

RESEARCH ARTICLE

## Prevalence and Antibiogram of Methicillin Sensitive and Methicillin Resistant *Staphylococcus aureus* Isolated from Pus Samples in a Tertiary Care Teaching Hospital

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### Abstract

*Staphylococcus aureus* is an important pathogen causing bacterial infection of skin lesions, such as burns, traumatic lesions, diabetic ulcers and post operative infections. The most common organism isolated from wound infections in our hospital is *Staphylococcus* and there have been several reports of MRSA isolates from pus samples during previous years. The aim of the present work is to study the rate of Staphylococcal wound infection and also to know the Antibiogram of Methicillin Sensitive and Methicillin Resistant *Staphylococcus aureus*. *Staphylococcus aureus* isolated from pus samples were identified based on colony morphology, Gram stain, Catalase and Coagulase test. Antibiotic susceptibility testing by disc diffusion was performed by Kirby Bauer method as per CLSI guidelines. Out of 595 samples collected, 534 [89.7 %] yielded growth of one or more bacterial species. Among 534 culture positive samples, 152 yielded pure growth of only staphylococcal species. 40.3% of the wound infections were caused by *Staphylococcus* species and among them 81.7% were caused by *Staphylococcus aureus*. The overall rate of MRSA infection in the present study was 29.6%. Significant difference was observed in the susceptibility pattern of Methicillin Sensitive and Methicillin Resistant *Staphylococcus aureus*. MRSA continues to be a major threat in the health care setting. The increased level resistance pattern of Methicillin Resistant *Staphylococcus aureus* observed in our study may be due to the fact that ours is a tertiary care hospital with extensive usage of broad spectrum antibiotics leading to survival advantage and persistence of drug resistant pathogens.

**Keywords:** *Staphylococcus aureus*, MRSA, MSSA, Wound infections, Antibiotic Susceptibility pattern.

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(Received: 26 October 2018; accepted: 18 December 2018)

**Citation:** Sowmya Govindanahalli Shivappa, Raghavendra Rao Morubagal, Rashmi Padmanabha Mahale and Ranjitha Shankare Gowda, Prevalence and Antibiogram of Methicillin Sensitive and Methicillin Resistant *Staphylococcus aureus* Isolated from Pus Samples in a Tertiary Care Teaching Hospital, *J Pure Appl Microbiol.*, 2018; **12**(4):2297-2303. http://dx.doi.org/10.22207/JPAM.12.4.71

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## INTRODUCTION

Staphylococci are an important pathogen causing human disease. It has an exceptional ability to colonise, spread and cause outbreaks in hospitals. They can be cultured even from dried clinical samples after several months, and are very hardy bacteria.<sup>1</sup> Therefore, it is not surprising that even in spite of availability of effective antimicrobial agents, improved health care conditions and stringent hospital infection control measures, *Staphylococcus aureus* has established itself as a major human pathogen.

*Staphylococcus aureus* is a major pathogen causing secondary bacterial infection complicating pre-existing skin lesions, such as burns, traumatic lesions, diabetic ulcers, osteomyelitis and surgical site infections. Staphylococcus isolated in hospitals has shown an alarming tendency to develop resistance to antimicrobial drugs<sup>2</sup> and has been recognized as one of the important challenges in control of hospital infections.

Resistance to penicillin appeared in 1942. The emergence of infections due to Methicillin Resistant *Staphylococcus aureus* [MRSA] during late 1970s and 1980s world over<sup>3</sup> and their introduction and spread in hospitals increased the rate of hospital acquired infections in the hospitals. The emerging threat of vancomycin resistance among coagulase negative Staphylococcus and Enterococci poses a serious public health concern as vancomycin has been the drug of choice for treatment of resistant gram positive organisms.

