant

Table 3: MRSA antibiotic resistance pattern in various clinical samples

Clinical sample	Ampicillin (%)	Amikacin (%)	Ciprofloxacin (%)	Cephalexin (%)	Cephotaxime (%)	Cotrimoxazol (%)	Erithromycin (%)	Gentamycin (%)	Linezolid (%)	Oxacillin (%)	Penicillium G (%)	Vancomycin (%)
Pus	66	9.3	47.4	60.8	8.2	21.6	57.7	12.4	4.1	67.0	63.9	70.1
Urine	3.1	3.1	2.1	2.1	3.1	2.1	3.1	2.1	0	3.1	3.1	3.1
Nasal	3.1	0	1.0	1.0	1.0	2.1	3.1	0	0	4.1	3.1	4.1
Sputum	5.2	0	2.1	2.1	2.1	2.1	2.1	2.2	1.0	4.1	4.1	5.1
Eye	1.0	1.0	1.0	0	1.0	1.0	1.0	2.1	0	1.0	2.1	2.1
ET	4.1	1.0	4.1	3.1	4.1	4.1	4.1	0	2.1	4.1	3.1	4.1
Throat	0	0	0	0	3.1	4.1	1.0	4.1	0	4.1	0	4.1
Ear	1.0	0	1.0	1.0	1.0	0	1.0	1.0	0	1.0	1.0	1.0
Umbilical	1.0	0	1.0	1.0	0	1.0	0	0	0	1.0	3.1	3.1
Semen	3.1	1.0	0	0	0	2.1	2.1	0	0	2.1	1.0	3.1

MRSA: Methicillin-resistant *Staphylococcus aureus*

appearance of MDR toward many lifesaving drugs, pose a treat for many health care agencies in controlling MRSA.

The present study indicates the prevalence and antibiotic susceptibility patterns of various MRSA isolates obtained from various clinical samples ranging from urine, pus, sputum, semen, ET secretion, and swabs from vaginal, umbilical, nasal, throat, ear, and eye, umbilical were determined. We isolated 226 (70%) MSSA strains and 97 (30%) MRSA strains from 671 clinical specimens. Though the prevalence obtained in this study was slight than lower the prevalence of 41% reported in earlier studies in India by Joshi *et al*. 2013 [16] and 32.22% by Bilal Ahmad Mir *et al*. 2013 [17]. On the other hand, a study from Eritrea revealed low MRSA (9%) pre-valence [18] which is less than the prevalence observed in our study. The observed differences may be due to the time when the studies were conducted, the method of sampling, methods used in the laboratory and more importantly the different population studied and their antibiotic consumption pattern.

Out of 97 MRSA isolates, the highest number of MRSA isolates (78.4%) was obtained from wound pus samples, 3.1% from urine, 4.1% from sputum, 5.2% from eye swab, and 1.0% from ear swab. In females, the maximum number of MRSA isolates (87.5%) was obtained from pus samples, followed by urine (7.5%), and swabs from nasal (7.5%), and eye (2.5%). A similar observation was reported by Qureshi [21] from Pakistan reported a high isolation rate of up to 83% MRSA from pus. On the other hand, in males, the highest number of MRSA isolates of were obtained from pus samples (76.0%), followed by 7.4% from eye swab, 5.6% from sputum, and 5.6% from semen.

The percentage of MRSA isolates was significantly different between males and females. The prevalence rate of MRSA was higher in clinical samples collected from females (39.13%) when compared to MRSA strains isolated from males (68.9%).

Other recent studies reported that prevalence of MRSA was high in females (58.8%) [19] which may be due to the improved personal hygienic practice among females.

Here, we observed the considerable difference in the distribution of MRSA among different age groups. The maximum number of MRSA strains was isolated from the age group between 21 and 30 years (22.88%), followed by 31-40 years (20.62%) and 51-60 years (15.46%). The percentage of MRSA stains isolated among the age group 21-30 years is 3.5-fold higher than the strains isolated from the group aged between 61 and 70 years (6.19%). The recent study reported that number of MRSA (52%) were isolated from the age grouped between 50 and 60 years. The high prevalence of MRSA among the young people may be due to prolonged usage and over dosing of antibiotics.

The assessment of the antimicrobial susceptibility profile of the MRSA showed 7.22% and 28.87% MRSA strains were resistance to linezolid and vancomycin, respectively. The other contemporary reports state that all the MRSA isolated in major southern districts of Tamil Nadu are 100% susceptible to vancomycin, and most of them were susceptible to linezolid [12]. Similarly, lower resistance of MRSA strains toward to linezolid was also reported by Tsiodras [20]. As expected, a higher percentage of MRSA strains was resistant to oxacillin (100%), ampicillin (90.72), penicillium G (84.54%), erythromycin (76.29%), and cephalexin (71.13%). The rRecent study on the spectrum of antimicrobial resistance among MRSA reported the ciprofloxacin resistance was as high as 90% [21]. In contrast, here we reported that 59.79% of the MRSA strains resistant to ciprofloxacin and a further lower resistant rate to amikacin (15.46%). An increase of gentamicin resistance from 0% before 1996 to 80% after 1996 has been reported. However, it was reported that only 8% MRSA were resistance to gentamicin as against our present data [22,23].

CONCLUSION

The degree of resistance or sensitivity of MRSA toward commonly used antibiotics is recognized to be diverse from region to region. The regular surveillance of MRSA and *in vitro* susceptibility testing for every isolated clinical sample will be useful in selecting an appropriate antibiotic and limit the use/overdose of powerful antibiotics and might change the trends of antibiotic susceptibility pattern, especially in developing countries.

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