Research Article

Characterization of Methicillin resistant Staphylococcus aureus strains from clinical isolates in a tertiary care hospital of south India

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Abstract

Methicillin resistant staphylococcus aureus (MRSA) strains which are the most frequent cause of hospital acquired infections (HAI), are also currently encountered with increasing frequency in the community. Phenotypic detection of methicillin resistance is inadequate, due to environmental factors & heterogeneous resistant strains which may affect the phenotypic expression of resistance. Phenotypic methods for MRSA detection have been compared with the gold standard which is Polymerase Chain Reaction (PCR) for *mecA* gene. Discrepancies in detection have an adverse effect on patient management, thereby highlighting the importance of accuracy in diagnosis. Therefore rapid & accurate identification is essential for both implementation of infection control measures & prevention of nosocomial spread of the organism. **Materials & Methods:** 166 *S. aureus* isolates were studied out of a total of 677 staphylococcal samples. Methicillin resistance were detected using oxacillin disc diffusion (ODD), cefoxitin disc diffusion (CxDD), oxacillin screen agar (OSA) & PCR for *mecA* gene, using standard protocol. **Results:** Out of 166 *S. aureus* isolated, MRSA prevalence was seen in 26.5%. MRSA was identified in 44 (100%) by CxDD, 43 (98%) by OSA and 38 (86.4%) by ODD methods respectively. When these isolates were tested with molecular methods, the CxDD and PCR test results were comparable. However by antibiotic susceptibility test (AST), no strain was resistant to vancomycin, linezolid & teicoplanin. **Conclusion:** To reduce the prevalence of MRSA, regular surveillance of HAI & monitoring of AST is the need of the hour. Proper detection of all MRSA isolates with rapid & accurate methods must be done as a routine laboratory procedure.

Keywords: *S. aureus*, MRSA, phenotypic tests, AST, *mecA* gene.

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Introduction

Staphylococcus aureus (*S. aureus*) is a bacteria of significant importance because of its ability to cause a wide range of diseases and its capacity to adapt to diverse environmental forms. *S. aureus* is a gram positive organism that serves as an opportunistic pathogen & frequent colonizer of epithelium causing severe diseases in man & animals [1, 2].

The incidence of Methicillin Resistant Staphylococcus aureus (MRSA) in India ranges from 30 – 70%. MRSA strains harbour *mecA* gene which encodes a modified (Penicillin binding protein) PBP2a with low affinity for methicillin and all β-lactam antibiotics. Since MRSA are resistant to all β-lactam antibiotics, the therapeutic options are limited significantly, and therefore their accurate identification becomes important. Phenotypic expression of methicillin resistance may alter depending on the growth conditions of *S. aureus* which may affect the accuracy of the methods used to detect methicillin resistance [3].

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There are many methods available for the detection of MRSA. Cefoxitin is a potent inducer of *mecA* gene regulatory system. In recent years there are multiple published-reports which suggest the use of cefoxitin as surrogate marker for the detection of *mecA* gene mediated *S. aureus* resistance. Clinical Laboratory Standards Institute (CLSI) guidelines recommend cefoxitin to be used to identify MRSA, using a 30µg cefoxitin disc and a zone of ≤19 mm is considered as resistance strain [4].

The introduction of methicillin in 1960s had an important impact on the treatment of infections caused

by penicillinase producing *S. aureus*. Shortly later, MRSA strains with a PBP appeared and spread worldwide. Such resistance mechanism is due to production of a modified PBP2a with low affinity to β–lactam antibiotics as a result of the acquisition of a *mecA* gene. It can be difficult to detect MRSA because of the heterogeneous nature of methicillin resistance. The *mecA* gene is highly conserved among the Staphylococcal species and consequently, the detection of this gene by the PCR is considered as the “gold standard” for the detection of methicillin resistance in Staphylococci. The existence of the *mecA* gene in *S. aureus* characterizes methicillin resistance [5].