Wound infections are the most common type of pyogenic infections that contributes for prolonged hospital stays, significantly increasing the cost of medical care and also have an important role in the development of multi drug resistance. Staphylococcus is the most common organism causing wound infections and isolated from pus samples in our hospital and, there have been several reports of MRSA isolates from pus samples during previous years. Thus a study was taken up to detect the number of wound infections due to Staphylococci and also to study their susceptibility pattern which helps in better management of the infection. The main aim of the present study was to find out the rate of Staphylococcal wound infection and also to know the Antibiotic susceptibility pattern of Methicillin

Sensitive and Methicillin Resistant *Staphylococcus aureus* isolated from pus samples.

## Methodology

All patients admitted and/or attending surgery and orthopedics OPD with suspected microbial wound infection and the discharge, which yielded growth of staphylococci, were included in the present study.

## Sample collection

Pus from the wound was collected using two sterile swabs after cleaning the wound with sterile normal saline and surrounding skin with alcohol and transported immediately to Microbiology laboratory. Care was taken not to touch the wound edges inadvertently to avoid unnecessary skin contamination. A second sample was collected in all culture positives for staphylococci, for reproducibility of result, to confirm the presence of true infection and to rule out colonization and contamination.

## Direct smear study

Direct smears were made from the first swab and stained with Grams stain.<sup>4</sup> The smear was screened for the presence of inflammatory cells and the type of microbial flora.

## Culture

The second swab was inoculated onto Mac Conkey agar and Blood agar. Inoculated plates were incubated aerobically at 37°C for complete 24 hours.

## Identification of Staphylococci<sup>5</sup>

Cream to white, opaque Colonies on blood agar showing Gram positive cocci arranged in clusters in Grams stain smear were further subjected to additional tests such as catalase test [to differentiate from streptococci], Hugh leifson's oxidative fermentative test, susceptibility to Furazolidone 100mg disc and resistance to Bacitracin 0.04 units [to differentiate from micrococci], and coagulase test to identify coagulase positive and coagulase negative staphylococcus species.

## Antibiotic susceptibility testing

The staphylococcal strains were subjected for antibiotic susceptibility testing by employing Kirby Bauer disc diffusion technique against cefoxitin [30µg], penicillin[10 units], ampicillin[10µg], cefazolin [30µg], ciprofloxacin [5µg], gentamicin [10µg], erythromycin [15µg],

clindamycin [2µg] and linezolid [30µg] discs.<sup>6</sup> All the discs were procured commercially [Hi-media Laboratories limited].

The methicillin resistant staphylococcal strains were tested for vancomycin susceptibility by "Macrodilution broth susceptibility method". The MIC [Minimum Inhibitory Concentration] of the test strain was interpreted as the lowest concentration of vancomycin at which there was no visible staphylococcal growth judged by lack of turbidity and interpreted according to CLSI guidelines.<sup>7</sup> ATCC *S.aureus* 25923 was used as control strain for the susceptibility testing.

All the statistical analysis was performed using the software SPSS for Windows (Statistical Presentation System Software, SPSS Inc, New York) version 22.

**Table 1.** Age-Sex distribution

S.No.	Age/Sex	Male		Female		Total	
		No.	%	No.	%	No.	%
1	0-9	18	4.2	9	5.3	27	4.5
2	10-19	34	8.0	9	5.3	43	7.2
3	20-29	55	12.9	22	12.9	77	12.9
4	30-39	72	16.9	14	8.2	86	14.5
5	40-49	64	15.1	36	21.2	100	16.8
6	50-59	80	18.8	33	19.4	113	19.0
7	>60	102	24.0	47	27.6	149	25.0
	Total	425	100	170	100	595	100

Contingency Coefficient =0.136; P<0.080 (NS)

among patients above 60 years. In this study it was observed that the Burns wound infection [M:F:: 1:1] and post operative wound infections [M:F:: 1.5:1] were more common among females compared to other clinical conditions.

Out of 595 samples collected, 534 [89.7 %] yielded growth of one or more bacterial species. 61 [10.3 %] samples did not yield growth of any bacterial species even after 48 hours of incubation. Out of these 61 samples, 19 samples showed presence of pus cells and bacteria in the direct smear examination. The result of correlation between direct smear study and culture isolates is shown in Table 2.

