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Evaluation and Comparison of Different Phenotypic Tests to Detect Methicillin Resistant *Staphylococcus aureus* and their Biofilm Production

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with high morbidity and mortality rates because of the development of multidrug antibiotic resistance. Rapid and accurate detection of methicillin resistant *Staphylococcus aureus* is an important role of clinical microbiology laboratories to avoid treatment failure. Hence the study was aimed out to evaluate different conventional phenotypic methods in detecting MRSA and to determine the prevalence of biofilm producers among MRSA. A total of 198 clinical samples were collected from a tertiary care hospital in Bangalore from July 2010 to January 2011 and subjected to MRSA screening by phenotypic methods using E- test MIC as standard. Subsequently biotyping and biofilm production was performed for confirmed MRSA isolates. Antibiotic susceptibility test by disc diffusion was also performed for all *S. aureus* isolates. Out of 153 *S. aureus* isolates, 42 (57.7%) were found to be methicillin resistant. The sensitivity and specificity for cefoxitin disk diffusion method were 100% and 99.10% respectively. The drug resistance patterns of MRSA isolates were found to be highly variable with high resistance to penicillin (100%), fusidic acid and cotrimoxazole (66.66%), pristinomycin (57.14%), rifampicin (50%) and mupirocin (47.61 %). Biotyping of MRSA isolates gave a typeability of 73.80 % and 61.90% of MRSA isolates have shown the potential to make biofilm. All phenotypic methods had high sensitivity and specificity for detection of MRSA. However, cefoxitin disk diffusion method in comparison to other methods had higher specificity. The present study reveals also reveals the emergence of vancomycin resistant isolates from this part of country and indicates the magnitude of antibiotic resistance in MRSA.

Key words: MRSA, cefoxitin, Biotyping, Biofilm.

INTRODUCTION

Staphylococcus aureus has long been recognized as a major pathogen of hospital acquired infections. Over the last decade, methicillin resistant *S. aureus* (MRSA) strains have become endemic in hospitals worldwide. In addition, it is now incipient community pathogen in many geographical regions[1]. Methicillin resistance is attributable to the *mecA* gene, encoding penicillin-binding protein (PBP)2a, which presents low affinity for β-lactam antimicrobials[2]. Heterogeneous resistance to methicillin also occurs among *S. aureus* isolates due to variations in the expression of the *mecA* gene, or alteration of constitutive PBPs[3]. MRSA, in addition to being methicillin resistant, most strains are also resistant to other β- lactam antibiotic, with the exception of glycopeptides antibiotics[4,5]. But in 1980s, because of widespread occurrence of MRSA, empiric therapy for Staphylococcal infections (particularly nosocomial sepsis) was changed to vancomycin in many health care institutions. As a consequence, selective pressure was established that eventually lead to the emergence of vancomycin resistant *S. aureus*[6]. To compound this problem further, *S. aureus* has the ability to colonize and form biofilms on implanted biomaterials. These biofilm structures are inherently resistant to antimicrobial challenge and difficult to eradicate from the infected host, as they can display susceptibilities towards antimicrobials of 10-1000 times less[7].

The prevalence of MRSA varies from hospital to hospital in various countries and is constantly soaring in many countries. In many American and European hospitals, the percentage of MRSA has ranged from 29% to 35%[8, 9]. The incidence of MRSA in India ranges from 30 to 70%[10, 11]. Information regarding MRSA prevalence is available from developed countries, which is inadequate in estimating the overall global distribution. This can be overcome by conducting regular epidemiological studies to know their changing trends. The detection of the methicillin resistance represents a real challenge for the routine clinical microbiology laboratories since molecular methods, the gold standard, are not available in most medical institutions. Thus, the objective of the study were (i) to evaluate the performance of conventional phenotypic methods in detecting MRSA using E- test MIC as gold standard method (ii) to detect the prevalence of biofilm producers among MRSA and (iii) to characterize the MRSA by biotyping.

MATERIALS AND METHODS

Study design

A total of 198 clinical specimens such as pus, blood, urine, throat swab, tissue bits were collected over a period of six months from July 2010 to January 2011 from Sri Bhagawan Mahaveer Jain hospital, a multispecialty tertiary care hospital in Bangalore, Karnataka. No duplicate specimens from the same patients and no environmental isolates were included in this study. The demographic information about the patients was obtained in a proforma designed for this purpose.

