

Isolation and Identification of *Staphylococcus aureus* from Skin and Soft Tissue Infection in Sepsis Cases, Odisha

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Staphylococcus aureus, a major gram positive bacterial pathogen that causes a wide spectrum of clinical infections, ranging from localized soft-tissue infections to life-threatening bacteremia and endocarditis. *S. aureus* infects tissues when the skin or mucosal barriers have been breached which leads to many different types of infections, including boils, carbuncles (a collection of boils) and abscesses. The present study envisaged the incidence of *S. aureus* in skin and soft tissue infection (SSTI), its susceptibility pattern to different antibiotics and molecular characterization isolated from three different hospitals of Odisha. Out of 1702 samples collected 1622(95.29%) were culture positive for different bacterial pathogens from which *S. aureus* was 356 (20.9%). The incidence rate of *S. aureus* among male and female group of patients was 57.5% and 42.2% respectively. The isolated *S. aureus* were resistant to most of the antibiotics such as azithromycin, penicillin, doxycycline, ciprofloxacin, tetracycline, gentamicin, ofloxacin, chloramphenicol, ampicillin and oxacillin. Further methicillin resistant *S. aureus* (MRSA)-95.8% were also identified from the *S. aureus* strains which were multi drug resistant and few were resistant to vancomycin (5%). The MRSA strains were confirmed genotypically by amplification of methicillin resistant (*mecA*- 63.3%) gene. Identification and antibiogram profile of *S. aureus* is highly essential for the treatment of sepsis patients in Odisha.

Keywords: *Staphylococcus aureus*, MRSA, Sepsis, Odisha.

Staphylococcus aureus is able to cause a large diversity of lethal infections in human because of a wide range of virulence factor that includes various toxin and enzymes¹. It has emerged as one of the most important human pathogen and has become a leading cause of hospital and community acquired infection². The introduction of penicillin greatly improved the prognosis of patients with severe staphylococcus infection, but after a few years resistance appeared

owing to production of β -lactamase. Methicillin was designed to resist β -lactamase degradation, but methicillin resistant *Staphylococcus aureus* (MRSA) strain that were resistant to all of β -lactam antibiotics were identified soon after methicillin was introduced³.

Sepsis causes millions of deaths globally each year.⁴In various sepsis cases skin and soft tissue infection (SSTI) caused by *Staphylococcus aureus* is common in hospitals. Approximately 7% to 10% of hospitalized patients were affected by SSTIs, and they are very common in the emergency care setting⁵. *S. aureus* associated with SSTIs frequently begin as minor boils or abscesses

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and may progress to severe infections involving muscle or bone and may disseminate to the lungs or heart valves (i.e. endocarditis)⁶. In the late 1990s, MRSA strains emerged in the community setting, without established high risk factor called community-associated (CA)-MRSA^{7,8}. SSTI due to CA-MRSA were more frequent among male⁹ and ranged from 16% to 44% of cases globally¹⁰. The incidence of MRSA varied from 25 per cent in western part of India¹¹ to 50 per cent in South India¹². Most SSTI are treated in outpatient settings with empiric antimicrobial therapy. Due to relative resistance of antibiotics which is difficult to treat and more virulent strains with capacity to adapt quickly to changing environment make the pathogens acquired in the hospital a matter of concern.¹³ Various studies across the globe have been consistent enough to show a predictable bacterial profile in the wound infections and few studies have attempted to estimate the number of *Staphylococcus aureus*- SSTIs; which makes an important observation for a clinician who intends to start empirical treatment to his patients, while laboratory culture reports are awaited.¹³

So far there is scanty report available on MRSA related to septic patients from Odisha. The present study has been envisaged to document the incidence of *S. aureus* (MRSA) strains in skin and soft tissue infections of septic patients from three different hospitals from this state.

MATERIALS AND METHODS

Study site

The skin and soft tissue infection like cellulitis, abscesses and soft tissue infection were included from the OPD and IPD of Capital Hospital, Bhubaneswar, District Headquarter hospital, Khurda and SCB Medical College and Hospital, Cuttack from March, 2015 to February, 2018. The informed consent were taken from patients/ their attendants before collection of samples. The pus samples were collected by sterile swabs in sterile vials in cold condition bearing the patients name, age, sex and were transported to microbiology division of Regional Medical Research Centre (RMRC), Bhubaneswar.

