

## Original Research Article

<https://doi.org/10.20546/ijcmas.2017.606.108>

## Study of Antibiotic Sensitivity Pattern of *Staphylococcus aureus* and Comparison of Cefoxitin and Oxacillin Discs for Detection of MRSA in a Tertiary Care Hospital

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### ABSTRACT

#### Keywords

MRSA, Cefoxitin, Oxacillin, Biochemical.

#### Article Info

##### Accepted:

17 May 2017

##### Available Online:

10 June 2017

*Staphylococcus aureus* is the commonly encountered pathogen isolated from clinical specimens. Methicillin resistant *Staphylococcus aureus* (MRSA) causes variety of human infections resulting in high rate of mortality and morbidity. The aim of the study was to screen for MRSA by disc diffusion method using cefoxitin and oxacillin discs and to determine antimicrobial susceptibility and resistance of *Staphylococcus aureus*. Two hundred *Staphylococcus aureus* were isolated from samples like pus, blood, sputum, vaginal swab, urine and body fluids received in microbiology department of Coimbatore medical college hospital. They were confirmed by microscopy, culture and biochemical reactions. MRSA were detected by disc diffusion test using cefoxitin (30µg) and oxacillin (1µg) discs. Majority of *Staphylococcus aureus* were isolated from pus samples (87.5%). Isolated *Staphylococcus aureus* strains were highly sensitive to linezolid (100%) and vancomycin (99%) and 100% resistant to penicillin. Cefoxitin disc detected higher percentage (26%) of MRSA than oxacillin disc (24%). Detection of MRSA is considered as very important for treating patients and to prevent its spread.

### Introduction

*Staphylococcus aureus* has the ability to asymptotically colonize the normal population either persistently or transiently. 30% of humans are likely to be nasal carriers. Person to person contact or contact with fomites plays a role in its transmission. Loss of normal skin barrier and presence of predisposing factors such as diabetes and HIV complicates infection. *Staphylococcus aureus* causes human infections such as furuncles, cellulitis, abscesses, toxic Shock syndrome, Staphylococcal scalded skin syndrome, endocarditis, pneumonia and

septicemia. Penicillin was the drug of choice to which *Staphylococcus aureus* developed resistance by producing the enzyme beta lactamase and hence Methicillin was introduced in 1959. In spite of this, MRSA appeared and was rapidly spreading in hospitals in 1961 (Poonam Sood Loomba *et al.*, 2010) Prolonged hospitalization, indiscriminate use of antibiotics and in dwelling medical devices are the cause for the appearance and spread of MRSA. The nosocomial multi drug resistant MRSA (HA-MRSA) strains have a high effect on patient

morbidity and mortality. Beta lactam agent's binds to PBP in cell wall of *Staphylococcus aureus* resulting in disruption of peptidoglycan synthesis and bacterial cell death. The *mec A* gene coding for PBP2a in cell wall of MRSA harboured by mobile SCC *mec* chromo some is responsible for methicillin resistance. Detection of MRSA can be performed by an oxacillin or cefoxitin disc diffusion test. Cefoxitin is a strong inducer of *mec A* gene and thus helps in detection of MRSA, specific methods for detecting antibiotic resistance in these pathogens accurately has become a significant tool in clinical diagnosis.

### **Materials and Methods**

The present study was conducted in Coimbatore Medical College Hospital, Coimbatore. The study period was for one year from September 2011 to August 2012. Before starting the study the ethical and research clearance was obtained from Ethical Committee of Coimbatore Medical College Hospital, Coimbatore. Total of 200 *Staphylococcus aureus* isolates from clinical samples including, pus, sputum, blood, vaginal swab and urine were included in the study. Samples were received from in patients who attended Coimbatore Medical College Hospital. The received samples were checked for proper labelling with name, age, sex and IP/OP number of the patient, date and time of collection of the sample and processed immediately. Blood samples sent in brain heart infusion broths were incubated for 18-24 hours and the sub cultured. All the above specimens were inoculated on to the nutrient agar, blood agar, and macconkey agar, and incubated at 37°C for 18-24 hours aerobically and observed after incubation. All the suspected colonies were identified by colony morphology, gram staining was done and the organism subjected to various biochemical tests to identify and characterize them. Further confirmation was done by slide and

tube coagulase test, catalase test and growth on mannitol salt agar.

The sensitivity to common antibiotics was done by Kirby Bauer Disc Diffusion method as recommended by CLSI. Control strains used were *Staphylococcus aureus* ATCC - 25923 and MRSA-ATCC - 43300.

