Original Research Article

The Trends of Distribution and Susceptibility profile of Methicillin resistant and Methicillin sensitive *Staphylococcus aureus* isolated from clinical samples at a tertiary care hospital

M Wajid[1*], Shazia Naaz[2], Saranya Mallamgunta[3]

[1]Associate Professor, Department of Microbiology, ESIC Medical College & Hospital, Sanathnagar, Hyderabad, India

[2]Assistant Professor, Department of Microbiology, ESIC Medical College & Hospital, Sanathnagar, Hyderabad, India

[3]Assistant Professor, Department of Microbiology, ESIC Medical College & Hospital, Sanathnagar, Hyderabad, India

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Abstract

Objective: Staphylococcus aureus [*S.aureus]* is known to be a pathogen of prime importance both in community and health care settings. This study aims at determining the distribution of Staphylococcus isolates both methicillin-sensitive *Staphylococcus aureus* [MSSA] as well as methicillin-resistant *Staphylococcus aureus* [MRSA] in the community as well as the health care settings.**Materials and methods:** This retrospective study was conducted at a tertiary care hospital in Hyderabad, South India between Jan 2020 – December 2020. Isolation and phenotypic identification of *S. aureus* was done using standard microbiological methods at the bacteriology laboratory, in the Microbiology department. Demographics, categorical variables, and antibiotic susceptibility patterns were compared between MRSA and MSSA along with a comparison of resistance patterns between Community-acquired MSSA and MRSA [CA-MSSA, CA-MRSA] and Hospital-acquired MSSA and MRSA [CA – MSSA, HA-MSSA] respectively.**Results:** About 139 (13.3%) isolates were positive for *Staphylococcus aureus*. Most of the positive isolates were received from males 56.1 % and between 41-60 years. The majority of the isolates were from pus (88%) followed by blood (32%). The prevalent isolate was MSSA (52%) with a predominance of CA-MSSA (39%). The resistance pattern was seen among *S.aureus* isolates to beta-lactams, quinolones, tetracyclines followed by macrolides.**Conclusion**: Constant monitoring of institutional antimicrobial susceptibility patterns and refurbishing the antibiogram accordingly is a pressing priority to prevent the further spread of antimicrobial resistance in the community and health care settings.

Keywords: MRSA, MSSA, Community, Hospital, Prevalence

Introduction

Staphylococcus aureus is a highly pathogenic organism, it was first identified in the year 1880 by Ogston in the purulent fluid from a leg abscess and was formally isolated by Rosenbach later.[1] It causes a wide range of infections in Humans from mild skin infections such as abscesses to severe systemic diseases such as pneumonia, bacteremia, and osteomyelitis. Infections are common both in the hospital-acquired setting as well as community setting. Because of the emergence of multi-drug resistant strains in Staphylococcus such as MRSA (Methicillin-Resistant Staphylococcus aureus) treatment remains challenging.[2] Scottish scientist Alexander Fleming discovered Penicillin as a crude extract of P. rubens in 1928. [3] Purified penicillin was used in medicine in the 1940s but Penicillin-resistant staphylococcus strains began emerging shortly after the introduction of penicillin. MRSA was identified 1 year after development of semi-synthetic penicillins with anti-staphylococcal activity around 1960.[4] Methicillin resistance is determined by the mecA gene, which encodes the penicillin-binding protein PBP 2A which has a low affinity for β-lactams, resulting in resistance to this entire class of antibiotics. The mecA is a 21- to 60-kb length gene and is a part of a mobile genetic element staphylococcal chromosome cassette mec (SCCmec), encoding resistance to non-β-lactam antibiotics such as Tn554, pUB110, and pT181. [5,6] MRSA has been recognized as a pathogen of global concern. MRSA infections were originally acquired only from hospital settings (HA-MRSA), but in the 1990s it has spread rapidly in the community (CA-MRSA) in Australia and the United States of America, and subsequently across the world.[7,8] According to US Centers for disease control, MRSA or MSSA infection can be categorized as Community-acquired infections when the patient has no history of hospitalization or residence in a long-term care facility within the year before infection or has no, hospitalization 48 hr before culture.[9] MRSA became prevalent in the community and healthcare settings it has rapidly spread globally, But the proportion of Staphylococcus aureus showing methicillin resistance was found to be lower compared to methicillin-susceptible isolates, although the rate varies worldwide. In the United Kingdom, they noted a declining trend in MRSA. The reason for the serial rises and falls of specific strain types remains poorly understood.[10-12] Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group conducted a study from January 2008 to December 2009 by the in 15 Indian tertiary care centers found an overall prevalence rate of 59% MSSA and 41% of MRSA in inpatients and outpatients.[13] Another study conducted in

