Original Article

Comparative Evaluation of Oxacillin and Cefoxitin disk Diffusion Method in Detection of Methicillin-resistant *Staphylococcus Aureus* (MRSA) Isolates from a Tertiary Care Hospital in North India

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Abstract

Background: Methicillin-resistant *S. aureus* (MRSA), is one of the most common gram positive nosocomial pathogen, responsible for causing variety of human infections that may range from minor skin disease to life-threatening infections. In the present era of antibiotic resistance, the emergence of multi-drug resistant organism is becoming more common. Laboratory detection of MRSA can be done by using either oxacillin or cefoxitin antibiotic dis diffusion tests. Present study was planned to compare both antibiotics for routine laboratory detection of MRSA isolates in our hospital.

Aim: To compare oxacillin and cefoxitin disc diffusion methods in detection of MRSA isolates from indoor patients of our hospital.

Materials and Methods: Oxacillin and cefoxitin disc diffusion methods were compared for detection of MRSA strains in *S. aureus* isolates from various clinical specimens sent from different indoor departments of the hospital was done for a period of one year.

Results: Out of a total of 100 *S. aureus* isolates 31(31%), were found to be MRSA, detection of MRSA was found to be 100% by cefoxitin disc diffusion method and oxacillin E test compared to oxacillin disc diffusion method (93.5%) alone. Multi-drug resistance was seen in 75% of MRSA isolates compared to around 30% in MSSA isolates. All isolates were found to be vancomycin sensitive.

Conclusion: Cefoxitin disc diffusion is more sensitive than oxacillin for routine laboratory detection of MRSA isolates in clinical settings.

Keywords: MRSA, oxacillin, cefoxitin disc diffusion method, *Staphylococcus aureus*, North-India

INTRODUCTION

Global emergence of multi-drug resistance (MDR) in bacterial isolates has become a silent pandemic, affecting public health for common diseases e.g. urinary tract infections, superficial soft tissue infections, tonsillitis etc. MDR has emerged in almost every genus and species of commonly isolated aerobic bacteria. Methicilliin-Resistant *Staphylococcus aureus* (MRSA) is an MDR strain of *Staphylococcus aureus,* resistant to penicillins, cephalosporins, carbapenems and macrolides. Methicillin was first introduced in 1959 to treat *S.aureus* infections resistant to penicillin.[1] First case of MRSA in humans' was reported in England.[2] Since then, it has emerged as a major cause of hospital acquired infections worldwide.[3] A recent study by Klevens et al. has showed that deaths from MRSA infections in the U.S. have eclipsed the number of deaths caused by HIV/AIDS on an annual basis. These investigators estimated that MRSA caused 94,000 invasive infections and over 18,000 deaths in 2005.[4] Many MRSA isolates are sensitive to only glycopeptides and even decreases susceptibility to them is emerging.[5] The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to

the hospital etc. are the possible predisposing factors of MRSA emergence, and important reservoirs of MRSA in hospitals/institutions are infected or colonized patients and transient hand carriage on the hands of health care workers is the predominant mode for patient-to-patient transmission.[6] Two antibiotic discs namely oxacillin and cefoxitin are used for routine detection of MRSA isolates in clinical settings. Therefore, a study was planned to compare the two methods with a confirmatory oxacillin E test to know their sensitivity in laboratory detection of MRSA isolates.

MATERIALS AND METHODS

A prospective study from 1st January 2011 to 31st December 2011 was conducted in the Department of Microbiology of a tertiary care hospital in North- India. A total of one hundred *S. aureus* isolates from clinical samples from indoor patients admitted in different departments of the hospital, were subjected to MRSA screening, using conventional microbiological methods. Specimens included pus, sputum, genital specimen (high vaginal swab, semen, and urethral discharge), urine, devices (urinary catheter, central venous line), blood and body fluids. All specimens were handled and processed aseptically. The standard microbiological methods were followed in this study during culture and antibiotic sensitivity test following universal precaution All isolates were identified by conventional methods including colony morphology, Gram staining, catalase test, coagulase test (tube & slide) and DNase test.[7]

All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance based on Kirby-Bauer disk diffusion method using oxacillin discs (1 µg) obtained from Hi-Media Laboratories Pvt. Ltd. The isolates were considered methicillin resistant if the zone of inhibition was 12 mm or less (Figure 1).

Cefoxitin is reported to be more sensitive in detection MRSA strains, therefore all suspected MRSA strains were cross checked by Cefoxitin disc diffusion test, using 30 µg disc. An inhibition zone of ≤21 mm was taken as MRSA.[8] E test for detection of MIC for oxacillin of MRSA isolates was also performed using Hi comb strips (Himedia, Mumbai) (Figure 2).

