ORIGINAL ARTICLE

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *STAPHYLOCOCCUS AUREUS* ON CLINICAL ISOLATES AND EFFICACY OF LABORATORY TESTS TO DIAGNOSE MRSA: A MULTI-CENTRE STUDY

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Background: The global problem of increasing trend in antimicrobial resistance is particularly pressing in the developing countries, where the Methicillin-Resistant *Staphylococcus aureus* (MRSA) is often the severe casual agent in hospital-acquired infections. **Methods**: This multi-centre surveillance prospective study was planned to define the magnitude of problem of MRSA among clinical isolates from four teaching hospitals of Lahore Pakistan; Mayo, Services, Jinnah and Shaikh Zayed Hospitals during April 2006–March 2008. Identification of organisms was done by the standard Microbiology methods. MRSA isolates identified on Kirby-Bauer disc diffusion were further evaluated by minimum inhibitory concentration on BD Phoenix™ system and detection of *mec*A gene by pulsed-field gel electrophoresis (PFGE) PCR. **Results**: Of the total 1,102 *S. aureus* isolates, oxacillin resistance was found in 462 on disc diffusion and 420 on MIC while *mec*A gene was detected from 307 strains. The prevalence of MRSA among *S. aureus* isolates was 41.9%, 38.1% and 27.9% on disc diffusion, MIC, and *mec*A gene detection respectively. Hospital acquired-MRSA strains were multi drug resistant while community acquired-MRSA showed susceptibility to clindamycin (63%), ciprofloxacin (24.2%) and SMZ/TMP (3.9%). In diagnosing MRSA, the sensitivity and specificity rates of disc diffusion test were 100% and 83.7% while MIC 96.2% and 93.3% respectively. **Conclusion:** There is an increasing trend in emergence MRSA and the conventional method of antimicrobial susceptibility testing showed false positive tests. This is the reason of misuse of vancomycin by physicians which may further increase MRSA in Pakistan. Therefore, molecular diagnostic facilities are recommended to avoid false-susceptible results.

Keywords: *S. aureus*, MRSA, *mec*A gene, MIC

INTRODUCTION

Staphylococci are widespread pathogens and are frequently associated with hospital acquired infections. Methicillin was introduced in 1960 as the first beta-lactamase-resistant penicillin and first case of methiclin resistant *Staphylococcus aureus* (MRSA) was reported in 1961, but outbreaks of MRSA infections were reported in Europe soon thereafter.[1] Healthcare-associated MRSA is a major cause of nosocomial infections worldwide, with significant attributable morbidity and mortality in addition to pronounced healthcare costs.[2] MRSA is also a major nosocomial pathogen in Pakistan and is emerging in the community.[3]

The *mec*A gene is responsible for resistance of nafcillin (and to methicillin or oxacillin) and resides on the chromosomes; therefore, the resistance is independent to ß-latamase production. The genetic origins of methicillin resistant in *S. aureus* have led to a greater understanding of the epidemiology of MRSA. However, due to some of the test conditions, errors may occur during the detection of hetroresistant bacteria.

The objectives of the study were to see the susceptibility pattern of *S. aureus* isolates against various brands of commonly used antibiotics in four teaching hospitals of Lahore, Pakistan, to determine the prevalence of MRSA among clinical *S. aureus* isolates on Kirby-Bauer disc diffusion method, and minimum inhibitory concentration (MIC), to detect *mec*A gene by polymerase chain reaction (PCR) test from MRSA identified on disc diffusion and MIC, and to evaluate efficacy of disc diffusion and MIC by comparing with *mec*A gene detection considering it as gold standard.