**Correspondence*
Dr.M Wajid
Associate Professor, Department of Microbiology, ESIC Medical College & Hospital, Sanathnagar, Hyderabad, India
E-mail: drmd.wajid@gmail.com

a tertiary care hospital in Bangalore in the year 2013 found MRSA prevalence rate of 23% among their staphylococcal isolates.[14] Mostly, MRSA strains and MSSA strains depict distinct microbiological and therapeutical features. MRSA strains are resistant to multiple classes and MSSA is found to be more susceptible to other antibiotics. However, recent studies have reported the emergence of higher antibiotic resistance in MSSA for other antibiotics.[11,15] Understanding the prevalence of MRSA and MSSA and their antibiotic resistance pattern in Community and health care settings is necessary for appropriate antibiotic treatment and effective infection control measures. The present study was aimed to study the distribution of MRSA ad MSSA in various samples and to know their antibiotic profile in community acquired and hospital acquired staphylococcal infections.

Materials and Methods

This is a retrospective study carried out in a tertiary care hospital in Hyderabad, India carried out after prior ethical approval. The study was carried out from January 2020 to December 2020.

Isolation& Identification of *S. aureus*

Various clinical samples such as pus, blood, respiratory specimens (sputum,Broncheoalveolar lavage -BAL and Endotracheal (ET) secretions), and other relevant samples that were received to the bacteriology laboratory were processed according to standard microbiological guidelines. [16]

5% Sheep Blood Agar (SBA- Himedia) and MacConkey Agar (MCA) plates were streaked and incubated aerobically for 18–24 h at 37 ℃. Isolates were identified with the help of phenotypic characters and were further confirmed with the aid of biochemical reactions such as catalase, tube and slide coagulase, mannitol fermentation test, and gram stain.

Antibiotic susceptibility testing : It was performed using Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute [CLSI] guidelines. [17] Various antibiotics such as ampicillin [10 µg],cefoxitin [30 µg], clindamycin [2 µg], erythromycin [15 µg], levofloxacin [5 µg], linezolid [30 µg], penicillin [10 U], tetracycline [30 µg], tigecyclin [15 µg] and vancomycin [30 µg] were tested.

Detection of MRSA: All the isolates of *Staphylococcus aureus* were tested as per standard protocols given by CLSI using cefoxitin (30 µg; HiMedia, India) disc diffusion test.[17] A zone of inhibition of ≤ 21 mm was taken as methicillin-resistant and ≥22mm was taken as methicillin-sensitive. Staphylococcus aureus ATCC 25923 and ATCC 43300 were used for quality control purposes.

Definitions: **CA-MRSA** is said to have occurred when the patient has no history of surgery, hospitalization, or residence in a long-term care facility within the year before infection, has no percutaneous device or indwelling catheter, has not undergone dialysis within the previous year, hospitalization <48 h before MRSA culture, or has no history of previous MRSA infection or colonization.[18]

HA-MRSA: Hospital-associated MRSA is defined as the isolate cultured from clinical specimens obtained 72 hrs after a patient's hospital admission or whose sources of isolation were associated with risk factors for HA-MRSA infection (recent hospitalization, recent surgery, residence in a long-term care facility, drug use, etc) within a year before isolation of the strain. [19]

Statistical Analysis: Data analysis was done using a statistical calculator of the chi-square test. Categorical variables were analyzed. Antibiogram was analyzed using WHONET software. The Chi-square test was used for comparison in the difference of antibiotic sensitivity concerning CA-MSSA and HA-MSSA was done by using chi-square test, where a test was considered significant with a p-value < 0.05.