Bacteriological analysis

The samples were immediately inoculated on MacConkey, blood Agar and nutrient Agar (Hi

Media, Mumbai) plates and incubated at 37°C for 24hrs. Blood Agar plates were incubated in aerobic and anaerobic conditions. Significant colonies with beta hemolysis in blood agar plates were picked up for gram's staining and biochemical tests were done for confirmation of bacterial pathogens. Identification of *S. aureus* isolates was done based on colony morphology and biochemical tests like catalase, coagulase test (slide and tube), etc. Pure isolates of *S. aureus* were further confirmed by manitol salt agar test and chromogenic agar media (Hi Media, Mumbai) was used for the rapid detection of MRSA strains¹⁴.

Antimicrobial susceptibility

Antibiotic susceptibility analysis was performed by modified Kirby Bauer's Disk diffusion technique (1966) with commercially available antibiotic disc (Hi Media, Mumbai). Characterization of stains as susceptible, intermediately resistant or resistant was done as based on the size of the inhibition zone according to the manufacturer's instruction which matched the interpretive criteria recommended by WHO¹⁵. The antibiotics used were penicillin(P, 10 µg), azithromycin(AZM, 15 µg), ciprofloxacin(CIP, 5 µg), tetracycline(T, 30 µg), gentamicin (G, 10 µg), doxycycline (DO, 30 µg), vancomycin (VA, 30 µg), ofloxacin(OF, 5 µg), chloramphenicol(C, 30 µg), ampicillin(A, 10 µg), ceftiofloxim (CX, 30 µg) and oxacillin(OX, 1 µg).

MIC by E-Test

The E-Test (Hi Media Laboratories Pvt, Ltd, Mumbai, India) for determining oxacillin MICs were performed on Muller-Hinton agar plate with 2% NaCl as per instruction and plates were incubated at 35°C for 24hrs. Similar procedure was followed for MIC of vancomycin and reading was taken after 24hrs of incubation as per the criteria provided by the CLSI guidelines (CLSI 2005).

The detail is as follows:

MIC of oxacillin > 4 µg/ml- resistant strains (MRSA);

MIC < 2 µg/ml- sensitive *Staphylococcus aureus* (MSSA).

MIC of vancomycin > 16 µg/ml- resistant *Staphylococcus aureus* (VRSA);

MIC (4-8 µg/ml)-intermediate *Staphylococcus aureus* (VISA);

MIC < 2 µg/ml-sensitive *Staphylococcus aureus* (VSSA) strains.

PCR amplification

Polymerase Chain Reaction was performed for molecular confirmation of MRSA strains by amplification of methicillin resistant (*mecA*) gene in a thermal cycler as described by Stegger et al, (2012)¹⁶. DNA amplification was carried out for 30 cycles according to the following protocol: denaturation at 94°C for 30s, annealing at 55°C for 30s, and extension at 72°C for 1 min. with a final extension at 72°C for 5 min. The PCR products were analysed in 2% agarose gel stained with ethidium bromide and visualized under UV light.

RESULTS

Out of 1702 samples collected, 1622 (95.29%) were culture positive for different bacterial pathogens from which *S. aureus* was 365 (20.9%). *S. aureus* isolated from different categories were, soft tissue infection-195(12.02%), abscess-33(2.03%) and cellulitis-137(8.5%). MRSA strains isolates was 350 (95.8%) from the total *S. aureus* isolates (Table 1).

In this study the clinical characterization for the positive cases of *S. aureus* in soft tissue infection was cellulitis with inflammation, wound infection, scalded skin, and folliculitis; in abscess cases infection of sebaceous cyst with pus discharge was observed. Most of the patients were from the community attending the OPD of the hospitals and few were indoor patients admitted in case of severe condition. Maximum *Staphylococcus aureus* infected cases were seen in the age group of 30-40 years in both male-90 (24.6%) and female-73(20%) followed by age group 50-60 years then 40-50 years. Least cases were found in age group 18-30 years. (Table 2). Most of the infections were associated with single pathogens and few were with multiple pathogens. Poly microbial infection was isolated from 362 (17.2%) cases but the coexistence of *Staphylococcus aureus* with different gram negative bacteria were seen in few cases.