A swab was submerged in bacterial suspension and was inoculated onto, Mueller Hinton agar plate. The surface of the plate is swabbed in three directions so that the reiseven and complete distribution of the inoculum. Within 15 minutes of inoculation antibiotic discs were applied using sterile forceps. The antimicrobial discs used were procured from Himedia. The drugs oxacillin (1µg), cefoxitin (30µg), penicillin (10u), linezolid (30µg), vancomycin (30µg), doxycycline (30µg), amoxycylavulanic acid (30µg,10µg), cephelexin (30µg), cotrimoxazole (25µg), cefotaxime (30µg), amikacin (30µg), and ciprofloxacin (5µg) were pressed down to ensure complete contact with the agar surface and incubated at 37°C for 24 hrs after which the zone of inhibition was measured by using zone measuring scale and interpreted as per the CLSI standards. In oxacillin disc diffusion test (Nicole Broekema *et al.*, 2009) zone diameter of 13mm or more was taken as sensitive, 11 to 12mm was taken as intermediate sensitive and 10mm or less is considered as MRSA. In cefoxitin disc diffusion test zone diameter of 22mm or more was taken as sensitive and 21mm or less was considered as resistant. These resistant isolates were considered as MRSA.

### **Results and Discussion**

The study was performed during the period from September 2011 to August 2012 at Department of Microbiology, Coimbatore Medical College Hospital. The study included 200 *Staphylococcus aureus* isolates from

samples like pus, blood, sputum, vaginal swab, urine and body fluids. Among 200 *Staphylococcus aureus* isolates the sample wise distribution was as follows: Pus constituted 175 (87.5%), urine 10 (5%), blood 6 (3%), sputum 4 (2%), vaginal swab 3 (1.5%) and synovial fluid. 2 (1%), the above observation shows that *Staphylococcus aureus* was isolated maximally from pus samples (87.5%) and only few were isolated from urine, blood, sputum, vaginal swab and other body fluids.

As evident by table 1, out of the 200 *Staphylococcus aureus* isolates 100% were sensitive to linezolid and 99% were sensitive to vancomycin. 77 % were sensitive to amikacin, 73% were sensitive to doxycycline, 69% were sensitive to cotrimoxazole, 68.5% were sensitive cephalixin, 66.5% were sensitive to amoxicillin clavulanic acid, 64% were sensitive to cefotaxime, 59% were sensitive to ciprofloxacin. *Staphylococcus*

*aureus* strains were highly sensitive to linezolid and vancomycin. Moderate level sensitivity was seen in amikacin, doxycycline, cotrimoxazole, cephalixin, amoxycillin clavulanic acid, cefotaxime and ciprofloxacin.

Out of the 200 isolates 100% were resistant to penicillin G, 33.5% were resistant to ciprofloxacin, 33.5% were resistant to amoxycillin clavulanic acid, 27.5% were resistant to cephalixin, 27.5% were resistant to cotrimoxazole, 26.5% were resistant to cefotaxime, 24.5% were resistant to doxycycline, 20.5% were resistant to amikacin and 1% were resistant to ancomycin. *Staphylococcus aureus* isolates were 100% resistant to penicillin and 100% sensitive to linezolid. Moderate level of resistance were seen to amikacin, ciprofloxacin, doxycycline, co-trimoxazole, cephalixin, cefotaxime and amoxycillin clavulanic acid. Very minimal resistance was noted in vancomycin.

**Table.1** Antibiotic sensitivity pattern of *Staphylococcus aureus*

(n=200)

Drugs	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Linezolid	200(100%)	-	-
Vancomycin	198(99%)	-	2(1%)
Amikacin	154(77%)	5(2.5%)	41(20.5%)
Doxycycline	146(73%)	5(2.5%)	49(24.5%)
Cotrimoxazole	138(69%)	7(3.5%)	55 (27.5%)
Cephalixin	137(68.5%)	8(4%)	55 (27.5%)
Amoxyclavulanic acid	133(66.5%)	-	67(33.5%)
Cefotaxime	128(64%)	19(9.5%)	53(26.5%)
Ciprofloxacin	118(59%)	15(7.5%)	67(33.5%)
Penicillin G	-	-	200(100%)

**Table.2** Prevalence of MRSA among *Staphylococcus aureus* isolates

Total isolates	MRSA	MSSA
200	52(26%)	148(74%)

**Table.3** Detection of Methicillin resistance using Oxacillin and Cefoxitin disc diffusion test  
n=200

Disc diffusion test	Cefoxitin (30µg)disc	Oxacillin (1µg) disc
Resistant	52(26%)	48(24%)
Sensitive	148(74%)	152(76%)

**Fig.1** MRSA detection using cefoxitin and oxacillin discs



As cited in table 2, among 200 *Staphylococcus aureus* isolates, 74% were sensitive to methicillin and 26% were MRSA. As evident from table 3 among 200 isolates of *Staphylococcus aureus*, 26% were resistant and 74% were sensitive to cefoxitin whereas 24% were found to be resistant and 76% were sensitive to oxacillin as determined by disc diffusion method. Cefoxitin disc detected higher percentage of methicillin resistant *Staphylococcus aureus* by disc diffusion method.

