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Prevalence and Antimicrobial Susceptibility of Methicillin Resistant *Staphylococcus aureus* in Wound Infections in a Tertiary Care Hospital

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A B S T R A C T

Staphylococcus aureus is the most frequently isolated pathogen from wound infections and is a threat to the modern antibiotic era due to it propensity to undergo multidrug resistance. The aim of the study was to determine the prevalence of MRSA and to analyze the antibiotic susceptibility pattern of Staphylococcus aureus isolates in pus samples. Pus samples were subjected to Gram stain and culture and the organisms were identified by standard methods and antibiotic sensitivity testing was done. Methicillin Resistance in Staphylococcus aureus were identified by using Cefoxitin disc diffusion test (30 µg). A total of 230 pus samples were sent for culture, out of which 32.14% was *Staphylococcus aureus.* Among the *S. aureus* isolates, 50.79% was MRSA. The isolates showed resistance to Ciprofloxacin and Gentamicin but were sensitive to Linezolid and Vancomycin. The panel of infections caused by *Staphylococcus aureus* combined with the problem of antimicrobial resistance should evoke proper surveillance measures and preventive efforts in hospitals. This along with judicious antibiotic use will decrease the rates of antibiotic resistance in hospitals.

Introduction

Staphylococcus aureus is the noteworthy species of the genus Staphylococcus with a tendency to cause both nosocomial and community acquired infections worldwide [1].

The pathogen has been found to cause a plethora of diseases such as blood stream infections, skin and soft tissue infections, pneumonia and hospital acquired post-operative wound infections [2]. Though the diseases are treatable by the usage of antibiotics, emergence of multi drug resistant strains have been reported worldwide, thereby complicating the treatment of infections caused by *Staphylococcus aureus* [3].

Ever since its evolution in 1961, Methicillin resistant *Staphylococcus aureus* (MRSA) frequent causes life threatening infections and poses a threat for modern antimicrobial therapy [4]. They cause both nosocomial (Hospital acquired MRSA) infections and community acquired infections (Community acquired MRSA).

MRSA spreads more readily than other strains once introduced into hospitals, and are very difficult to eradicate once established [5]. In India, MRSA make up to 25-50% % of all infections [6]. Transmission of MRSA occurs primarily from colonized or infected

persons and fomites [7]. The mechanisms for the development of MRSA have been the *mec A* that codes for the penicillin-binding protein (PBP 2a) and has a low affinity for β-lactam antibiotics and the production of penicillinase enzyme mediated by the *bla* gene [8].

This study is important because the changing prevalence and antibiotic susceptibility pattern of MRSA in wound infections will guide the clinician to make better treatment modalities and lead to better clinical outcomes in patients.

Materials and Methods

Study setting and context

A cross sectional study was conducted among 230 patients of all age groups at the Department of Microbiology of Sree Balaji Medical College, Chennai from March 2017 to July 2017 for a period of five months.

Sample collection and processing

Pus samples from wound infections were collected from patients under aseptic precautions and transported to the Central Microbiology lab of the hospital, with minimal delay for processing.

The samples were inoculated on 5% blood agar base and mannitol salt agar. Gram stain showed the presence of Gram positive cocci in clusters with abundant pus cells. Inoculated plates were incubated at 35–37 °C for 18 to 24 hours aerobically. Bacterial colonies which show the typical characteristics of *S. aureus* such as betahemolytic colonies and colonies with golden yellow pigmentation on mannitol salt agar were subjected to further testing by Gram stain and biochemical tests catalase and coagulase. When the isolate was positive for both catalase and coagulase it was confirmedas S.aureus.

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was done using Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI, 2016) guidelines on Muller Hinton agar against the following antibiotics- Ampicillin (10 µg), Erythromycin (15 µg), Clindamycin (2 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Trimethoprim-Sulfamethoxazole [1.25/23.75 µg], Linezolid (30 µg) and Vancomycin (30 µg),. The growth suspension was prepared in 0.5 ml of the nutrient broth and the turbidity was adjusted to match that of 0.5 McFarland standard. A sterile swab was dipped into the inoculum and the excess of inoculum was removed by pressing it against the sides of the tube. Then the swab was applied as a lawn culture on Muller Hinton agar plate evenly. Antibiotic discs were placed after 15 minutes of inoculation and were incubated for 24 hours at 35–37 °C. The diameter of the zones of inhibition around the disc was measured using calipers.