MATERIAL AND METHODS

The study was conducted at Department of Bacteriology, Institute of Public Health Lahore and Department of Microbiology, Quaid-i-Azam University Islamabad, Pakistan from April 2006–March 2008. The routine clinical microbiology specimens were collected from four hospitals: Mayo, Services, Jinnah, and Shaikh Zayed Hospitals, Lahore, Pakistan. The specimens were processed within 2 hours of collection by the standard microbiology technique. Initial inoculation was made on sheep blood agar and mannitol salt agar. The plates were then incubated at 35 °C for 18–24 hours in aerobic atmosphere. The identification of *S. aureus* was made on the basis of colony morphology, Gram's staining, catalase and coagulase tests. The coagulase positive isolates were confirmed by identification of deoxyribonuclease (DNase) enzyme by performing

DNase test.[4] Antimicrobial drug susceptibility of the isolates was tested by the modified Kirby-Bauer technique and results were interpreted according to the Clinical Laboratory Standards Institute-CLSI Guideline (2007).[5] The antimicrobial susceptibility testing was performed for clindamycin, erythromycin, ciprofloxacin, penicillin G, ampicillin, gentamicin, sulfamethaxazole/ trimethoprim, vancomycin, cephalothin, rifampicin, tetracyclineand cephalothin. For antimicrobial susceptibility of oxacillin, microbial suspensions after comparing with 0.5 McFarland turbidity standards were streaked onto Mueller-Hinton agar supplemented with 4% sodium chloride as recommended method of inoculation by CLSI guideline 2007.[5] For oxacillin susceptibility testing, using oxacillin disc (1 µg) the zone size of ≤10 mm was considered resistant; a zone size of ≥13 mm was considered susceptible.

Antimicrobial susceptibility by MIC method was done on BD Biosciences, MD, USA Phoenix™ system.[6] The control strain *S. aureus* ATCC 29213 was used for the quality control for disc diffusion and MIC methods.[5]

Detection of *mec*A gene by PCR was performed by pulsed-field gel electrophoresis (PFGE) using primer 533 base pair (bp) fragment. When interpreting the results of the test, a positive outcome indicates the presence of the *mec*A gene.

RESULT

The distribution of total 1102 *S. aureus* isolates as follows; Mayo hospital 432 (39.2%), Services hospital 185 (16.79%), Jinnah hospital 273 (24.78%) and Shaikh Zayed hospital 212 (19.23%). The unit wise distribution of 1102 specimens as follows; ICU 162, Medical 225, Surgical 239, cardiac 44, Obs and Gynae 118, Paediatrics 136, neonatology 78 and OPD 100. Distribution of site of infection of total 1102 *S. aureus* isolates in four hospitals; Mayo, Services, Jinnah, and Shaikh Zayed is given in Table-1.

Table-1: Distribution of site of infection by *S. aureus* isolates in four hospitals (Total: 1,102)

	Hospital							
	Mayo		Services		Jinnah		Sh. Zayed	
Site of infection (Number)	No	%	No	%	No	%	No	%
BSI (153)	59	13.7	26	14.1	38	13.9	30	14.2
RTI (305)	117	27.1	52	28.1	77	28.2	59	27.8
UTI (213)	86	19.9	3 5	18.9	52	19.0	40	18.9
SSI (243)	95	22.0	41	22.2	60	22.0	47	22.2
Genital (57)	23	5.3	9	4.9	14	5.1	11	5.2
ENT & Eye (66)	26	6.0	11	5.9	16	5.9	13	6.1
Body Fluids (22)	9	2.1	4	2.2	5	1.8	4	1.9
Miscellaneous (43)	17	3.9	7	3.8	11	4.0	8	3.8
Total (1102)	432	100	185	100	273	100	212	100

BSI: Blood stream Infection; **RTI**: Respiratory Tract Infection; **UTI**: Urinary Tract Infection. **SSI**: Skin and oft tissue infection

The resistant pattern of 1102 *S. aureus* isolates on disc diffusion. There were 420 susceptible strains which had MIC's to oxacillin of <4 mg/L on Phoenix BD system. Table-2 shows antimicrobial susceptibility pattern of *S. aureus* on disc diffusion with MIC. There was a significant correlation between MRSA identified on disk diffusion and MIC (p<0.05). The *mec*A gene was detected in 307 strains identification was made by PCR from all MRSA isolated on MIC. The prevalence of MRSA strains among *S. aureus* isolates was 41.9%, 38.1% and 27.9% on disc diffusion, MIC, and *mec*A gene detection respectively. Table-3 summarises methicillin resistance among *S. aureus* isolates identified by three different tests; disc diffusion, MIC, and *mec*A gene detection. Of total 307 MRSA strains, 209 (68%) were hospital-acquired (HA-MRSA), and 98 (32%) community acquired (CA-MRSA).