Staphylococcal resistance was reported shortly after penicillin was introduced, and within approximately 5-6 years, 25% of community isolates were penicillin resistant. Although the rates are only approximate because they are based on reports from numerous locations, a clear correlation exists between the prevalence of penicillin resistant strains of *S. aureus* reported in hospitals and rates in the community. Strains of MRSA, which had been largely confined to hospitals and long-term care facilities, are emerging in the community. The changing epidemiology of MRSA bears striking similarity to the emergence of penicillinase mediated resistance in *S. aureus* since decades ago. Even though the origin (hospital or the community) of the emerging MRSA strains is not known, the prevalence of these strains in the community seems likely to increase substantially [6].

Resistance to penicillin is determined by the *mecA* gene, which encodes the low affinity PBP2a. Lately, new methicillin resistance gene, *mecC* has been discovered from humans, animals and food products. This new *mecA* homolog has been detected in bacteria from dairy cattle in England and humans in England, Scotland and Denmark. This newly identified protein has a ≤ 63% similarity with the PBP2a encoded by *mecA* [7].

Laboratory diagnosis and susceptibility testing are crucial steps in the treatment, control and prevention of MRSA infections. Hence methods used to detect MRSA in clinical samples should have high sensitivity and specificity with the results available within a short time. Various methods have evolved for rapid detection of MRSA but the optimal method remains controversial. The most commonly used methods in laboratories are culture and sensitivity test, oxacillin disc diffusion (ODD), mannitol salt agar (MSA), oxacillin screen agar (OSA), broth and agar dilution tests etc. All these are conventional phenotypic methods of MRSA

identification. Genotypic method is the polymerase chain reaction (PCR) based method for detecting *mecA* gene which remains the gold standard for MRSA [8].

The phenotypic methods in general are easier to perform and interpret, cost effective and are widely available, however less discriminatory. The genotypic methods are expensive and technically demanding, and more precise. Newer technologies involving sequencing of various genes are coming up as broadly applicable typing systems. Still there is no consensus regarding the single best method for detection of MRSA strains. Application of any identification method requires careful assessment of its suitability and an individual approach depending on the purpose of the study [9].

Aims and Objectives

1. To study the prevalence of MRSA in a tertiary care hospital.
2. To compare the various phenotypic methods for isolation of MRSA.
3. Detection of *mecA* gene by genotyping for confirmation of the isolated MRSA strains.

Materials & Methods

The study was conducted in our teaching hospital from Jan – Dec 2014. The study was commenced after getting the ethical clearance from the Institutional Ethics Committee.

From a total of 677 staphylococci studied from various clinical samples, 166 *S. aureus* strains were isolated, identified and characterized as per recommended standard protocol [10, 11].

All the isolates were tested for methicillin resistance by disc diffusion using oxacillin (1µg), cefoxitin (30µg), MSA & OSA methods [Himedia India].

The isolates were subjected to AST by Kirby Bauer disc diffusion method.

Antibiotics tested were penicillin (10 units), ampicillin (10µg), cephalexin (30µg), oxacillin (1µg), cefoxitin (30µg), erythromycin (15µg), clindamycin (2µg), ciprofloxacin (5µg), ofloxacin (10µg), gentamycin (10µg), amikacin (30µg), linezolid (30µg), vancomycin (30µg), cotrimoxazole (25mcg)& teicoplanin (30µg).

Zone diameters were measured as per CLSI criteria [11].

Oxacillin screen agar: Mueller Hinton agar (MHA) with 4% NaCl and oxacillin 6µg/ml was prepared. The 0.5 McFarland suspension of the test strains was inoculated as spots over the plates, incubated at 35^0C for 24 hours. The strains which were able to grow on this medium were designated as MRSA.

Oxacillin and cefoxitin disc diffusion test: MHA plates were overlaid with a saline suspension of the isolate (0.5 McFarland), cefoxitin (30µg) and oxacillin (1µg) were placed on the plates. After 24-48 hours of incubation at 35^0C, the plates were read using CLSI cut off points as reistant (<19mm cefoxitin; <10mm oxacillin).

Molecular detection of *mecA* gene by PCR: *S. aureus* DNA extraction was performed by using Gene Elute Genomic DNA kit (Sigma Aldrich). The primers used for detection of *mecA* gene were [12]:
*mecA*1: 5' – GTAGAAATGACTGAACGTCCGATAA
*mecA*2: 5' – CCAATTCCACATTGTTTCGGTCTAA

The target gene was amplified using the above set of primers by PCR in a 100µl of reaction mixture containing dNTPs (200µM), 2.5µM (each primers), 2.5U of Taq DNA polymerase (Bangalore Genei), 50mM KCl, 10mM Tris-HCl, 1.5mM MgCl2 & 0.01% gelatin.