Methicillin resistance was detected using a 30 µg Cefoxitin disc. Zone diameter was read after incubation at 35°C for a full 24 hours. Strains with zone diameter ≤21 mm for Cefoxitin were regarded as methicillin resistant. ATCC Staphylococcus strain 25923 was used as quality control.

Results and Discussion

Demographic characteristics of all study participants

A total of 230 pus samples were sent for aerobic culture and sensitivity to the Department of Microbiology. Among them, 182 (79.13%) samples were positive for growth and the remaining 48(20.87%) samples were negative for aerobic growth. Among the 182 culture positive pus samples,

the male patients had majority that is 102(56.04%) and 80(43.96%) were females. Maximum cases which were positive for culture were in the age group of 41-60 years (62, 34.07%)

Out of the 182culture positive pus samples, 168 (92.31%) were monomicrobial and 14(7.69%) were polymicrobial. So the total numbers of isolates from positive pus samples were 196.

Prevalence and characteristics of *Staphylococcus aureus*

Out of 196 culture positive isolates, 63(32.14%) were *Staphylococcus aureus*. Males had a higher isolation rate of *S. aureus* than females (45, 71.43%) versus (18, 28.57 %). Rate of isolation of *S. aureus* was the highest in the age group of 41-60 years (23, 36.51%).

Prevalence and characteristics of Methicillin resistant *Staphylococcus aureus*

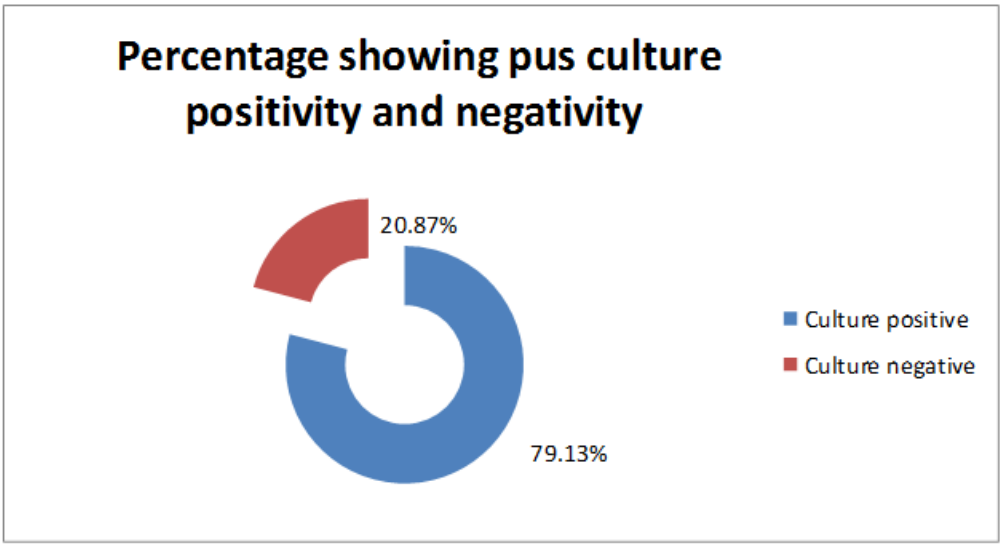
Among the 63 *Staphylococcus aureus*, 32(50.79%) were MRSA and the rest 31 were Methicillin sensitive *Staphylococcus aureus* (49.21%). Males predominated with a percent of 62.5% and females 37.5%. All isolated *Staphylococcus aureus* were sensitive to Vancomycin and Linezolid