Results

A total of 5826 clinical samples were processed for culture and sensitivity testing from January to December 2020. Bacterial growth was obtained in 1042 (17.8%) samples out of which 139 (13.3%) isolates of the positive cultures, was that of *Staphylococcus aureus.*

Of these 139 samples, 78(56.11%) were obtained from male patients and 61(43.8%) from female patients (Table 1). The isolation rate was highest in patients aged between 41-60 years followed by those between 21-40 years of age (Table 2). The maximum numbers of isolates were obtained from pus samples followed by blood cultures.

Observed in susceptibility profiles of CA- MRSA, and HA-MRSA (Table 5).

Table 1: Gender wise distribution [n=139]

Sex	Patients with culture positive for Staphylococcus aureus
Males	78(56.1%)
Females	61 (43.8%)

Table 2: Age wise distribution [n=139]

Age group	Culture positives
0-20 years	8(5.75%)
21-40 years	42(30.21%)
41-60 years	56(40.28%)
61-80 years	29(20.86%)
>80 years	4(2.87%)

Distribution of culture-positive samples received from Inpatient & Outpatient with increasingly higher samples received from Inpatient (IP) rather than Outpatient (OPD) (Fig 1).

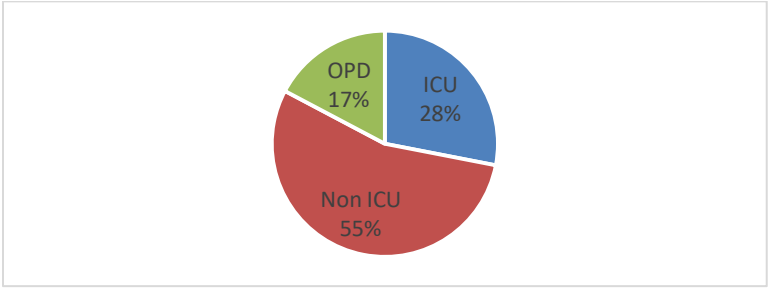

Fig 1: Distribution of culture positive samples received from In patient & Out patient

A significant difference was not observed with regards to samples collected from CA- MRSA, HA - MRSA, CA- MSSA, and HA – MSSA (Table 3). A significant difference between MSSA and MRSA resistance shown against penicillin, ampicillin, clindamycin, erythromycin, tetracycline was observed [p<0.05] (Table 4/Fig2). CA-MSSA and HA-MSSA showed a significant difference in susceptibility profiles to penicillin, ampicillin, clindamycin, erythromycin, tigecycline, and levofloxacin. A significant difference was not

Table 3: Isolation rate of *Staphylococcus aureus* from various clinical samples

Sample	Culture positives	MSSA [n=72]		Chi square value	P value	MRSA[n=67]		Chi square value	P value
		CA- MSSA	HA -MSSA			CA-MRSA	HA - MRSA		
Blood	32[23%]	10	4	0.3997	.843	7	11	5.068	**.079**
Pus	88[63.3%]	37	11			28	12		
Respiratory specimens	19[13.6%]	7	3			5	4		
Total [n=139]		54 [39%]	18[13%]			40[29%]	27[19%]		

Table 3: Significant difference was not observed with regards to samples collected from CA- MRSA and HA - MRSA & CA- MSSA and HA - MSSA

Table 4: Antibiogram of *Staphylococcus aureus*

Antibiotic	Susceptiblity No [%]		Resistance No [%]		P value
	MSSA (n=72)	MRSA(n=67)	MSSA (n=72)	MRSA(n=67)	
PENICILLIN	21 [29.1%]	7 [15.4%]	51 [71%]	60[93 .7%]	0.005*
AMPICILLIN	25 [34.7%]	10[10.9%]	47 [66%]	57 [89%]	0.01*
CLINDAMYCIN	54 [75%]	29 [43.2%]	18 [26%]	38 [56.7%]	0.001*
ERYTHROMYCIN	50 [69.4%]	23 [34.3%]	22 [31%]	44 [65.6%]	0.01*
TIGECYCLIN	68 [94.4%]	58 [86.5%]	4 [5.6%]	9 [13.4%]	>0.05
TETRACYCLIN	32 [44.4%]	49 [73.1%]	40[56.3%]	18 [26.8%]	0.004
LEVOFLOXACIN	32 [44.4%]	20 [29.8%]	40 [56.3%]	47[70.1%]	>0.05
VANCOMYCIN	72[100%]	67[100%]	0[0%]	0[0%]	N/A
LINEZOLID	70 [97.2%]	56 [83.5%]	2 [2.8%]	11 [16.4%]	>0.05
Chi Square test for individual antibiotics done depicting P value for each * Significant p < 0.05, N/A - Not applicable					