Further, the antibiotic susceptibility pattern of methicillin resistant *S. aureus* strains was determined on the day of their isolation by the modified Kirby Bauer disc diffusion method on Muller Hinton agar, using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials. The antibiotics used were penicillin-G (10 units); ampicillin (10 µg); cloxacillin (30 µg); cephalexin (30 µg); cephotaxime (30 µg); erythromycin (15 µg); gentamycin (10 µg); amikacin (30 µg); netillin (30 µg); ciprofloxacin (5 µg); ofloxacin (5 µg); norfloxacin (10 µg); co-trimoxazole (25 µg); vancomycin (30 µg); linezolid (30 µg). Finally, the data were recorded and analyzed at the completion of the study as per

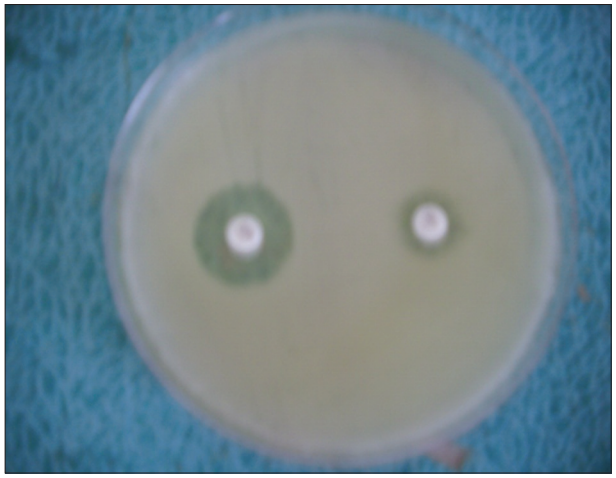

Figure 1: Comparative zone difference in an MRSA isolate by disc diffusion method Cefoxitin (30 µg)-Left disc and Oxacillin (1 µg)-Right disc

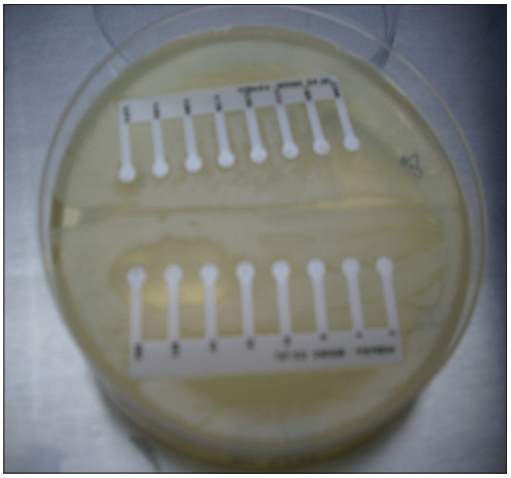

Figure 2: Hi comb Oxacillin MIC test of MRSA strains

Table 1: Age and gender distribution of MRSA isolates

Age groups (years)	Total No. of MRSA N (%)	Male	Female	Male Female ratio	P value
0-10	0	0	0	0	N.A.
11-20	1 (3.2)	1	0	N.A.	N.A.
21-30	8 (25.8)	2	6	1:3	0.419
31-40	10 (32.3)	5	5	1:1	1.000
41-50	6 (19.3)	2	4	1:2	0.653
51-60	5 (16.2)	4	1	4:1	0.171
61-70	1 (3.2)	1	0	N.A.	N.A
Total	31 (100)	15	16	1:1.1	

recommendations of the CLSI.[8] *S. aureus* ATCC 29213 was used as reference strain for the standardization of antibiotic susceptibility testing. Chi-square test and Fisher exact test were used to calculate p value, while comparing various parameters between MRSA and MSSA. P<0.05 was taken as statistically significant.

RESULTS

Out of a total 100 isolates of *S. aureus,* 31 were found to be MRSA strains by oxacillin E test method. Male female distribution of these isolates was 1:1.1. Maximum number of isolates were found in 31-40 years age group (32.2%) followed by 21-30 (25.8%), 41-50 (19.3%), 51-60 (16.1%) and 3.2% in 11-20 and 61-70 years age group (Table 1). Most of the MRSA isolates were from pus specimens 23 (74.2%) followed by urine 7 (22.6%) and only 1 (3.2%) from sputum and blood specimen each. Prevalence of MRSA isolates was maximum in urine (41.2%) followed by sputum (33.3%) and pus (29.5%) (Table 2).

Table 2: Prevalence of MRSA isolates from different clinical samples

Clinical samples	No. of *S.aureus* n=100	No. of MRSA n=31	Prevalence of MRSA
Pus	78	23 (74.2)	29.5
Urine	17	7 (22.6)	41.2
Sputum	3	1 (3.2)	33.3
Blood	2	0	0

Table 3: Oxacillin MIC range in MRSA isolates

Oxacillin MIC value (µg/ml)	MRSA isolate (n=31) N (%)
4	2 (6.5)
8	4 (12.9)
16	6 (19.4)
32	5 (16.1)
64	3 (9.7)
128	5 (16.1)
256	6 (19.4)

Table 4: Comparison of two phenotypic methods with E-test (Hi comb MIC test) for detection of MRSA isolates