Isolation and identification of *Staphylococci* from clinical specimens

A preliminary gram staining was performed to determine the likely organism present. The samples were inoculated onto blood agar, MacConkey's agar and mannitol salt agar (Hi-Media, India). Urine samples were inoculated onto Cysteine Lactose Electrolyte Deficient agar (Hi-Media, India). Plates were incubated at 37° C for 18- 24 hours. *S. aureus* was identified and differentiated from related organisms on the basis of colony morphology, Gram staining, catalase test, slide and tube coagulase test and mannitol fermentation[12]. *S. aureus* ATCC 25923 was used as control strain.

Antibiotic susceptibility test by Kirby Bauer method

The isolates were subjected to susceptibility testing by Kirby Bauer disc diffusion method on Mueller- Hinton agar plates and the results were interpreted according to the guidelines of the CLSI[13]. The antibiotics used were penicillin (P), 10U; erythromycin (E), 15µg; mupirocin (Mu), 5µg; fusidic acid (Fc), 30µg; pristinomycin (Pm), 15µg; linezolid (Lz), 30µg; vancomycin (V), 30µg; teicoplanin (Te), 30µg; rifampin (R), 5µg; chloramphenicol (C), 30µg; cotrimoxazole (Co), 30µg; ciprofloxacin (Cf), 5µg; gentamicin (G), 30µg; amikacin (Ak), 30µg, and tetracycline (T), 30µg (Hi Media, India). *S. aureus* ATCC 25923 was used as control strain.

Detection of methicillin resistance by phenotypic methods

All the *S. aureus* isolates were subsequently tested for methicillin resistance by oxacillin screen agar test and by cefoxitin (30 µg) discs.

Oxacillin agar screen

This test was carried out according to the CLSI guidelines[14] (CLSI, 2006b). A McFarland 0.5 suspension was spotted onto Mueller-hinton agar containing 4% (w/v) NaCl and 6 mg oxacillin ml^{-1}, and incubated at 35°C for 24 h.

Cefoxitin disc diffusion test
All the isolates were subjected to cefoxitin disc diffusion test using a 30 µg disc. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture was done on MHA plate. Plate was incubated at 37° C for 18 hours and zone diameter was measured. An inhibition zone diameter of ≤19 mm was reported as oxacillin resistant and ≥ 20mm was considered as oxacillin sensitive[15].

Determination of MIC by E- test
MIC for oxacillin and vancomycin were determined with the E-test strips (Hi-media, India) using 0.5 McFarland inoculum according to the manufacturer's instructions. The oxacillin and vancomycin E-test strip was placed onto Mueller- Hinton agar plate supplemented with 2% NaCl, and the plate was incubated at 35°C for 24 hrs. According to CLSI standards, *S. aureus* isolates with oxacillin MICs of ≤2 µg/ml and ≥4 µg/ml are defined as methicillin-susceptible S. *aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). *S. aureus* isolates with vancomycin MICs of ≤2 µg/ml were considered susceptible. Vancomycin intermediate *S. aureus* (VISA) was defined by MICs of 4 to 8 µg/ml, and vancomycin resistant *S. aureus* (VRSA) by MICs of ≥16µg/ml[16]. Mueller-Hinton agar plates without antimicrobial were used as controls of bacterial growth. *S. aureus* ATCC 25923 was used as control strain.

Determination of MAR index
The MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested[17].

Biotyping of the MRSA isolates: All MRSA were characterized into 4 groups by biotyping[18] as shown in the Table 1.

Detection of biofilm formation by congo red agar method
Slime production by 42 MRSA clinical isolates was studied by congo red agar method[19]. Briefly, Brain heart infusion agar supplemented with 5% sucrose and Congo red (0.08 g/l) was prepared. Congo red was prepared as a concentrated aqueous solution and autoclaved at 121°C for 15 minutes separately from other media constituents and was then added when the agar had cooled to 55°C. Plates were inoculated and incubated aerobically for 24 to 48 hours. Biofilm production was indicated by black colonies with a dry crystalline consistency whereas biofilm non-producers remain pink, though occasional darkening at the center of the colony was observed. A darkening of colonies with absence of crystalline colony morphology indicates an indeterminate biofilm production.