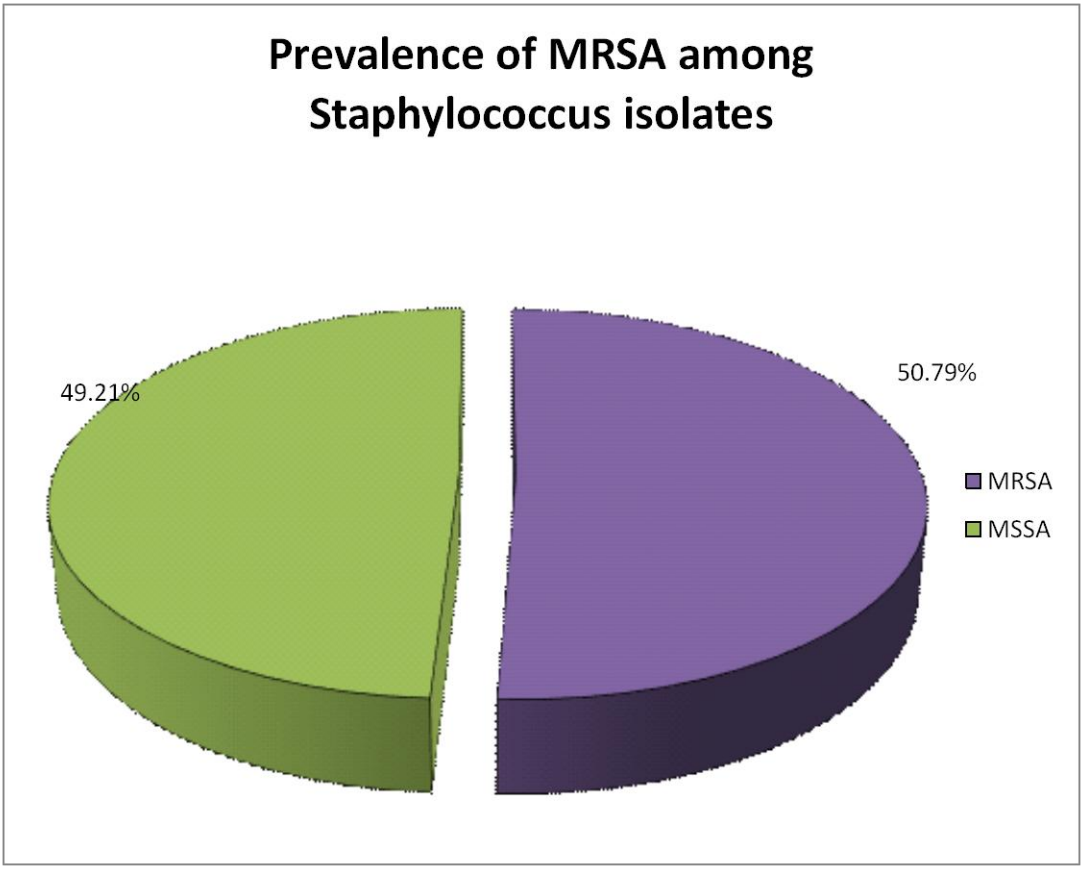
Among the total 230 samples sent, 182 (79.13%) were culture positive and 48 (20.87%) were culture negative. 168 (92.31%) were monomicrobial and 14 (7.69%) were polymicrobial. So the total numbers of isolates from positive pus samples were 196.

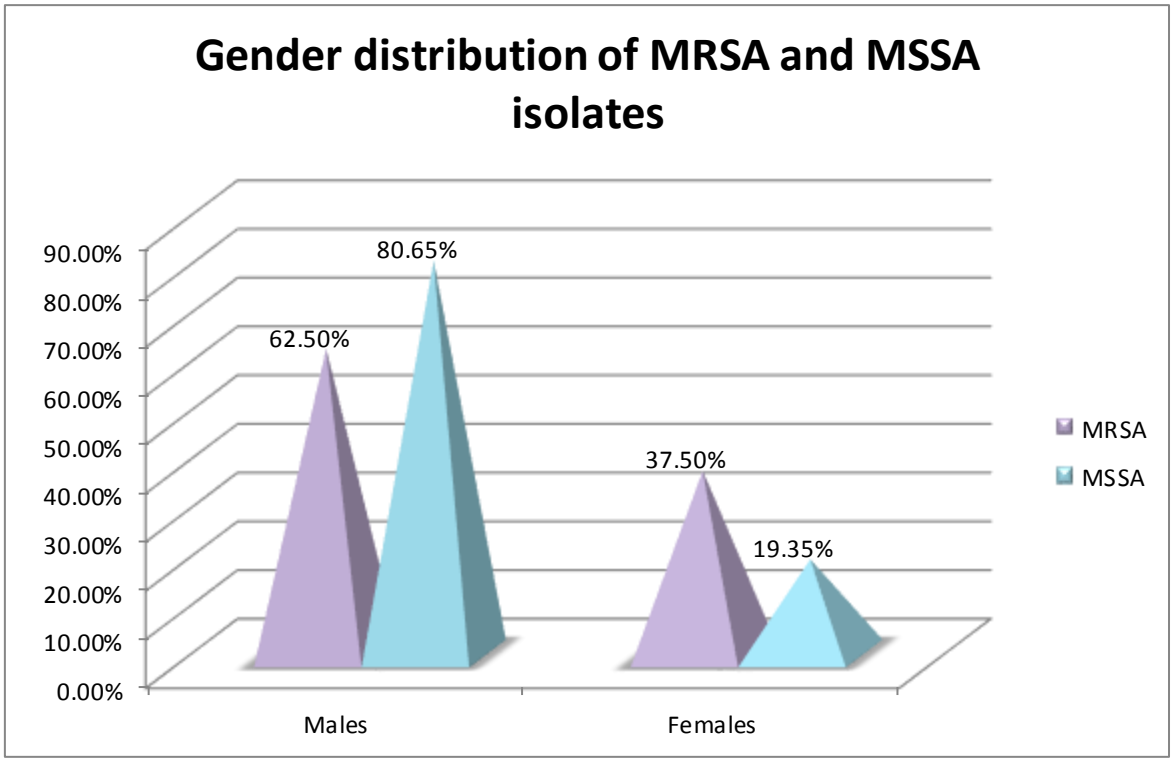
Out of the 196 culture positive isolates,63 (32.14%) were *Staphylococcus aureus*. *Trojan et al.*, reported 21 % *Staphylococcus aureus* isolates in pus isolates which is lower compared to our study. Muluye *et al.*, reported 32.9% which is in accordance of our study [10, 11].

