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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF DIFFERENT METHODS FOR THE DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AND ITS ANTIBIOTIC SUSCEPTIBILITY PATTERN

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ABSTRACT

Background& Objectives: Methicillin resistant *Staphylococcus aureus* (MRSA) is majorly responsible for a variety of infections in healthcare setting and in general community. These infections range from mild skin boils or pimples to severe life threatening infection of deep tissues, lungs etc. MRSA is of concern not only because of its resistance to methicillin but also because it is generally resistant to many other chemotherapeutic agents like cephalosporin, macrolides and aminoglycosides. MRSA must be detected and treated aggressively to prevent secondary infections. This study will help to determine the simple, most specific and sensitive method for the detection of MRSA, which could be carried out in routine microbiology laboratory.

Methods: Strains of *Staphylococcus aureus* isolated from different clinical specimens were included in the study, which were screened for methicillin resistance. Screening with oxacillin and cefoxitin antibiotic discs, oxacillin MIC using commercial E test strip and detection of *mecA* gene product PBP2a using Latex agglutination test was carried out. Antibiotic susceptibility test for all MRSA strains was carried out by Kirby Bauer Disc diffusion method as per CLSI guidelines to determine their sensitivity pattern against an array of antibiotics. Multiplex PCR of confirmed MRSA strains was carried out to study resistance genes.

Results: In the present study 70 (74.468 %) *S.aureus* isolates were found to be methicillin resistant by AST with oxacillin (1µg) as well as cefoxitin (30µg), latex agglutination test identified 89 (94.680 %) as MRSA, whereas by oxacillin MIC by E-test only 55 (58.510 %) isolates were found to be MRSA and 74 (78.723%) carried the *mecA* gene which was determined by the PCR.

Interpretation and Conclusion: Results of AST with both Oxacillin (1µg) and cefoxitin (30µg) were somewhat similar to that of PCR results, which is considered as gold standard for detection of methicillin resistance genes. The other investigated methods like latex agglutination, oxacillin E-test may not be appropriate because of the relatively lower levels of concordance with PCR results.

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INTRODUCTION

Staphylococcus aureus is one of the most common human pathogens in both hospital and community setup. It is a major nosocomial pathogen worldwide. It is responsible for causing various skin infections like boils, carbuncles, furuncles and impetigo, but after gaining access to the blood, may also be a major cause of endocarditis, osteomyelitis, pneumonia, toxic shock syndrome and septicaemia (There and Wadhai, 2013). Methicillin resistant *Staphylococcus aureus* is resistant to all β lactam antibiotics and also other groups of antibiotics including aminoglycoside, macrolide, lincosamides etc. (Cookson and Phillips, 1998).

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Resistance is often due to horizontal gene transfer, encountered in hospitals and healthcare institutions, where the selective pressures for resistance are maximum. Overuse and misuse of antibiotics is a major factor that leads to more drug resistant strains (Chambers and DeLeo, 2009). The most important mechanism of resistance to penicillin is production of encoded by *blaZ* gene which inactivates penicillin by hydrolysis of its beta lactam ring. Methicillin resistance in staphylococci is mediated by *mecA* gene, which encodes for a 78 kDa protein, penicillin-binding protein 2a (PBP2a) an altered form of the normal penicillin binding protein,which has reduced affinity for the beta lactam antibiotics including the penicillinase-resistant penicillin (Chambers, 1997). Besides this, staphylococcal strains show resistance to aminoglycosides due to genes encoding for aminoglycoside modifying enzymes (AMEs). AMEs are classified into acetyltransferase (AAC), aminoglycoside phosphotransferase (APH), and

aminoglycoside nucleotidyltransferase (ANT). Among staphylococcal strains, the most commonly found AME is aac(6')/aph(2") encoded by the aac(6')/aph(2") gene. In addition, APH(3')-III is encoded by aph(3')- IIIa gene and the ANT(4')-I by ant(4')-Ia gene, are also found, (Choi *et al*., 2003). Resistance to tetracyclines and erythromycin is also prevalent among the strains of MRSA. Tetracycline resistanceis usually due to the presence of tetracycline resistance (*tet*) genes. The main mechanisms of resistance to tetracycline in *S. aureus* is predominantly due to *tetK* and *tetM* genes (Schmitz *et al*., 2001). Three different resistance mechanisms for macrolide antibiotics have been described among staphylococci. The main mechanism involves target-site modification followed with methylation of the ribosome. Second resistance mechanism involves an efflux system that results in resistance to macrolides and streptogramin B antibiotics. The third mechanism involves inactivation of antibiotics by the enzymes acetyltransferase, nucleotidyltranferase, phosphotransferase and hydrolase (Johnston *et al*., 1998).