Statistical analysis
Statistical package for social sciences (SPSS) version 18 was used to analyze our data. Clinical outcomes were compared statistically using Chi- square test and Student's t- test. P <0.05 was taken as "statistically significant".

Table 1. Biotyping pattern of MRSA isolates

Test biotype	Pattern of results forming biotype			
	A	B	C	D
Tween 80 hydrolysis	-	-	+	+
Urease production	-	+	-	+
Pigmentation on Tween 80 agar	cream	buff	variable	gold
Gentamicin susceptibility	S	R	S	R

Table 2. Proficiency of techniques used for MRSA detection

Proficiency testing on total 153 *S. aureus* isolates	Oxacillin screen agar	Cefoxitin disc diffusion	MIC (E- test)
True positive	58	43	42
False positive	16	1	-
True negative	111	111	-
False negative	0	0	-
Sensitivity rate	100	100	100
Specificity rate	87.40	99.10	100

RESULTS

A total of 153(77.27 %) *Staphylococcus aureus* isolates were isolated from a total of 198 clinical specimens. Of the total 153 isolates, 109 (71.24 %) and 44 (28.75 %) of *S. aureus* were isolated from males and females respectively. The mean age of the study group was 40 years with an age span from <1 to 90 years old. The age group 0-10 years included 13 (8.49 %); 10- 20 years, 8 (5.22 %); 20- 30 years, 31(20.26 %); 30- 40 years, 15 (9.80 %); 40- 50 years, 18(11.76 %); 50- 60 years, 34 (22.22 %); 60- 70 years, 22 (14.37 %); 70- 80 years,10 (6.53 %) and 80- 90 years, 2(1.30 %). It was noted that the extreme of ages were more prone to get *S. aureus* infection. The difference was statistically significant (p<0.0001).

Out of the 153 *S. aureus* isolates, 110 isolates were from pus, 13 from sputum, 11 from ear swab, 9 from blood, 5 from urine and 5 from tissue bits. Of the total 153 *S. aureus* isolates, 94.11 % isolates were coagulase positive and 5.88 % isolates were coagulase negative.

By using oxacillin E-test method, of the 153 isolates 42(27.45%) isolates were MRSA whereas oxacillin screen agar showed 58 MRSA positive isolates. The sensitivity and specificity of oxacillin screen agar were 100% and 87.40%, respectively. Forty three *S. aureus* isolates were MRSA by cefoxitin disk diffusion method. The sensitivity and specificity for cefoxitin disk diffusion method were 100% and 99.10% respectively (Table 2). All the methods showed similar sensitivity although specificity of oxacillin screen agar seemed to be lower than other methods. Out of 42 MRSA, 90.47% MRSA isolates were from coagulase positive *S. aureus* whereas 9.52% MRSA isolates were from coagulase negative *S. aureus.*

The prevalence of MRSA was significantly different among various clinical specimens (p< 0.0001) and was found that 71.42 %of these isolates were from pus sample, followed by ear swab and blood (9.52 %).The occurrence of MRSA was significant (t=2.970, df=6, p=0.025) between male (76.19%) and female (23.80%). Our study showed the highest percentage of MRSA (26.19%) occurrence in patients with age group 20- 30 years while the least was in the 70- 80 years group (4.76%). The difference was statistically significant (p< 0.0001).

Characteristics of antibiotic resistance in MRSA isolates

The results of antibiotic resistant rates and patterns of methicillin resistant isolates (n=42) are shown in **Table 3 and 4.** Ninety percent of MRSA isolates were observed to be resistant to ≥3 drugs other than penicillin and were considered as multidrug-resistant (MDR). The drug resistance patterns of MRSA isolates were found to be highly variable with high resistance to penicillin (100%), fusidic acid and cotrimoxazole (66.66%), pristinomycin (57.14%), rifampicin (50%) and mupirocin (47.61 %). MRSA isolates recorded 92.85 % sensitivity to vancomycin, followed by 80.95% to amikacin, 78.57 % to linezolid and teicoplanin. Coexisting resistance to different antibiotics (expect penicillin) with methicillin was significantly higher in methicillin resistant isolates compared to methicillin sensitive isolates (p<0.0178). Significant difference is also seen in individual antibiotics between MRSA and MSSA isolates (p<0.0001). Overall, the *S. aureus* were resistant from two to fourteen antibiotics of fifteen antibiotics tested generating multiple antibiotic resistance index (MAR) range from 0.266 to 0.933.