Among 534 culture positive samples, 152 yielded pure growth of only staphylococcal species, 88 showed mixed growth of staphylococci with other gram positive and gram negative

## RESULTS

In the present study, 595 pus samples were collected from clinically suspected wound infection cases. Clinical diagnoses of the cases selected in the present study were diabetic foot, cellulitis, traumatic wound, osteomyelitis, non-healing ulcer, post-operative wound infection, gangrene and burns.

Out of the 595 subjects, 425 [71.4%] were male patients and 170 [28.6%] were female patients. The male to female ratio in our present study was 2.5:1. The age and sex distribution of the samples is depicted in Table 1.

152 cases of diabetic foot and 122 cases of cellulitis were the predominant clinical cases in the present study. These were found more

bacteria and 294 samples yielded growth of bacterial species other than staphylococci.

Among the 216 samples in which only gram positive cocci was seen in the direct smear study 152 yielded pure growth of staphylococci, 23 samples showed mixed growth of staphylococci and other Gram positive bacteria, 30 samples yielded the growth of other gram positive cocci and in 11 samples there was no bacterial growth.

Out of 95 samples in which mixed bacterial flora of gram positive cocci and other bacteria was seen, 65 samples showed mixed growth of staphylococcal species and other bacteria. 27 yielded growth of bacteria other than staphylococci and 3 did not yield growth of any aerobic bacteria.

In the present study overall rate of wound infection based on direct microscopic examination

**Table 2.** Association between Direct smear study and culture

S. No.	Direct smear study	Culture isolates					
			Pure growth of Staph	staph+other isolates	Other isolates	No growth	Total
1	PC & GPC	No.	152	23	30	11	216
		%	70.4	10.6	13.9	5.1	100
2	PC, GNB & GPC	No.	-	65	27	3	95
		%	-	68.4	28.4	3.2	100
3	PC & GNB	No.	-	-	237	5	242
		%	-	-	97.9	2.1	100
4	Few PC, No org	No.	-	-	-	32	32
		%	-	-	-	100	100
5	No PC, No Org	No.	-	-	-	10	10
		%	-	-	-	100	100
Total		No.	152	88	294	61	595
		%	25.5	14.8	49.4	10.3	100

Contingency Coefficient =0.799; P<0.000 (Highly Significant)

finding was 98.3%. Staphylococcal wound infection rate was 40.3% and wound infections due to bacteria other than staphylococci were 49.4%.

Among various clinical conditions, osteomyelitis [50.6%] and burns [50%] has yielded the highest staphylococcal growth and traumatic wounds have yielded the lowest [31.2%] growth.

In the present study, among 240 staphylococcal isolates, 196 [81.7%] were *Staphylococcus aureus*. The high rate of *Staphylococcus aureus* infection in the present study was observed among the cases of osteomyelitis [95%] followed by gangrene [90%].

**Table 3.** Distribution of *Staphylococcus aureus* among clinical samples

S. No.	Clinical diagnosis	<i>S. aureus</i>	
		No.	%
1	Diabetic foot	42	85.7
2	Cellulitis	48	81.4
3	Traumatic wound	18	62.1
4	Osteomyelitis	38	95.0
5	Non healing ulcer	24	85.7
6	Post op infection	11	64.7
7	Gangrene	9	90.0
8	Burns	6	75.0
	Total	196	81.7

Contingency coefficient = 0.257;  
P value = 0.017 (Highly significant)

The distribution of *Staphylococcus aureus* isolated from different clinical cases is shown in Table 3.