The efficacy rate of disc diffusion test in our study was found 85.9%. The sensitivity rate of disc diffusion and MIC was found 100% while specificity rate was 80.5 and 85.8% respectively. The positive productive value (PPV) of disc diffusion and MIC in present study was 66.5 and 73.1 % respectively and negative predictive value (NPV) was 100% (Table-4). The Pearson correlation between MRSA identified on disc diffusion and minimum inhibitory concentration was determined and it was found that both antimicrobial susceptibility-testing methods had positively significant correlation (p<0.05). Similar results found on application of Chi Square test on MRSA detection by *mec*A gene detection, MIC and disc diffusion.

Table-2: Antimicrobial susceptibility pattern of *S. aureus* on disc diffusion with MIC

	Antimicrobial Susceptibility Test			
	Disc Diffusion		MIC	
Name of Antibiotic	Sensitive No (%)	Resistant No (%)	Sensitive No (%)	Resistant No (%)
Oxacillin	0 (0)	462 (100)	42 (9.1)	420 (90.9)
Clindamycin	291 (63.0)	171 (37.0)	213 (46.1)	249 (53.9)
Erythromycin	8 (1.7)	454 (98.3)	43 (9.3)	419 (90.7)
Ciprofloxacin	112 (24.2)	350 (75.8)	211 (45.7)	251 (54.3)
TMP/SMZ	18 (3.9)	444 (96.1)	82 (17.7)	380 (82.3)
Penicillin	0 (0)	462 (100)	0 (0)	462 (100)
Gentamicin	11 (2.4)	451 (97.6)	84 (18.2)	378 (81.8)
Vancomycin	462 (100)	0 (0)	462 (100)	0 (0)
Rifampicin	440 (95.2)	22 (4.8)	440 (95.2)	22 (4.8)
Tetracycline	0 (0)	462 (100)	40 (8.7)	422 (91.3)
Ampicillin	0 (0)	462 (100)	28 (6.1)	434 (93.9)
Cephalothin	35 (7.6)	427 (92.4)	42 (9.1)	420 (90.9)

Table-3: Methicillin resistance among *Staphylococcus aureus* isolates identified by three different tests; disc diffusion, MIC, and *mec*A gene detection

Strains Isolated	Test used for the detection of MRSA					
	Disc Diffusion		MIC		*mec*A detection	
	No	%	No.	%	No.	%
MSSA	640	58.1	682	61.9	795	72.1
MRSA	462	41.9	420	38.1	307	27.9

Table-4: Disc diffusion and MIC in diagnosing MRSA by Gold Standard *mec*A gene detection

Proficiency Testing (On total 1102 *Staph. aureus* strains)	Laboratory techniques for detection of MRSA	
	Disc diffusion (DD)	MIC BD Phoenix™
True positive	307 (27.9%)	307 (27.9%)
False positive	155 (33.5%)	113 (26.9%)
True negative	795 (72.1%)	795 (72.1%)
False negative	0.0 (0%)	0.0 (0%)
Sensitivity rate	100.0%	100.0 %
Specificity rate	83.7%	87.6%
Positive predictive value	66.5%	73.1%
Negative predictive value	100.0%	100.0%
Efficacy rate	85.9%	89.7%

DISCUSSION

The problem of MRSA is not only restricted to industrialised countries but an alarming increase in MRSA infections was also found in Pakistan in the last decade. The first MRSA case was emerged in Pakistan in 1989[7] and later continuous increase in its prevalence was reported, (Table-5).[7–14]

Table-5: Increasing trends of prevalence of MRSA among *S. aureus* isolates in Pakistan 1989–2007