Table 3. Resistance rates of other antibiotics tested in methicillin resistant isolates (n= 42).

Antibiotic tested	% resistance
Penicillin	42 (100)
Mupirocin	20(47.61)
Fusidic acid	28(66.66)
Linezolid	9 (21.42)
Pristinomycin	24(57.14)
Teicoplanin	9(21.42)
Vancomycin	3(7.14)
Rifampicin	21(50)
Co-trimoxazole	28(66.66)
Tetracycline	15(35.71)
Chloramphenicol	11(26.19)
Gentamicin	17(40.47)
Erythromycin	16 (38.09)
Ciprofloxacin	22(52.38)
Amikacin	8(19.04)

Table 4. Resistance pattern of methicillin resistant isolates (*n* = 42)

Resistant to	Resistance patterns (*n*)	Total
Eleven drugs and above	P Mu Fc Lz Pm Te V R Co T C G E Cf (1) P Mu Fc Lz Pm Te R Co T G E (2) P Mu Fc Lz Pm R Co T G E Cf Ak (1) P Mu Fc Lz Pm Te R Co T C E Cf (1) P Mu Fc Lz Pm Te V R Co T C E (1) P Mu Fc Pm R Co T C G E Cf Ak (1)	7
Nine drugs	P Mu Te Co T G E Cf Ak (1) P Fc Pm Co T G E Cf Ak(1) P Fc Pm R T C E Cf Ak (1) P Mu Fc Pm R Co T C G (1) P Mu Fc Lz Pm V R T Cf (1) P Fc Pm R Co C E Cf Ak(1)	6
Seven drugs	P Mu Fc Lz Pm R Co (1) P Fc Pm Te Co G Cf (1) P Pm Te R Co G Cf (1)	3
Six drugs	P Co T G E Cf (1) P Fc Lz R Co Cf (1) P Pm Co C Cf Ak(2) P Pm Co T E Cf (1) P Fc Co C G Cf (2)	7
Five drugs	P Pm G E Cf (2) P Mu Fc R Co (4)	6
Four drugs	P Pm Co Cf (1) P Fc Te T (1) P Fc G E (1) P Mu Fc Pm (1) P Co G Cf (1) P Mu Fc R (3)	8
Three drugs	P Fc Cf (1)	1
Less than 3 drugs	P Pm (2) P Mu (1) P Co (1)	4

Antibiotics key: P, penicillin; Mu, mupirocin; Pm, pristinamycin ; Lz, linezolid; Fc, fucidic acid; V, vancomycin; Te, teicoplanin; E, erythromycin; G, gentamicin; Ak, amikacin; Cf, ciprofloxacin; R, rifampicin; Co, co-trimoxazole; C, chloramphenicol; T, tetracycline.

MIC by E- test

The oxacillin E-test revealed that 72.54 % of the isolates had MICs of ≤2 µg/ml whereas 7.18 % (11/153) had values ≥ 512 µg/ml, 8.49% (13/ 153) had MIC of 256µg/ml and 11.76% (18/153) had values ≥64 µg/ml. Three isolates (1.96%) were found to be vancomycin resistant by disc diffusion and by E- test. Out of 38 methicillin resistant coagulase *S. aureus* isolates, one isolate showed vancomycin MIC of 32µg/ml (VRSA) and the other 8 µg/ml (VISA). Out of 4 methicillin resistant coagulase negative *S. aureus*, one isolate showed vancomycin MIC of 8 µg /ml (VISA). None of the MSSA isolates showed vancomycin resistance. All 3 vancomycin resistant isolates were also found to be teicoplanin, mupirocin, linezolid and fusidic acid resistant. The age of the patients infected with vancomycin resistant *Staphylococci* ranged from 50 to 63 years; of the three vancomycin resistant isolates, two were from males and one was from female patient.