Males had a higher isolation rate of *S. aureus* than females (45, 71.43%) versus (18, 28.57 %). Rate of isolation of *S. aureus* was the highest in the 41-60 years age group (44.44%).

This could be because as age increases, there is lowered immunity and poor wound healing and the presence of co morbidities such as hypertension and diabetes.




Prevalence of MRSA among Staphylococcus isolates
49.21%
50.79%
MRSA
MSSA


Gender distribution of MRSA and MSSA isolates
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
62.50%
80.65%
37.50%
19.35%
Males
Females
MRSA
MSSA

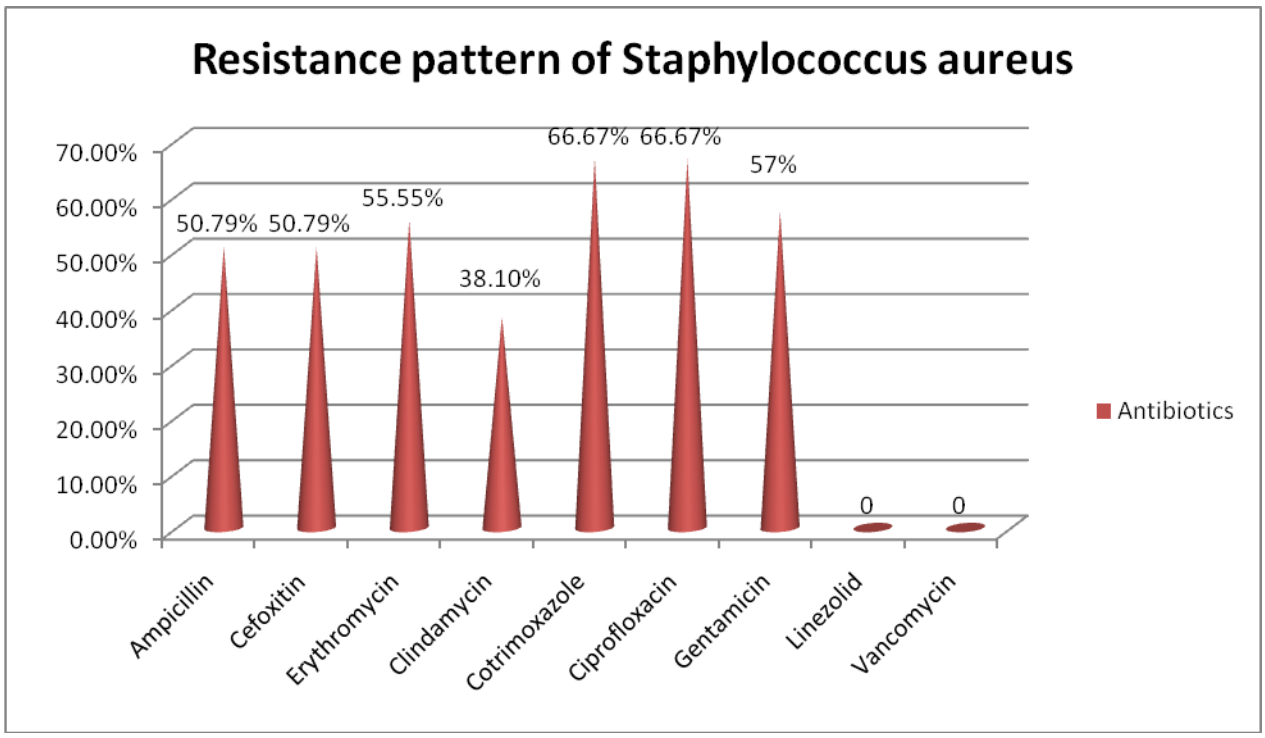


Table.1 Age wise distribution of MRSA isolates

Age group	**MRSA Percent**	**MSSA**
Less than 20 years	10(31.25%)	6 (19.35%)
21-40 years	7(21.88%)	9 (29.03%)
41-60 years	12(37.50%)	11 (35.48%)
61-80 years	3(9.37%)	5 (16.13%)

Table.2 Department wise distribution of MRSA

Ward	**MRSA prevalence(n=32)**	**MSSA(n=31)**
Surgery	12 (37.50%)	9 (29.03%)
Orthopaedics	7 (21.87%)	13 (41.94%)
Obstetrics & Gynaecology	4 (12.50%)	5 (16.13%)
Paediatrics	2 (6.25%)	1 (3.23%)
Urology	3 (9.38%)	2 (6.45%)
Intensive care unit	3 (9.38%)	0
Dermatology	1(3.12%)	1(3.23 %)

Table.3 Comparison of prevalence of *Staphylococcus aureus*

Author	Percent of S.aureus in wound infections	Publication
Our study	32.14%	-
Trojan et al.,	21 %	International Journal of Microbiology, vol. 2016, Article ID 9302692, 4 pages, 2016.
Muluye et al.,	32.9%	BMC Research Notes2014;**7**:619

Table.4 Comparison of prevalence of MRSA

Author	Percent of MRSA in wound infections	Publication
Our study	50.79%	-
Harshan et al.,	29.7%	Int.J.Curr.Microbiol.App.Sci (2015) 4(11): 718-723
Salurai et al.,	19%	International Journal of Microbiology, vol. 2017, Article ID 2529085, 5 pages, 2017
Arti Tyagi et al.,	44%	JIACM 2008; 9(1): 33-5
Tiwari et al.,	69.1%	Journal of Infection in Developing Countries, vol. 3, no. 9, pp. 681–684, 2009

Among the 63 *Staphylococcus aureus*, 32(50.79%) were MRSA and the rest 31 were Methicillin sensitive *Staphylococcus aureus*(49.21%).Males predominated with a percent of 62.5% and females 37.5%.