**Antibiotic susceptibility**

In our study, out of 196 *Staphylococcus aureus*, 58 [29.6%] were methicillin resistant and 138 [70.4%] were methicillin sensitive. Significant difference was observed in the susceptibility pattern of Methicillin Sensitive and Methicillin Resistant *Staphylococcus aureus* (Table 4)

Methicillin Resistant *Staphylococcus aureus* were completely resistant to both penicillin and ampicillin [100%]. 57[98.3%] MRSA were resistant to cefazolin and gentamicin. 55[94.8%] MRSA isolates were resistant to ciprofloxacin, 53[91.4%] were resistant to erythromycin and 31[53.5%] were resistant to clindamycin. Thus in the present study, 31 strains of MRSA were found to be Multi Drug Resistant showing resistance to erythromycin, clindamycin, ciprofloxacin, gentamicin, cefazolin and penicillin group of drugs.

Out of 138 methicillin sensitive *S.aureus*, 35[25.4%] strains were sensitive to penicillin and ampicillin, while 136[98.6 %] were susceptible to cefazolin. The susceptibility rate of MSSA to gentamicin, ciprofloxacin, erythromycin and clindamycin was 92%, 97.1%, 94.9% and 97.8% respectively. 3 MSSA strains showed inducible resistance to clindamycin. All staphylococci in our study were sensitive to linezolid and vancomycin.

**Table 4.** Antibiogram of *Staphylococcus aureus* isolates

S. No.	Drugs	MRSA [n=58]				MSSA [n=138]				CC	P value
		Sensitive		Resistant		Sensitive		Resistant			
		No.	%	No.	%	No.	%	No.	%		
1	Penicillin	0	-	58	100	35	25.4	103	74.6	0.289	.000
2	Ampicillin	0	-	58	100	35	25.4	103	74.6	0.289	.000
3	Cefazolin	1	1.7	57	98.3	136	98.6	2	1.4	0.694	.000
4	Gentamycin	1	1.7	57	98.3	127	92	11	8	0.655	.000
5	Ciprofloxacin	3	5.2	55	94.8	134	97.1	4	2.9	0.675	.000
6	Erythromycin	5	8.6	53	91.4	131	94.9	7	5.1	0.65	.000
7	Clindamycin	27	46.6	31	53.5	135	97.8	3	2.2	0.532	.000
8	Linezolid	58	100	0	-	138	100	0	-	-	-
9	Vancomycin	58	100	0	-	138	100	0	-	-	-

**Table 5.** Comparison of Resistance pattern of MRSA isolates by various studies

S. No.	Author	Resistance pattern of MRSA isolates [%]							
		P	A	Cz	G	Cf	E	Cd	Va
1	Uma Chaudhary et al <sup>11</sup>	100	100	96.8	38.2	5.7	42	6.3	0
2	Anvikar et al <sup>18</sup>	100	100	-	98.9	-	95.9	-	-
3	Udaya Shankar et al <sup>19</sup>	-	-	-	-	95.8	-	-	-
4	Hanumanthappa et al <sup>20</sup>	-	100	-	81.39	65.11	93.02	-	0
5	Pulimood et al <sup>21</sup>	-	-	-	85.5	90	-	-	0
6	Kakru et al <sup>12</sup>	48	43	38	38	40	42	-	0
7	Anupurba et al <sup>13</sup>	100	100	88.7	10.3	84.1	-	-	0
8	Majumder et al <sup>22</sup>	94.1	-	-	70.3	22.8	33.9	-	-
9	Vidhani et al <sup>14</sup>	-	-	79.5	79	33	22.3	-	0
10	Present study	100	100	98.3	98.3	94.8	91.4	53.5	0

## DISCUSSION

The discovery and development of miracle antibiotics is undoubtedly one of the utmost advances of modern medicine. But, unfortunately, the emergence of multidrug resistant bacteria, is threatening the effectiveness of many of these antimicrobial agents.

When Penicillin was first introduced in early 1940s, almost all staphylococci were susceptible. By 1942, there were few isolated reports of penicillin resistance among *S.aureus* isolates, by 1950s almost 50% of all strains were resistant to penicillin. In 1960, penicillinase resistant drugs such as methicillin, oxacillin, nafcillin etc., were introduced. By late 1970s Methicillin resistant strains of staphylococci emerged and they have become increasingly more established as nosocomial pathogens.