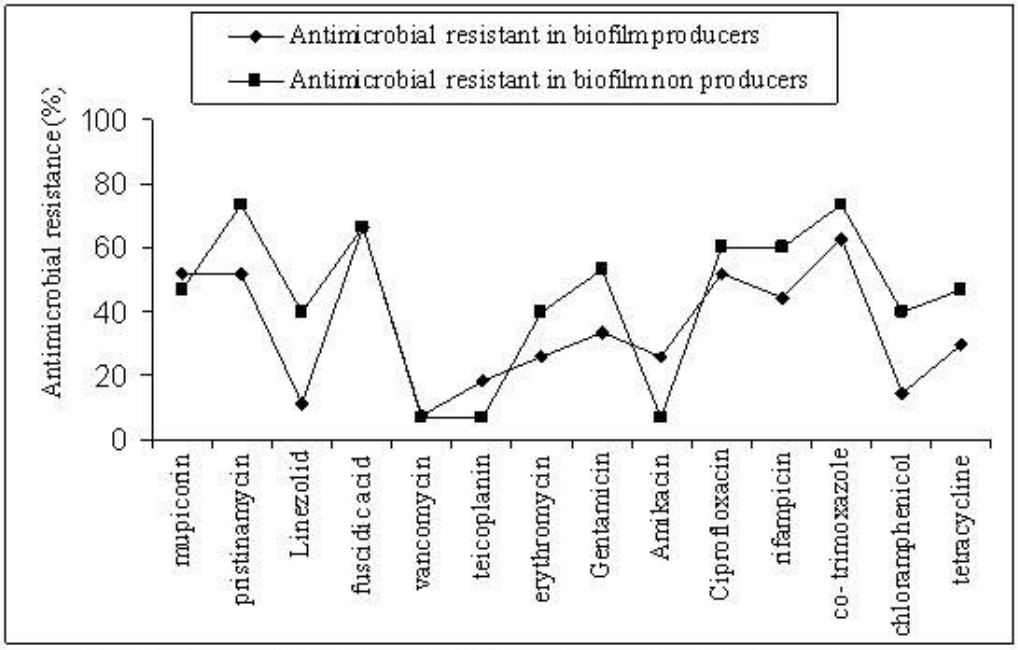

Fig. 1: Antimicrobial resistance of biofilm producers and nonbiofilm producers

Biotyping of the MRSA isolates

By Biotyping the isolates were divided into four groups (A-D). Typeability by biotyping was found to be 73.80 %. Maximum number of isolates belonged to group C (30.95 %) followed by group A (26.19 %), group B (9.52 %) and group D (7.14 %). 26.19 % of the isolates could not be categorized into any of the above mentioned groups and hence were called non typeable group. Tween 80 hydrolysis was of particular value in those gentamicin resistant strains arbitrarily designated B and D, as they had similar antibiograms. Although the B strains were more highly resistant to gentamicin they were difficult to distinguish by routine sensitivity testing.

Biofilm producing phenotype of MRSA isolates

Among the 42 MRSA isolates, 27 (61.90 %) showed black colonies with dry crystalline consistency whereas 16 (38.09 %) isolates showed pink colored colonies with mucoid appearance. Maximum biofilm producers were from pus sample. We found high resistance pattern among biofilm producers in comparison with non- biofilm producers (Figure1). Ciprofloxacin was found effective against biofilm producers. Among 3 vancomycin resistant isolates, the isolate with vancomycin MIC of 32 µg/ ml was also a biofilm producer.

DISCUSSION

MRSA is a major nosocomial pathogen causing significant morbidity and mortality[20]. In India, the significance of MRSA had been recognized relatively late and epidemic strains of these MRSA are usually resistant to several antibiotics. During the past 15 years, the appearance and world-wide spread of many such clones have caused major therapeutic problems in many hospitals[11].