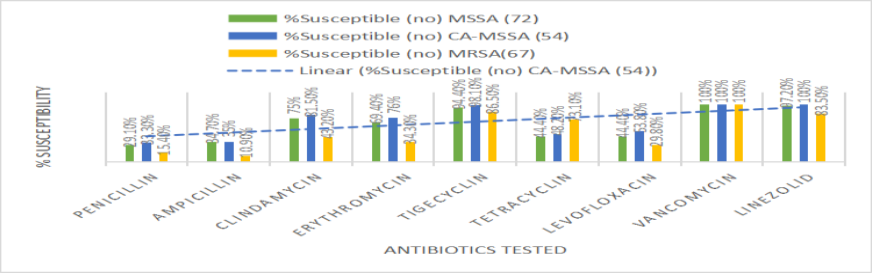

Fig 2: Susceptibility pattern of *Staphylococcus aureus*

Image 2: The susceptibility pattern and trend of MRSA , MSSA and CA-MSSA is shown. The trend line for CA-MSSA depicts the linear increase in susceptibility to higher end antibiotics

Table 5: Resistance Profile of Community and Hospital Acquired MSSA and MRSA

Antibiotic	Resistance No [%]					
	MSSA [72]		*P value*	MRSA[67]		*P value*
	CA-MSSA (n=54)	HA- MSSA (n=18)		CA-MRSA (n=40)	HA-MRSA (n=27)	
PENICILLIN	36 [66.6%]	14 [77.7%]	>0.05	35 [87.5%]	25 [92.5%]	>0.05
AMPICILLIN	35 [65%]	12[66%]	>0.05	34[85%]	23[85%]	>0.05
CLINDAMYCIN	10 [18.5%]	8 [44%]	0.02	25[62.5%]	16[59.3%]	>0.05
ERYTHROMYCIN	13 [24%]	9 [50%]	0.03	27 [67.5%]	17 [63%]	>0.05
TIGECYCLIN	1 [1.9%]	3 [16.6%]	0.01	4 [10%]	5 [18.5%]	>0.05
TETRACYCLIN	28 [51.8%]	12 [66.6%]	>0.05	10 [25%]	8 [30%]	>0.05
LEVOFLOXACIN	25 [46.2%]	15 [83.3%]	0.006	27 [67.5%]	20 [74%]	>0.05
VANCOMYCIN	0[0%]	0[0%]	N/A	0[0%]	0[0%]	>0.05
LINEZOLID	0[0%]	2 [11.1%]	N/A	4[10.0%]	7 [25.9%]	>0.05

Significant difference is observed where p > 0.05 for various antibiotics.

N/A – *p value* not applicable

Discussion

Antimicrobial-resistant among bacteria has been observed to spread rampantly especially in the 21st century. *S. aureus* has proven to be a nasty bug posing challenges with increasing resistance to the line of drugs available and creating difficulties even after the introduction of new classes of antimicrobial agents which is usually followed by the appearance reduction in susceptibility. [20] The present study shows a higher predominance of MSSA in comparison to MRSA. Limited data regarding antibiotic susceptibility profile is available in the Indian literature search. [23]

In the present study majority of the samples were isolated from males in comparison to females with the predominant age group being 41-60 years. Preeja et al determined that incidence of HA-MSSA was more predominant amongst 41-60 years and a predominance amongst males. [23] Another Indian study depicted a male/female ratio of 2.29. [24] 48% and 52% of the strains isolated from various samples were MRSA and MSSA respectively. This study shows a higher prevalence of MSSA with a predominance of CA-MSSA (39%). Various Indian studies show a predominance of MSSA in comparison to MRSA (Table 6).