The Male to Female ratio in our study was 2.5:1. The increased rate of wound infections among males could be due to their outdoor occupation, more prone for injuries and infections occur due to exposure to contaminated environment. A similar observation has been made by Siddiqi et al<sup>8</sup> who have reported a male to female ratio of 2.6:1.

Out of 595 cases, 149 [25.1%] were found in the sixth decade. Majority of these were cases of diabetic foot wounds and cellulitis. This is because of diminishing immunity as age advances and also the possibility of underlying hormonal abnormalities.

The direct gram stain smear study of the samples helps to confirm the presence of true infection, to identify the type and number of bacteria causing infection. It also gives a clue

to the clinician to initiate an effective empirical antibiotic treatment pending culture report. Absence of inflammatory cells would suggest faulty collection of the sample or an indication of absence of pyogenic infection.

In the present study, Gram stained direct smear study revealed presence of infection in 98.3% of samples. 96.8% correlation between the gram stained smear finding and culture was observed in the present study. De et al<sup>9</sup> also has observed a correlation rate of 87.3% which is comparable with that of the present study. Thus the observations of this study and that of other workers confirm that there is no replacement for a gram stained direct smear study in clinical microbiology.

In this study, 81.7% of the wound infections were caused by *Staphylococcus aureus*. Dang et al<sup>10</sup> have reported 79% infection with *S.aureus*.

The overall rate of MRSA infection in our study was 29.6%. The MRSA infection rate is consistent with Uma Choudhary et al<sup>11</sup> [26.8%] and Kakru et al<sup>12</sup> [33%]. Higher MRSA infection rate is reported by Anupurba et al<sup>13</sup> [52.5%], Vidhani et al<sup>14,15</sup> [51.6% and 42.02%] and Mohanty et al<sup>16</sup> [38.56%]. The higher rate in their studies has been attributed to fact that the study group was from a tertiary care multispecialty center with no admission policy and indiscriminate use of antibiotics.

The multidrug resistance of MRSA to a wide range of antibiotics is well known. The antibiotic susceptibility results showed that all MRSA isolates were significantly highly resistant to antibiotics than MSSA isolates [ $P < 0.00$ ]. The resistance pattern of MRSA to  $\beta$  lactams like penicillin and ampicillin was 100% in our study. Similar findings were seen in the studies by Gupta et al,<sup>17</sup> Uma choudhary et al,<sup>11</sup> Anupurba et al<sup>13</sup> and Anvikar et al<sup>18</sup> (Table 5).

In the present study, 91.4% of MRSA isolates were resistant to erythromycin. Similar rate of resistance was observed in studies of Anvikar et al<sup>18</sup> [95.9%], Hanumanthappa et al<sup>20</sup> [93.02%] and Gupta et al<sup>17</sup> [100%]. 39.7% [23 isolates] of MRSA isolates in present study were resistant to clindamycin. A consistent result was reported by Thouvez et al<sup>23</sup> [30%]. A low percentage of 6.3% was reported by Uma Choudhary et al<sup>11</sup> and they

have opined that clindamycin can be used as first line drug for treatment of MRSA infections.

Linezolid, the oxazolidinone has shown 100% efficacy against *Staphylococcus aureus* in the present study. Similar consistent activity of linezolid against MRSA has also been shown by Stevens et al.<sup>24</sup> However, linezolid resistance in *S.aureus* has been reported by Tsiodras et al.<sup>25</sup> As linezolid's antibacterial activity is comparable with that of vancomycin in the present study, linezolid can be used as another option to vancomycin in treating MRSA infections.

## CONCLUSION

The observations of the present study conclude that staphylococcal infections are the most common cause of wound infections among hospitalized patients and continue to be a growing thorn in the Health care system. Despite the latest reports that Gram Negative bacteria have overtaken staphylococci as the leading cause of nosocomial infections, MRSA continues to be the major threat in the health care setting. The results of this study would not only contribute a great deal in the prevention of such infections but also help us in formulating an antibiotic policy for the treatment of Staphylococcal lesions in our hospital.

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