Studies show that the epidemiology of MRSA over different parts of India is not uniform. The present study reports 27.45 % MRSA among *S. aureus* isolates. Few earlier studies have reported comparable prevalence of 24% in Chandigarh[21], 31.1% in a multicenter study in Tamilnadu[11], 24% in Vellore[22]. In contrast, variable prevalence of 80.89% in Indore[10], 52.9% in Assam[23] and 19.56% in Nagpur[8] have also been reported. This discrepancy could be due to difference in the study design, population and geographical distribution and the variation is probably due to differential clonal expansion and drug pressure in community.

MRSA isolates were predominantly isolated from pus (71.42 %), a similar finding was reported by Anupurba *et al*. (2003)[24]. In the current study, MRSA infection was found more in males (76.19%). Similar male preponderance among MRSA isolates was found in other studies[23]. Male patient predominance may be due to the fact that they are involved in numerous outdoor

activities and their work too exposes them to an increased chance of infection.

Testing of oxacillin (methicillin) resistance in *S. aureus* has been a challenge for clinical laboratories in recent years. Several studies have been showed that detection of *mecA* gene is a gold standard method for diagnosis of MRSA in clinical microbiology laboratories[25]. However, most laboratories especially in developing countries are not in position to perform molecular methods. In the present study, we evaluated different phenotypic methods for the detection of MRSA. We used E-test MIC as a gold standard method for detection of MRSA. The E-test method has the advantages of being easy to perform as a disk diffusion test and approaches the accuracy of PCR for *mecA*. There are many studies comparing E test MIC with broth dilution and PCR methods with has yielded satisfactory results[26]. The presence of resistance in *S. aureus* isolate on an oxacillin screen agar plate generally means that the isolates are *mecA* gene positive. However heteroresistant *mecA* positive strains are not detected due to low expression of resistance. Oxacillin agar screen generally does not detect borderline resistant strains, when studies have included heteroresistant strains the test has been shown to perform less well[27]. The isolates which were resistant to oxacillin but sensitive to cefoxitin were also negative by MIC in our study. The high false positivity of oxacillin disc diffusion method in this study could be due to hyper production of β-lactamases which may lead to phenotypic expression of oxacillin resistance, while they appear oxacillin resistant but do not posses the usual genetic mechanism for such resistance. Probably these isolates under antibiotic pressure may evolve into fully resistant isolates subsequently.

Recently CLSI has replaced oxacillin with cefoxitin for detection of MRSA[25]. Many studied have reported that the results of cefoxitin disk diffusion tests correlate better with the presence of *mecA* than do the results of oxacillin disk diffusion test[26]. Several studies have shown that cefoxitin disk diffusion method to be reliable method for detection of MRSA and the results were found to be in concordance with the PCR *mecA* gene detection method[27-29]. The cefoxitin disc diffusion method yielded the greatest efficiency as mentioned by earlier studies its results were easy to read in both transmitted and reflected lights[30]. Cefoxitin is a better inducer of the expression of the *mec*A gene; this could explain why heterogeneous MRSA populations that variably express the *mec*A gene are better detected by disk diffusion with cefoxitin than with oxacillin, which is a weak inductor of PBP2a production[26].

Methicillin resistance in *S. aureus* restricts therapeutic options for clinical isolates and the incidence of MRSA is escalating in India. Antibiogram analysis has been found to be a good epidemiological marker for MRSA phenotyping. In our study, all the MRSA isolates were resistant to penicillin. But the significant and clinical relevant observation of this study is the moderate resistance shown by MRSA to other conventional antibiotics and a high percentage of multidrug resistant MRSA isolates.

Majority of the MRSA isolates were resistant to fusidic acid, cotrimoxazole, pristinomycin, ciprofloxacin, rifampicin and mupirocin. Considerable variations were found in the resistance profiles among MRSA isolated from different countries. The high level resistance of the isolates in the present study to penicillin, fusidic acid, cotrimoxazole, pristinomycin, ciprofloxacin, rifampicin and mupirocin can be attributed to the fact that these broad spectrum antibiotics are frequently used in treatment of common infections. Monotherapy is associated with increased resistance as compared to combination therapy. Therefore, combination treatment is advisable and proven to be beneficial in treatment and eradication of MRSA strains[31].