Harshan et al., reported MRSA prevalence of 29.7% which is lower and *Salurai et al.,* reported a percent of 19% [12,13] while *ArtiTyagi et al.,*[14]reported a prevalence of 44% and *Tiwari et al.,* 69.1% which is higher when compared with our study[15].

Again the rate of isolation of MRSA was highest in 41-60 years age group (37.50 %).

The difference in the prevalence of MRSA in different studies might be due to the difference in duration of the studies, healthcare facilities and hygiene measures followed by the hospital, implementation of rigid infection control precautions and the use of antibiotics, which may vary from one hospital to the other.

The prevalence of MRSA was the highest in the surgery wards where there is need to do daily dressings for the post-operative patients and the tendency to use antibiotics to prevent infection.

The Staphylococcal isolates showed high resistance to Ciprofloxacin (66.67%) and Cotrimoxazole (66.67%) followed by Gentamicin (57%) and Erythromycin (55.55%). *Bhattacharya et al.,* also reported a high resistance to Cotrimoxazole and Gentamicin [16]. But all the isolates were

sensitive to Linezolid and Vancomycin which is in accordance with *Rajaduraipandi et al.*, [17] and *Negi et al.*,[18] but Vancomycin resistance have been reported by other authors [19,20].

Our study shows that MRSA has been on the higher end in wound infections in our hospital. The MRSA could be prevented by identifying and screening MRSA carriers. Preventing colonisation and infection remains the most effective way to control the spread of MRSA and simple measures such as patient isolation, strict enforcement of hand washing will pave way towards reducing the spread of this pathogen. Vancomycin is the drug of choice of MRSA isolates. With such limited treatment options, we should develop a strong antibiotic policy to maximize the judicious use of antibiotics, with strict infection control measures, along with proper training and awareness alert of the nurses and other health care professionals to decrease the spread the spread of MRSA and cut down the rates of antimicrobial resistance.

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