MRSA is a major cause of hospital and community acquired infections, pneumonia, and staphylococcal scalded skin syndrome. In the present study 200 samples were processed and results were analysed. In this study majority of the *Staphylococcus aureus* isolates, (87.5%) were from pus samples while 5%, were from urine, 2% were from sputum, 3%, were from blood, 1.5% were from vaginal swab and 1% were from synovial fluid. This is supported by the study of Vidyapai *et al.*, (2011) who has isolated

181 (76.3%) of *Staphylococcus aureus* in pus samples followed by 28 (11.81%) from urine, 17 (7.17%) from respiratory specimen, 9 (3.79%) from blood and 2 (0.84%) from body fluids. This also correlates with the study conducted by Anupurba *et al.*, (2003), in which, they have reported 381 (69.39%) of *Staphylococcus aureus* in pus samples followed by 59 (10.74%) from urine, 25 (4.55%) from high vaginal swab, 27 (4.91%) from body fluids, and sputum 23 (4.18%). Lakari Saikia *et al.*, (2009) have reported 46.67% of *Staphylococcus aureus* from pus and 42.86% from sputum.

The present study showed multi drug resistant pattern of *Staphylococcus aureus* as amikacin 20.5%, ciprofloxacin 33.5%, doxycycline 24.5%, cotrimoxazole 27.5%, and cephelexin 27.5 % cefotaxime 26.5 %, penicillin G 100%, vancomycin 1% and amoxy clavulanic acid 33.5 %. The present study showed 100% sensitivity to linezolid. In accordance with present study, Shilpa Arora *et al.*, (2010) has reported antimicrobial resistance of



*Staphylococcus aureus* as amikacin (22%), ciprofloxacin (52.8%), cephalexin (56.8%) and penicillin (78.4%). *Staphylococcus aureus* was 99.2% sensitive to linezolid and 100% sensitive to vancomycin. Adebayo Shittu *et al.*, (2006) in his study of 227 *Staphylococcus aureus* isolates has reported that 70 isolates (30.8%) were resistance to cotrimoxazole, 68 isolates (30%) were resistant to tetracycline. Vidhani *et al.*, (2001) has documented 87% of *Staphylococcus aureus* isolates resistant to amoxicillin clavulanic acid, 100% resistant to penicillin and 78.5% resistant to cefotaxime.

The present study showed 26% of MRSA among 200 *Staphylococcus aureus* isolates. The above data correlates with the result of Vidyapai *et al.*, (2011) who has documented 29.1% MRSA. This is in accordance with study of Gupta *et al.*, (2009) who has documented 25% of MRSA among 200 *Staphylococcus aureus* isolates. Pal (2010) has documented 31.60% of MRSA, Oommen (2010) has reported 28.7% of MRSA. Jadhav Savita Vivek *et al.*, (2011) have reported 32.5% of MRSA. In contrary Anupurba *et al.*, (2003) has reported 54.8% of MRSA in their study.

Presence of pre disposing factors such as prolonged hospital stay and antibiotic in take as evidenced by Mathanraj *et al.*, (2009) may be the reason for high MRSA report among in patients, invasive procedures and use of resistant antibiotics results in bacteremia by MRSA (Waness, 2010). By disc diffusion method the present study showed 26% of MRSA using cefoxitin disc and 24% of MRSA by oxacillin disc. Similarly Shilpa Arora *et al.*, (2010) have detected 46% of MRSA by cefoxitindisc diffusion method and 40.4% of MRSA by oxacillin disc diffusion method. This shows that cefoxitin is superior to oxacillin in detecting MRSA (Fig. 1).

In conclusion, among the 200 *Staphylococcus aureus* isolates 26% were methicillin resistant. MRSA infection in surgical site is commonly noted. Multi drug resistance to commonly used drugs like ciprofloxacin, amikacin, doxycycline and cotrimoxazole are to be noted with concern. *Staphylococcus aureus* is a leading cause of hospital acquired infections including pneumonia, endocarditis, bacteremia, and surgical wound infections. The problem is exacerbated by the ability of the MRSA to colonize the individuals years together and infect them frequently. Hence detection of methicillin resistance in *Staphylococcus aureus* is very important for treating patients and to prevent its spread.

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#### **How to cite this article:**

Aruna, V., S. Nirmala and Rajendran, K.R. 2017. Study of Antibiotic Sensitivity Pattern of *Staphylococcus aureus* and Comparison of Cefoxitin and Oxacillin Discs for Detection of MRSA in a Tertiary Care Hospital. *Int.J.Curr.Microbiol.App.Sci.* 6(6): 921-926.  
doi: <https://doi.org/10.20546/ijcmas.2017.606.108>