It has been found that erythromycin resistance in *Staphylococcus aureus* is caused mostly by *ermA* or *ermC*, where as *msrA* and *ermB* are rare (Lina *et al*., 1999). A number of methods recommended by CLSI are used for the detection of MRSA. Conventional methods for the detection of MRSA include oxacillin disc diffusion, cefoxitin disc diffusion method, oxacillin MIC etc. (CLSI, 2008). All these phenotypic methods are prone to error due to heterogeneous nature of methicillin resistance and dependence on environmental condition, inoculum size, incubation time , temperature, pH of the medium, salt concentration of the medium, and exposure to beta lactam antibiotics etc., (Datta, *et al*., 2011; Mohanasoundaram and Lalitha., 2008). Polymerase chain reaction (PCR) can be considered as rapid and reliable tool for detection of MRSA and therefore is specified as gold standard technique for detection of MRSA (Pournajaf *et al*., 2014). However, it cannot be carried out in small laboratories as it requires expertise and costly equipment. This study was carried out to evaluate different methods for the detection of MRSA like sensitivity testing with oxacillin and cefoxitin, Minimum Inhibitory Concentration (MIC) using Oxacillin E test strip, Latex agglutination test for *mecA* gene product PBP2a. Also, sensitivity test to an array of antibiotics by Kirby Bauer method was done. Multiplex PCR was carried out to see the relation between antibiotic susceptibility patterns and the antibiotic resistance genes in MRSA.

MATERIALS AND METHODS

The study was conducted over a period of three years from October 2013 to October 2016 in the Microbiology Department of Sir H.N. Medical Research Society. A total of 250 strains of *Staphylococcus aureus* were isolated from different clinical specimens and identified based on colony characteristics and biochemical reactions. Screening of MRSA strains was done based on the sensitivity testing by Oxacillin and Cefoxitin sensitivity disc, Mueller Hinton Agar (MHA) (Himedia Laboratories, Mumbai, India) plate supplemented with 4% NaCl was swabbed with the suspension of test strain whose turbidity was adjusted to 0.5 Mac Farland standard. A 1µg Oxacillin disc and a 30µg Cefoxitin disc was placed on the MHA plate and incubated at 37°C for 18-24 hours. Further, these screened isolates were further subjected to various test for detection of MRSA.

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) was carried out using commercial Oxacillin E Test strip (Biomerieux) as per manufacturer's instructions.

Latex agglutination method

Detection of *mecA* gene product PBP2a was carried out using commercial kit (Biomerieux Slidex MRSA agglutination kit) as per the manufacturer's instructions.

Antibiotic Sensitivity Pattern

AST was carried out by Kirby Bauer disc diffusion method as per CLSI guidelines. The antibiotics tested were Ampicillin/ Sulbactum (10/10mcg), Cefazolin (30 mcg), Cefdinir (5 mcg), Cefuroxime (30mcg), Clarithromycin (15 mcg), Erythromycin (15 mcg), Linezolid (30 mcg), Tetracycline (15 mcg), Vancomycin (30 mcg), Chloramphenicol (30 mcg), Clindamycin (2 mcg), Levofloxacin (5 mcg), Penicillin G (10 units), Rifampicin (5 mcg) and Roxithromycin (30 mcg), Gentamicin (10mcg),Co Trimoxazole (25mcg).