Table 6: Comparison of isolation and susceptibility pattern of Staphylococcus aureus with other studies

Author	MSSA	MRSA	% Antibiotics susceptibility – MSSA						
			Erythromycin	Clindamycin	Tetracyclin	Levofloxacin	Vancomycin	Tigecyclin	Linizolid
Present study 2021	52% CA-MSSA-39% HA-MSSA-18%	48% CA-MRSA-29% HA-MRSA-19%	69.4%	75%	44.4%	44.4%	100%	94.4%	97.2%
Preeja et al 2021 [23]	CA-MSSA 71.8% HA-MSSA 28.20%	NA	56.1%	79.3%	97.4%	89.8%	100%	99%	100%
Ganesan et al 2021 [25]	MSSA -52%	CA-MRSA-48%	-	-	-	-	-	-	-
Gurung et al 2020 [26]	25%	75%	53.9%	76.9%	92.3%	-	100%	-	-
Senthil Kumar et al 2019 [22]	53%	47% CA-MRSA -35% HA-MRSA -9%	69%	71%	75%	77%	100%	-	-
C Bouchiat 2015 [24]	47.8%	52.2%	45.7%	-	80.4%	-	-		

Studies were done by Eshawara et al, Chatterjee et al, and Senthil Kumar et al stating a prevalence of 54%,52% and 47% MRSA isolate which is in concordance with our study. [14,21,22] No resistance was observed towards vancomycin. MRSA both CA-MRSA and HA-MRSA were susceptible to vancomycin linezolid, tigecycline which is similar to a study done by Singh et al and Adwan et al. Multidrug-resistant CA-MRSA has been reported by various Indian studies as well as worldwide.

In the present study, it was found CA-MSSA and HA-MSSA were susceptible to various antibiotics, and CA-MSSA showed increased susceptibility to clindamycin, erythromycin, and tigecycline compared to HA-MSSA. Increasing resistance to macrolides was observed in the HA-MSSA. These findings are of prime importance as macrolides play a key role in the treatment of Staphylococcal infections. [24]

Both CA-MSSA, HA-MSSA, CA- MRSA, and HA- MRSA showed resistance to quinolones, ampicillin, penicillin, tetracyclines. These findings are in concordance with a study done by Senthil Kumar et al and Preeja et al. [22, 23] Higher resistance towards tetracycline was noted in this study may be due to frequent use of doxycycline and tetracycline for treatment in our setup.MSSA isolates resistant to three or more classes of antibiotics were found. The aforementioned isolates were resistant to penicillin (71%), ampicillin (66%), levofloxacin (56.3%), clindamycin (26%), erythromycin (31%). Aggarwal S et al & Preeja et al reported multidrug-resistant MSSA isolates in their study. [11,23] A comparison of susceptibility patterns of MRSA and MSSA in comparison to other studies has been depicted in table 6.CA-MSSA and HA-MSSA showed a significant difference in susceptibility profiles to clindamycin, erythromycin, tigecycline, and levofloxacin which showed a significantly higher resistance in HA-MSSA isolates. Bouchiat C et al determined that resistance in CA –MSSA and HA -MSSA against erythromycin 43.8% and 41.7% and against ciprofloxacin 56.3% and 50%, respectively, which is not significant.[24] These findings are contrary to the present study. Many factors play a vital role in deciding the resistance pattern of an organism, both organism, and environment dependant. Therefore, constant scrutiny and monitoring of the antimicrobial susceptibility patterns of *S. aureus* is relevant to decipher the new emerging trends of resistance and aid in the management of both community-acquired and hospital-acquired infections.

Conclusion

This study revealed an increasing trend towards MSSA, especially HA-MSSA portraying an increasing trend of resistance towards a certain group of antimicrobials. Pertinent follow-up with constant surveillance and updated antibiogram is the need of the hour to understand the ever-changing trend of susceptibility of *S.aureus* and keep a check on the further rise of antimicrobial resistance pattern.

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