Our study reveals a high rate (47.61%) of mupirocin resistance when compared to overall resistance rates (2- 14%) reported in the literature[32,33] but similar prevalence was reported by Vasquez *et al*. (2000)[34] and Orrett (2008)[35]. This could provide a substrate for more widespread resistance if selective pressure was applied by increasing mupirocin use. Testing for mupirocin resistance is not routine at most institutions. The significant rate of mupirocin resistance highlights the need for baseline testing and subsequent monitoring for mupirocin resistance before implementing infection-control strategies that rely heavily on mupirocin for MRSA decolonization.

Qureshi *et al.* (2004)[36]and Kandle *et al.*(2003)[37] had reported 97.8 & 91 % resistance to gentamicin. The present study revealed high percentage of sensitivity to gentamicin. This was a rather unexpected result because earlier MRSA were known to be resistant to gentamicin and so the use of gentamicin gradually decreased until it was no longer used for therapy, while the use of fluoroquinolones and macrolides increased. This change in antibiotic trend would have led to the development of gentamicin sensitive phenotype.

In the present study 73.80% of isolates were sensitive to chloramphenicol and similar results (80%) have been reported by Baddour *et al.* (2006)[38]. Similar to the findings of our study Udo *et al.* (2006)[39] found

high prevalence of isolates resistant to erythromycin, ciprofloxacin and fusidic acid.

The continued increase in the incidence of MRSA has led to widespread use of vancomycin for the treatment of *Staphylococcal* infections. VISA and VRSA strains have been reported recently from various parts of the country[40,41]. In our study, 7.14% of isolates were resistance to vancomycin. Present study showed 2 isolates having MIC of 8ug/ml for vancomycin which is borderline between susceptible and resistant values. Occurrence of VISA is mainly due to alteration in gene expression caused by gene induction or accumulation of multiple mutations which finally lead to intermediate levels of glycopeptide resistance[42]. Arise of VISA may be a pointer towards emerging low level vancomycin resistance in *S. aureus*.

Biofilms constitute reservoir of pathogens and are associated with resistance to antimicrobial agents and chronic infections[9]. In the present study, 61.90% of MRSA isolates have shown the potential to make biofilm, which highlights the high prevalence of resistant microorganism in our set up. In our study biofilm producing MRSA showed high resistance to almost all the groups of antibiotics compared to the biofilm non- producers which correlate with other studies[43]. As a consequence of biofilm development it is said that the ability of organism to transfer genes horizontally will be enhanced within these micro communities thus facilitating the spread of antibiotic resistance. High colonizing capacity combined with its resistance to multiple drugs, contributes to the organism's survival and further dissemination in the hospital setting.

Epidemiological typing for MRSA by phenotypic methods poses a problem due to changing pattern every year. In the present study 30.95% of MRSA belonged to biotype C. Twenty six percent of the isolates were nontypeable. This implies that biotyping alone cannot be used for typing purposes. The major advantages of biotyping are the simplicity, quickness, and reproducibility especially with the modified biotyping method used in this study. However, strain discrimination is limited with the use of biotyping only. Antibiogram and biotyping can be used together in discriminating or in distinguishing closely related strains. The antibiogram on its own cannot be used in monitoring the spread and determination of origin of MRSA within the hospital community because of loss or gain of antibiotic resistance due to loss or gain of plasmid DNA. Biotyping is a method worth adopting by medical microbiology reference laboratories in developing countries where the cost of applying today molecular biological methods in typing of isolates could be too expensive. Further there is a need to develop a local set of MRSA phages pertaining to a particular area so as to increase the typeability by phage typing.

CONCLUSION

Our study revealed that cefoxitin disk diffusion method had a high sensitivity and specificity compared to other methods for detection MRSA. This method can be preferred in clinical microbiology laboratories because it is easy to perform, do not require special technique, media preparation and finally more cost-effective in comparison to other methods. The study had demonstrated a high prevalence rate of MRSA with high rate of resistance to commonly used anti-staphylococcal agents. A large proportion of these MRSA were found to be multidrug resistant. These findings call for urgent attention whereby strict antibiotic policy should be enforced to curtail irrational use of antibiotics with its attendant evolution of resistant strains of *S. aureus*. There is need for continuous monitoring of antibiotic susceptibility pattern of all *S. aureus* isolates for selection of appropriate therapy.

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