DNA extraction

A modified method for DNA extraction was carried out based on extraction of DNA from whole blood (John M. S. Bartlett and Anne White).Overnight grown culture of the isolatesin nutrient broth was centrifuged at 13,000 rpm/1min to which 50µl of 10mg/ml of lysozyme was added and incubated at 37°C for 1hour. Then 450 µl of lysis buffer (tris EDTA (10Mm) pH8 (5ml)+SDS (0.1g)) was added and incubated at RT for 4-5 min. After which 250 µl of sodium perchlorate (5M) was added, mixed thoroughly and heated at 65°C for 15min. To this 600 µl of chilled chloroform was added & mixed by inversion with force for minimum 30 min and then centrifuged at 13,000 rpm/20min,from this mixture 500 µl of the top aqueous supernatant was removed in a fresh tube using a cut tip to prevent shearing of the DNA and two volumes of chilled absolute ethanol was added. The tubes were then incubated overnight at -20°C for precipitation. Centrifugation was carried out at 13,000 rpm/5 min. The supernatant was discarded and the pellet was washed with 70% ethanol after which the centrifugation was carried out again and the pellet obtained was suspended in 100 µl of TE (1mL) + RNase (2 µl) and kept at 70°C for 10 min for dissolution of the DNA pellet and for RNA degradation.

Multiplex PCR

Multiplex PCR was carried out for resistance genes to different antibiotics. Primers for the genes were selected based on the references in the literature. The primers for the genes were grouped into three groups depending on their optimal conditions (Table 1). Multiplex PCR was standardized using a positive control ATCC MRSA 43300. The primers selected for the genes *16S rDNA*, *mecA, femA, aac(6')/ aph(2''), aph(3')-IIIa* were based on the study of Choi *et al*., 2003. *blaZ* primer was selected from the work of Martineau *et al*., 2000,whereas primers for the genes *erm(A), erm(B), tet(K) and tet(M)* were selected as described by (Strommenger *et al*., 2003).

PCR method for Group A

The amplification was carried out in 25ul reaction mixture [(2.5 ml of 10x reaction buffer with 2mM $MgCl_2$ (Thermo

Fisher)]; 100 µM of each deoxynucleoside triphosphate (dNTPs) (Thermo Scientific), 0.5mM $MgCl_2$; 10pmol of primer for *16S rDNA, mecA,* 15 pmol for *femA and* 20 pmol for *blaZ* primer) and DNA template, brought up to a 25µl final volume with distilled water. 3U of Taq polymerase (Thermo Scientific) and 10 ng DNA template 20 ng was added.

72°C for 5 min was applied in a thermal cycler (Techne TC=512). Then 10 µl of amplified products were mixed with 2 µl of ethidium bromide (Himedia) and loaded on a 4% agarose gel along with Gene Ruler ^{TM} 50 bp DNA Ladder (Thermo Scientific); electrophoresis was performed and bands visualized in UVItec Gel Documentation System.

Table 1. Primer sequences used in Multiplex PCR and predicted product size

Group for Multiplex PCR	Gene	Primer Sequence (5'-3')	Amplified Product Size (bp)
Group A	*16S rDNA*	5'-CAG CTC GTG TCG TGA GAT GT-3'	
		5'-AAT CAT TTG TCC CAC CTT CG-3'	420
		5'-CCTAGTAAAGCTCCGGAA-3'	
	mecA	5'-CTAGTCCATTCGGTCCA-3'	314
		5'-ACTTCAACACCTGCTGCTTTC-3'	
	blaZ	5'-TGACCACTTTTATCAGCAACC-3'	173
		5' AAAAAAGCACATAACAAGCG 3'	
	femA	5' GATAAAGAAGAAACCAGCAG 3'	132
		5'-GAAGTACGCAGAAGAGA-3'	
	aac(6')/ aph(2'')	5'-ACATGGCAAGCTCTAGGA-3'	491
Group B		5'-AAG CGG TAA ACC CCT CTG A-3'	
	erm(A)	5'-TTC GCA AAT CCC TTC TCA AC-3'	190
		5'-CTATCTGATTGTTGAAGAAGGATT-3'	
	erm(B)	5'-GTTTACTCTTGGTTTAGGATGAAA-3'	142
		5'-GTA GCG ACA ATA GGT AAT AGT-3'	
	tet(K)	5'-GTA GTG ACA ATA AAC CTC CTA-3'	360
Group C		5'-AAATACCGCTGCGTA-3'	
	aph(3')-IIIa	5'-CATACTCTTCCGAGCAA-3'	242
		5'-AGT GGA GCG ATT ACA GAA-3'	
	tet(M)	5'-CAT ATG TCC TGG CGT GTC TA-3'	158