Test methods	Detected as MRSA	Sensitivity (%)	Specificity (%)
Oxacillin (1 µg) disc diffusion	29	93.54	100
Cefoxitin (30 µg) disc diffusion	31	100	100
Hi Comb MIC test	31	100	100

Out of total 31 MRSA isolates, only 29 MRSA were detected by using oxacillin (1µg) disc compared to 31 MRSA by cefoxitin (30 µg) disc diffusion test. E-test (Hi comb MIC test) was also done to know the MIC value for oxacillin in total 31 MRSA isolates. MIC range for oxacillin was between 2 µg/ml to 256 µg/ml for MRSA strains. Majority of MRSA isolates (83.9%) had MIC in range of 16-256 µg/ml (Table 3). The sensitivity and specificity of these two phenotypic tests was compared with E-test. Sensitivity of cefoxitin disc diffusion method was 100% compared to 93.5% for Oxacillin disc diffusion method (Table 4).

The susceptibility pattern of antibiotics showed that all MRSA isolates were significantly less sensitivity to antibiotics as compared to MSSA. The values were statistically significant as P-value was <0.05 for every antibiotics. Vancomycin was 100% sensitive in both MSSA as well as MRSA. Out of 31 MRSA isolates, 12(67.7%) were sensitive to amikacin, followed by 14(45.2%) to gentamicin, 11(35.5%) to ciprofloxacin, 10(32.3%) to Ceftazidime and only 9(29%) to erythromycin. Whereas, out of 69 MSSA isolates 62(89.9%) were sensitive to amikacin followed by 58(84.1%) to erythromycin, 54(78.3%) to ciprofloxacin, 52(75.3%) to ceftadizime and 44(66.7%) to gentamicin (Table 5).

DISCUSSION

In this study, we isolated 31(31%) MRSA out of 100 *S.aureus* isolates from various clinical specimens from patients admitted in different departments of our hospital. Out of 31 MRSA isolates, 15 were from male and 16 from female cases, so it can be inferred that, there is no gender predilection in acquisition of infection by an MRSA isolate. Prevalence rate of MRSA was found to be 31% in our study, which is in accordance with the findings of studies from Anbumani N et al from Chennai (31%)[9] and Mehta AA et al from Mumbai (31.8%)[10] whereas, few studies from India has reported high prevalence rate of MRSA as compared to this study such as 46% by Arora S et al from Amritsar[11], 48.72% by Deepa S et al from Mysore, South India[12], 51.6% by Vidhani S et

Table 5: Comparative analysis of antibiotic susceptibility pattern of MRSA and MSSA isolates

Name of antibiotics	MRSA (n=31) N (%)	MSSA (n=69) N (%)	P value
Amikacin (30 µg)	21 (67.7)	62 (89.9)	0.009*
Gentamicin (10 µg)	14 (45.2)	46 (66.7)	0.049*
Ciprofloxacin (5 µg)	11 (35.3)	54 (78.3)	<0.001*
Ceftazidime (30 µg)	10 (32.3)	52 (75.3)	<0.001*
Erythromycin (15 µg)	9 (29)	58 (84.1)	<0.001*
Vancomycin (30 µg)	31 (100)	69 (100)	NA

al from New Delhi[13], 54.85% by Anupurba S et al from Banaras Hindu University[6]. Tahnkiwale et al from Nagpur[14] reported a lower prevalence rate of 19.5%, while Verma et al from Indore[15] reported a very high rate of 80.8% of MRSA compared to our study. Above studies clearly show that prevalence of MRSA varies from one setting to other and our hospital being a newer one, the prevalence rate has been found at lower levels and it might increase with time.

In the present study, comparison of two phenotypic methods proved that cefoxitin (30 µg) disc diffusion method is better than oxacillin (1 µg) disc diffusion method, in screening of MRSA strains in daily routine laboratory procedures.

Another statistically significant (p<.0.001-.03) finding of this study has been that, all MRSA isolates were significantly less sensitive to first line antibiotics erythromycin, gentamicin, ciprofloxacin, ceftazidime and amikacin as compared to MSSA; similar findings have been reported previously by other workers.[6,9,10,11,13-16] However, all *S. aureus* isolates were sensitive to vancomycin.

CONCLUSION

Methicillin-resistant *S. aureus* is one of the most common causes of nosocomial pathogen responsible for causing variety of human infections that may range from minor skin disease to life-threatening infections. In the present era of antibiotic resistance, the emergence of multi-drug resistant organism is becoming more common, therefore early detection is most important for treatment, prevention and control of such organisms.

Our study reconfirmed that, screening of every *S. aureus* isolate by cefoxitin disc diffusion test is necessary for the early detection, treatment, prevention and control of MRSA strains in hospital environment. Moreover, MRSA is a multidrug resistant organism, therefore AST of such isolates become important and our study shows high level of muti-drug resistance in nearly 75% of MRSA isolates. Nevertheless, regular monitoring of hospital environment, personnel and patients for MRSA strains, should be done to keep prevalence of this notorious pathogen under check.

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