Studies conducted in Pakistan (Reference)	Study period	MRSA Prevalence (%)
Ashiq and Tareen, (1989)[7]	1989	5.0
Qureshi and Hannan, (1991)[8]	1991	13.8
Siddique *et al.* (1999)[9]	1999	22.0
Latif, (2000)[10]	2000	29.0
Hafiz *et al.* (2002)[11]	2002	42.0
Safdar *et al.* (2003)[12]	2003	65.0
Anwar *et al.* (2004)[12]	2004	19.5
Bukhari *et al.* (2004)[13]	2004	38.6
Perwaiz *et al* (2007)[14]	2004–5	43.0

The overall prevalence of MRSA among *S. aureus* was documented as 42% in Pakistan. This figure varies widely from 2–61%.[11] There is an increasing trend of MRSA in big cities. In Karachi the prevalence was 5% in 1989[7] and 7.5% in 2002[11]. Our results of antimicrobial susceptibility pattern on disc diffusion test and MIC were comparable to similar studies.[17,18] The 73.1% true positive MRSA strains on *mec*A gene detection and 26.9% false on MIC technique reported in current study were similar to the local study Zeeshan *et al.*[17] The PPV of disc diffusion and MIC in the present study was less than the values reported earlier while negative predictive value was similar to Geha *et al.*[18] The low PPV can be explained because of discordant isolates could not be induced due to phenotypic resistant which can be explained by the heterogeneous expression of resistance and the variables that influence this expression, i.e., *p*H, temperature, and salt concentration. Other factors associated with low PPV may be the variation of incubation temperatures, the concentration of sodium chloride (NaCl) in Mueller-Hinton agar plates and inoculums used by using cotton swab.

We found 155 MRSA strains on disc diffusion failed to prove MRSA because of negative *mec*A gene on PCR similar to other study on *mec*A gene detection conducted by Robert *et al.*[19,20] This can be explained as hyper production of ß-lactamase, production of Penicillin binding protein (PBP) with altered binding capacity and/ or other factors. Some strains resistant to β-lactam agents were found susceptible to other antibiotics like clindamycin, rifampicin, and trimethoprim-sulfamethoxazole, as reported by Nevet *et al.*[21]

In current study the methicillin resistance on disc diffusion was comparable to other studies[13,22] The emergence of resistance on MIC (<4 mg/ml) was also found comparable to Hafiz et al10, and lower than Araj et al23 where they reported 64.5%. All MRSA on disc diffusion were found multiple drug resistant, e.g., 100% resistance found in penicillin, ampicillin and erythromycin. This is in agreement with previous studies.[24,25] Macroloid resistance seen in our study is higher than Hafiz *et al.*[11] All MRSA strains were sensitive to glycopeptides-vancomycin similar to other studies.[26,27] In contrary to our study, an alarming figure of 4% Vancomycin resistant *Staphylococcus aureus* (VRSA) has also been reported by Bukahri *et al.*[13] Due to the emergence of resistance of *S. aureus* strains to glycopeptides, there is need to find out a good alternative of vancomycin. Daptomycin, a lipopeptide antibiotic, has broad activity against Gram-positive organisms similar to Vancomycin; however, its mechanism of action differs resulting in interference with cell membrane transport and a more rapid bactericidal activity. There were 155 false MRSA found on conventional method of antimicrobial susceptibility testing which was commonly used in all hospitals. The excessive use of glycopeptide to such false positive MRSA cases leads to its resistance. Therefore, molecular diagnosis of MRSA is cost effective.

For many years MRSA has been considered a typical nosocomial pathogen. In recent years, its epidemiology has radically changed, now observed even more frequently in community.[28–30] This can be explained due to misuse of antibiotics which is a major concern of interaction by health agencies.

CONCLUSION

There is an increase in the prevalence of MRSA among *S. aureus* isolates. Oxacillin resistance on MIC was the

more reliable than disc diffusion for defining MRSA isolates. Both phenotypic tests of methicillin resistance in *S. aureus* strains created false-susceptible results. These problems can be avoided using a *mec*A gene-based detection system, as the presence of the *mec*A gene was proved the hallmark for identification of MRSA strains. Establishment of molecular diagnostic laboratory in secondary and tertiary units is urgently required. Although it is an expensive technique but it is cost effective.

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