Table 2. The rate of antibiotic resistance by AST

Sr.no	Antibiotic	Resistant	Intermediate	Sensitive
1	Ampicillin/Sulbactum(10/10mcg)	63(67.02%)	14(14.893%)	17(18.085%)
2	Cefazolin (30mcg)	5 (4.255%)	3(3.191%)	86(91.489%)
3	Cefdinir(5mcg)	55(58.510%)	14(14.893%)	25(26.595%)
4	Cefuroxime(30mcg)	13(13.829%)	10(10.638%)	71(75.531%)
5	Clarithromycin(15mcg)	48(51.063%)	2(2.127%)	44(46.808%)
6	Erythromycin(15mcg)	45(47.872%)	12(12.765%)	37(39.361%)
7	Linezolid(30mcg)	2(2.127%)	0.000	92(97.872%)
8	Tetracycline(30mcg)	3(3.191%)	3(3.191%)	88(93.617%)
9	Vancomycin(30mcg)	2(2.127%)	0.000	92(97.872%)
10	Chloramphenicol(30mcg)	0.000	1(1.063%)	93(98.936%)
11	Clindamycin(2mcg)	13(13.829%)	7(7.446%)	74(78.723%)
12	Levofloxacin(5mcg)	1(1.063%)	7(7.446%)	86(91.489%)
13	Penicillin G(10units)	91(96.808%)	0.000	3(3.191%)
14	Rifampicin(5mcg)	0.000	0.000	94(100%)
15	Roxithromycin(30mcg)	50(53.191%)	13(13.829%)	31(32.978)
16	Gentamicin (10mcg)	33(35.106%)	27(28.723%)	34(36.170)
17	Co Trimoxazole (25mcg)	20(21.276%)	4(4.255%)	70(74.468%)

Reaction mixtures were subjected to initial denaturation at 95°C for 5 min followed by 30 cycles of 45 sec at 95°C, 1 min at 54°C and 45 sec at 72 °C. A final extension step at 72°C for 5 min was applied in a thermal cycler (Techne TC=512).

PCR method for Group B : The amplification was carried out in 25ul reaction mixture. The reaction mixture [(2.5 ml of 10x reaction buffer with 2mM $MgCl_2$ (Thermo Scientific)]; 100 µM of each dNTPs, 0.5 mM $MgCl_2$; 20 pmol of each primer] and 10 ng DNA template, and brought up to a 25 µl final volume with distilled water and cycling conditions were same as for Group A.

PCR method for Group C: The amplification was carried out in 25ul reaction mixture [(2.5 ml of 10x reaction buffer with 2mM $MgCl_2$ (Thermo Scientific)]; 100 µM of dNTPs; 15pmol of each primer and template DNA, and brought up to a 25µl final volume with distilled water. 2U of Taq polymerase was added. Reaction mixtures were subjected to initial denaturation at 95°C for 5 min followed by 30 cycles of 45 sec at 95°C, 1 min at 51°C and 45 sec at 72°C. A final elongation step at

RESULTS

Among 250 *S.aureus* isolates screened, 94 isolates were found to be resistant to either Oxacillin (1µg) or cefoxitin (30µg) or both, and were considered to be MRSA in this preliminary screening.

Oxacillin and Cefoxitin Disc Susceptibility Test: Out of the 94 isolates, 70 (74.468%) isolates showed resistance to both oxacillin (1µg) and cefoxitin (30µg), 13 (13.829%) were found resistant to only oxacillin and 11(11.702%) isolates were found to be resistant to only cefoxitin.

MIC by using Oxacillin E test strips: By using E-test method for oxacillin, 55 (58.510%) isolates were identified as MRSA i.e. having MIC ≥ 4µg/ml and 39 (41.489%) isolates were sensitive i.e. having MIC < 4µg/ml.

Latex agglutination method for the detection of presence of *mecA* gene product PBP2a: Latex agglutination test for *mecA* gene product PBP2a identified 89 isolates as MRSA.