Staphylococcus aureus were resistant to most of the antibiotics such as azithromycin, penicillin, doxycycline, ciprofloxacin, tetracycline, gentamicin, ofloxacin, chloramphenicol, ampicillin and oxacillin. Ninety percent of the *Staphylococcus aureus* isolated were methicillin resistant *S. aureus* (MRSA) strains as they were resistant to oxacillin

as per CLSI guidelines and vancomycin resistant strain were 20.1% (Table-3). The MIC test for oxacillin and vancomycin was performed on 200 MRSA confirmed strains which showed 120(60%) were resistant for oxacillin and 80(40%) were sensitive. Out of total MRSA strain (200), 90(45%) of the MRSA strains were sensitive to vancomycin, 100(50%)-intermediate and 10(5%) were resistant. PCR amplification on 260 *S. aureus* positive strains revealed that 164(63.3%) were positive for *mecA* gene.

DISCUSSION

Staphylococcus aureus is an important human pathogen and is implicated in a wide variety of infections such as skin and soft-tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis.¹⁷ In most of the sepsis case it was seen that *S. aureus* infection starts from small skin sites and further progresses to severe infections involving muscles or bone and may disseminate to lungs or heart. The cause of transmission of *S. aureus* infection could be by direct contact with infected patients, colonized subjects or a contaminated environment as most of the *S. aureus* were isolated generally from OPD patients. Pain and fever were invariably present in infection involving lower extremities; and in blood infection an increase in WBC count was observed. In most of the skin tissue infection, abscesses and ulcer cases the recurrence of lesions was frequent as observed in our study.

Effective antibacterial therapy may improve cure rate especially among patients with larger/severe infection. Over the past 20 years, the incidences of both community-acquired (CA) and hospital-acquired (HA) *S. aureus* infections have increased, while antibiotic treatment is increasingly hampered by the spread of *S. aureus* strains that are resistant to multiple antibiotics, including methicillin.¹⁸ As per our study the infection rate was more in male population than the female which was similar to the research carried out in United States¹⁹. The predominance of mono-microbial infections from chronic wound infections was observed which was similar to the findings reported from Banaras Hindu University, Varanasi²⁰. Incidence of MRSA strains isolates was 350 (95.8%) from the total *S.*

aureus isolates and coagulase negative *S. aureus* was 10.2%. Similar types of findings were seen in clinical cases in Iraq hospitals where MRSA

percentage isolated was 95.2% and coagulase negative *S. aureus* (CoNS) was 4.7%²¹.

According to the antibiotic status of this study doxycycline and vancomycin were highly

Table 1. Prevalence of *Staphylococcus aureus* from different SSTI patients

Category of SSTI cases	Total cases	Culture+ve	<i>S. aureus</i>	MRSA
Soft tissue Infection	510(30.08%)	501(29.4%)	195(12.02%)	190(52%)
Abscess	252(14.8%)	216(12.7%)	33(2.03%)	30(8.2%)
Cellulitis	940(55.23%)	905(53.2%)	137 (8.5%)	130(35.6%)
Total	1702	1622(95.29%)	365 (20.9%)	350(95.8%)

Table 2. Age group wise distribution of *S. aureus* positive cases

Age	Gender	
	Male	Female
>18 - <30	6 (1.6%)	9(2.5%)
>30- <40	90 (24.6%)	73(20%)
>40 - <50	39(10.%)	13(3.5%)
>50 - <60	76(20.8%)	59(16.2%)
Total	211(57.8%)	154(42.2%)

sensitive drugs against MRSA isolates but were resistant to most of the other antibiotics. All of the MSSA strains were sensitive to azithromycin, penicillin, chloramphenicol, doxycycline, tetracycline, gentamicin, ofloxacin, vancomycin, ampicillin, cefoxitin and oxacillin and showed least sensitive to ciprofloxacin. The MIC results confirmed the presence of MRSA and VRSA strains as these were resistant to both oxacillin and vancomycin, similar results were reported

Table 3. Resistance and sensitivity Profile (%) of *S.aureus* to different antibiotics

Antibiotics	Strain (MRSA n=721) (MSSA n=77)	Resistant (%)	Sensitive (%)	P value
Azithromycin	MRSA	65	35	2E-06
	MSSA	14.7	85.3	
Penicillin	MRSA	97.4	2.6	-
	MSSA	23.6	76.4	
Ciprofloxacin	MRSA	86.1	13.9	-
	MSSA	47.3	52.7	
Chloramphenicol	MRSA	59	41	0.0011
	MSSA	27.3	72.7	
Doxycycline	MRSA	31.2	68.8	0.00497
	MSSA	4.1	95.5	
Ofloxacin	MRSA	76.1	23.9	2.6E-05
	MSSA	38.3	61.7	
Tetracycline	MRSA	61.5	38.5	1.4E-05
	MSSA	14.8	85.2	
Vancomycin	MRSA	20.1	79.9	-
	MSSA	100	0	
Ampicillin	MRSA	65.9	34.1	1E-06
	MSSA	16.5	83.5	
Gentamicin	MRSA	56.6	43.4	1E-06
	MSSA	4.1	95.9	

MRSA and MSSA- methicillin resistant and sensitive *S. aureus*
(P<0.005 is Significant)

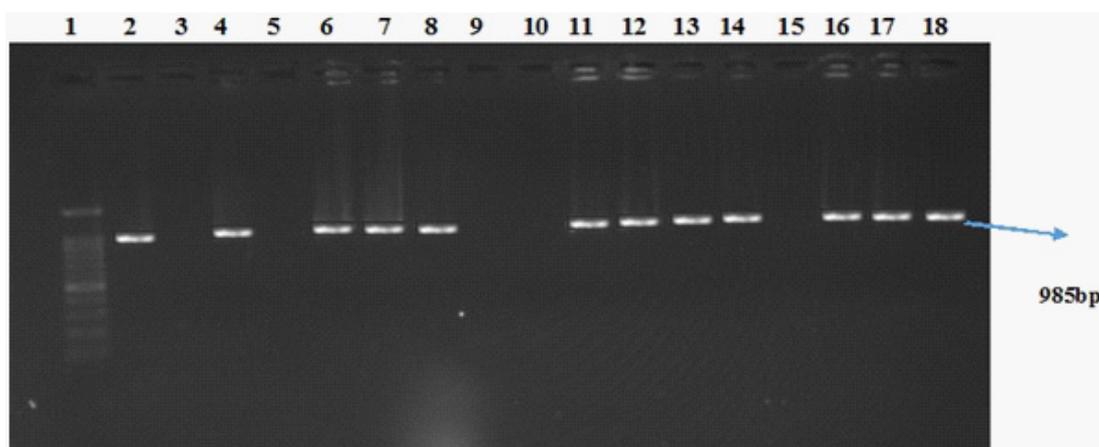


Fig. 1. Detection of the *mecA* gene (985bp) by polymerase chain reaction assay by 1.8% agarose gel electrophoresis. Lane 1: 100bp molecular weight marker; lanes 2: positive control (standard *S. aureus*) strain and Lane 3: negative control, Lane 4 -18: Samples

in an Indian teaching Hospital from Odisha by Dubey et al., 2013¹⁴. But MRSA strain which were 100% sensitive to vancomycin was reported from a surveillance study done in different hospitals of North and south India²². The resistance value that were observed in the *S. aureus* isolated strains by phenotypic methods was compared by PCR amplification for MRSA detection and it interpreted similar results.

The emergence and spread of antibiotic resistance remains a global public health concern. So a core function of all clinical laboratories have to determine the antibiotic susceptibility pattern of bacterial isolates which would guide the clinicians in treating the patient infected with MRSA. High clinical suspicion and appropriate microbiological tests are also essential for early diagnosis and proper administration of antibiotics to reduce morbidity rate. So, continuous surveillance on resistance pattern of MRSA isolates and molecular study of toxic genes are essential for the understanding of new and emerging trends of this infection. Thus, this type of study should be encouraged in different hospitals of Odisha.

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