Table 3. Comparison of antibiotic resistance by AST (Phenotypic method) and Multiplex PCR

Antibiotic	Antibiotic Resistance by AST	Presence of antibiotic resistance genes			
Penicillin G	91(96.808%)	*blaZ*		Negative isolates	
		87 (92.553%)		7 (7.446%)	
Gentamicin	33(35.106%)	*aac*(*6'*)/*aph*(*2"*)	*aph*(*3'-IIIa*)	Both genes	Negative isolates
		21 (22.340%)	12 (12.765%)	48 (51.063%)	13 (13.829%)
Erythromycin	45(47.872%)	*ermA*	*ermB*	Both genes	Negative isolates
		11 (11.702%)	3(4.16%)	1(2.08%)	79 (84.042%)
Tetracycline	3(3.191%)	*tetK*	*tetM*	Both genes	Negative isolates
		28 (29.787%)	14(14.893%)	24 (25.531%)	28 (29.787 %)

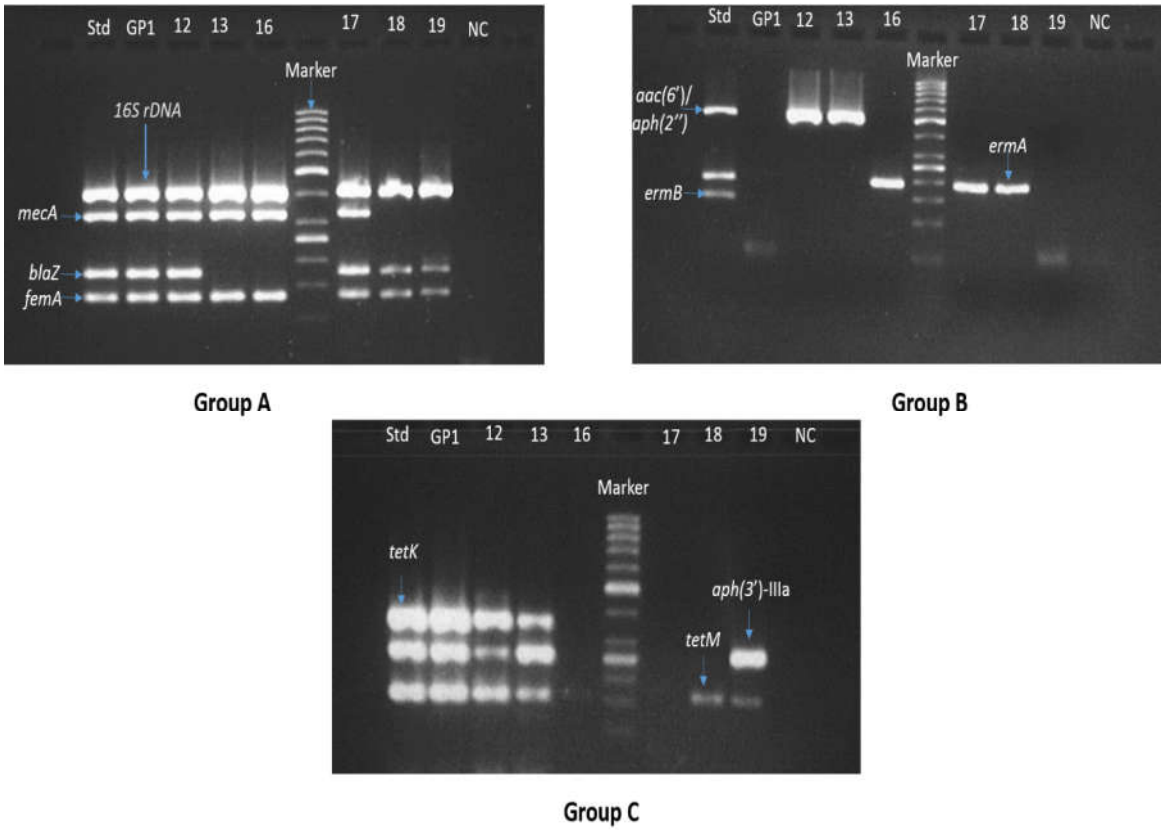

Fig.1. Multiplex PCR amplification products obtained for Groups A, B and C

Table 4. Comparative analysis for methicillin resistance in *S.aureus* isolates

AST with oxacillin(1µg)& cefoxitin(30µg)	Oxacillin E-test	Latex agglutination	PCR (*mecA* gene)
70 (74.468%)	55 (58.510%)	89 (94.680%)	74 (78.723%)

Antibiotic Susceptibility Pattern: By AST, maximum isolates were found to be sensitive to Rifampicin (5µg), Chloramphenicol (30µg), Vancomycin (30µg), and Levofloxacin (5µg). Maximum resistance was found for the drugs like Penicillin G (10 units), Ampicillin/Sulbactum (10/10µg), Cefdinir (5µg) etc. (Table 2)

Multiplex PCR: All 94 *S.aureus* isolates were found positive for *16S rDNA*. Out of 94 *S.aureus* isolates, 91(96.808%) showed presence of *femA* gene, which is a species specific marker and its gene product influences the level of methicillin resistance. *blaZ* gene was found in 92.5%. strains. *mecA* gene for methicillin resistance was detected in 78.7% isolates. Whereas *aac*(*6'*)/*aph*(*2"*)and *aph*(*3'-IIIa*) both the genes were present in majority (51.063%) of the isolates. Only *aac* (*6'*)/*aph*(*2"*) and *aph*(*3'-IIIa*) individually were present in 12.765% and 22.340% isolates respectively. *erm A* and *erm B* genes were detected in 11.702% and 4.16% isolates respectively. Of the total, 84.042% isolates were found to be negative for both the *erm* genes. Resistance to tetracycline conferred by the both *tetK* and *tetM* was detected in 25.531% and 44.680% isolates showed presence of at least one of the *tet* gene, (Table 3). All the phenotypic techniques like AST, oxacillin E-test, and latex agglutination applied for detection of MRSA showed variable results. However, PCR done to detect the methicillin resistance gene (*mecA*) was considered as standard and confirmatory test for identification of MRSA isolates .Results of AST with oxacillin (1µg) and cefoxitin (30µg) when considered together showed concordance with the PCR results (Table 4).

DISCUSSION

MRSA is responsible for several difficult-to-treat infections in human beings. MRSA is especially troublesome in hospitals and nursing homes, where patients with open wounds, invasive

devices, and weakened immune systems are at greater risk of nosocomial infection. Infection due to MRSA is becoming more prevalent due to multiple reasons, most common being overuse or misuse of antibiotics (Ventola, 2015). Phenotypic methods like antibiotic susceptibility testing routinely used in the laboratories for detection of MRSA are prone to errors and may not be accurate enough in identifying heterogenous strains. Inaccuracy in differentiating MRSA from borderline oxacillin-resistant *S. aureus* (BORSA) strains is also often encountered, (Sakoulas *et al*., 2001; Cavassini *et al*., 1999 and Gerberding *et al*., 1991). In the present study, different phenotypic methods like disc diffusion, E-test, latex agglutination were applied for detection of MRSA strains and their efficacy was confirmed by considering the PCR results of *mecA* gene as standard.

Methicillin resistance by AST with Oxacillin (1µg) and cefoxitin (30µg) both was detected in 74.468% *S.aureus* isolates. Many studies suggest drawbacks of using Oxacillin disc diffusion method in detecting heteroresistant MRSA and Cefoxitin being far more superior for accurate MRSA detection (Swenson *et al*., 2007; Unal *et al*., 1994 and Anand *et al*., 2007). Cefoxitin, a cephamycin, is a more potent inducer of the *mecA* regulatory system and an accurate surrogate marker for the detection of MRSA in the routine susceptibility testing (Brown *et al*., 2005). However, in our study both Oxacillin and Cefoxitin detected approximately same number of isolates 83 and 81 respectively (n=250) as MRSA during the screening. E test as well as latex agglutination test for detection for *mecA* gene product have advantage that they are easy to perform as they require no special equipment (Brown *et al*., 2005 and Louie *et al*., 2000). Identification based on oxacillin E-test showed only 58.510 % isolates as MRSA. Whereas, latex agglutination identified 94.680% as MRSA which were not in agreement with the *mecA* PCR results which identified 78.723% as MRSA. In E-test as well as other susceptibility tests, factors like incubation time, temperature, inoculum size have profound effect on resistance results. In Latex agglutination false positive may be due to weak reaction which can be misinterpreted.

Antibiotic susceptibility patterns of the MRSA isolates for a panel of antibiotics was also studied. The majority of the isolates were resistant to the beta lactam antibiotics like Penicilin G (96.808%), Ampicilin/Sulbactum (63.02%) and Cefdinir (58.510%). High percentage (97.872%) of MRSA strains were sensitive to Vancomycin (Glycopeptide) and Linezolid, which is similar to other studies and makes Vancomycin a drug of choice for treatment of MRSA infections (Hafeez and Aslam 2004; Al-Zoubi *et al*., 2015 and Panda *et al*., 2016). Variation in percentage of MRSA found sensitive to the macrolides (erythromycin, clarithromycin and roxithromycin) and lincosamide (clindamycin) was observed as also previously reported by (Hobul *et al*., 2013 and Panda *et al*., 2016). We observed high percentage of isolate sensitive to, tetracycline and trimethoprim-sulfamethoxazole, whereas only 36.170% isolates were sensitive to Gentamicin. Different sensitivity percentages have been reported for Gentamicin, tetracycline and trimethoprim-sulfamethoxazole in India and other countries (Rajaduraipandi *et al*., 2006; Hafeez and Aslam 2004). This difference, as explained by Al-Zoubi *et al*., 2015 stems from variations in geographical conditions. All the MRSA isolates were sensitive to Rifampicin and 98.936% showed sensitivity to chloramphenicol, such high sensitivity percentage of isolate to these antibiotics has been previously reported by (Elsahn *et al*., 2010 and Kaleem *et al*., 2010). In this study we also carried out multiplex PCR to detect the presence of antibiotic resistance genes and compared the results with the phenotypic susceptibility patterns for the respective antibiotic. *blaZ* gene was detected in 92.25% of strains and phenotypic resistance was observed in 96.808% isolates which is in concordance with the results obtained by Duran *et al*., 2012. Presence of at least one of aminoglycoside modifying enzyme genes *aac*(*6'*)/*aph*(*2"*) and *aph*(*3'-IIIa*) was observed in 86.170% of the isolates,out of which 51.063% isolates had both the *aac*(*6'*)/*aph*(*2"*) and *aph*(*3'-IIIa*).In our study *aac*(*6'*)/*aph*(*2"*) was detected in 73.404% isolates whereas, *aph*(*3'-IIIa*) was found in 63.829% isolates. Studies carried by Duran *et al*., 2012; Choi *et al*., 2003 have also reported *aac*(*6'*)/*aph*(*2"*) as the predominant aminoglycoside modifying enzyme genes. Discordance between the phenotypic and genotypic results for gentamicin may be due to external factors like incubation time, temperature, pH that affects the phenotypic methods like AST.

Erythromycin resistance conferred predominantly by *ermA* gene in the MRSA isolates was observed in 12.765% isolates, *ermB* was detected only in 4.255% isolates, and 47.872% isolates were found to be resistant by AST. The resistance may also be due to other genes like *ermC*, *msrA* which were not applied in the study (Schmitz *et al*., 2000). Some MRSA isolates in the present study were phenotypically resistant to erythromycin despite lacking *erm* genes, which are linked with a lack of *erm* genes in small plasmids (Jaglic *et al*., 2012; Cengiz *et al*., 2015). In present study phenotypic resistance to tetracycline was observed in only 3(3.191%) strains whereas, genotypically *tet* genes were detected in 70.021%. Out of all the strains 25.531% genes showed presence of both *tetK* and *tetM*. All these strains showing presence of the *tet* genes should be considered as potentially tetracycline resistant as genetic encoding is not always essentially expressed. However, as demonstrated by Trzcinski *et al*. (2000), phenotypic resistance to tetracycline, and minocycline can be improved when the isolates are incubated with sub-inhibitory concentrations of tetracyclines.

In conclusion, the results of our study suggest the use of both Oxacillin and Cefoxitin disc diffusion method for detection of MRSA, since the results this phenotypic method showed maximum concordance with the PCR results, which is considered as the gold standard for detection MRSA. Similar recommendation has been put forth by Matos *et al*., (2010) as well as Mimica *et al*. (2012). Besides being easy to perform the method is economical as compared to other phenotypic methods and can be carried out for accurate detection of MRSA in any diagnostic laboratory. Antibiotic susceptibility patterns and its comparison with the PCR gives a better idea for the choice of drug to combat the MRSA infections and the trend of resistance seen in the local isolates.

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