The procedure steps were as follows: Pre-denaturation for 4 minutes at 94^0C, denaturation for 45s at 94^0C: annealing for 45s at 55^0C, primer extension for 1min at 72^0C. Each step was repeated 30 times. For visualization, 10µl of PCR amplicon was loaded in 2% agarose gel with ethidium bromide. The band of amplified DNA was visualised under UV trans-illuminator. A 310 bp amplicon corresponds to the *mecA* gene shown in the fig [13].

Quality Control

1. *S. aureus* ATCC 25923 was used as standard control strain.
2. In-house strain of *S. aureus* showing sensitivity to cefoxitin.

The viability of the isolates, was maintained by periodic subculture on semi-solid nutrient agar.

Results

Out of the 677 Staphylococcal isolates from various clinical specimen, *S. aureus* was present in 166 samples (24.5%). Out of 166 *S. aureus*, MRSA was the isolate in 44 (26.5%).

Table 1 shows the Sample-wise distribution of MRSA: Pus 29 (65.9%), Blood 13 (29.5%) and ET tip 02 (4.6%). The maximum isolation of MRSA was from surgical departments (45.4%) – Surgery 14 (31.8%) & Orthopaedics 06 (13.6%).

Table 1. Sample-wise distribution of MRSA isolates:

	MED	SUR	PAED	ORTHO	OBG	DERMO	ENT	ICU	Total (44)
BLOOD	01	-	12	-	-	-	-	-	13 (29.5%)
PUS	-	14	02	06	04	01	01	01	29 (66.0%)
ET Tip	01	-	-	-	-	-	-	01	02 (4.5%)
%	4.5	31.8	31.8	13.6	09	2.4	2.4	4.5	

Table 2 shows the phenotypic & genotypic characteristics of MRSA isolates: MRSA detection by OSA & CxDD was 98% & 100% and by ODD method it was 86.4%; *mecA* gene detection by molecular methods gave comparable results with that of CxDD.

Table 2. Phenotypic and Genotypic characteristics of MRSA strains:

	MRSA (44)	%
Oxacillin Disc Diffusion	38	86.4
Cefoxitin Disc Diffusion	44	100
Oxacillin Screen Agar	43	98
mecA gene by PCR	44	100

Table 3 shows the antibiotic susceptibility pattern of the MRSA strains: The isolates were highly resistant to cefoxitin (100%), ampicillin (95.5%), cephalexin (95.5%), ciprofloxacin & gentamycin (75%) each & erythromycin (68%); and were moderately resistant to cotrimoxazole (48%) & clindamycin (52%). All the strains were 100% sensitive to linezolid, teicoplanin & vancomycin.

Table 3. Antibiotic Susceptibility Pattern of MRSA strains:

	Amp	E	Co	Cd	Lz	Tei	Va	Cx	Cp	Cf	Of	G
Sensitive	02	14	23	21	44	43	44	-	02	11	14	11
%	4.5	32	52	48	100	98	100	-	4.5	25	32	25
Resistant	42	30	21	23	-	01	-	44	42	33	30	33
%	95.5	68	48	52	-	02	-	100	95.5	75	68	75

Antibiotics: Ampicillin, Erythromycin, Cotrimoxazole, Clindamycin, Linezolid, Teicoplanin, Vancomycin, Cefoxitin, Cephalexin, Ciprofloxacin, Ofloxacin, Gentamicin

Table 4. Statistical analysis of data in different studies:

	MRSA (%)	ODD (%)	CxDD (%)	OSA (%)	mecA (%)
Present Study 2015	**26.5**	**86.4**	**100**	**98**	**100**
Manju Pillai 2014	37.5	93	98	98	100
James John 2012	60	65	76	68	76
Pramodhini 2011	36.4	90	100	100	100
Kumar S 2009	56	85	100	96	98
Murakami K 1991	53	98	98	98	100

Oxacillin disc diffusion (ODD), Cefoxitin disc diffusion (CxDD), Oxacillin screen agar (OSA).

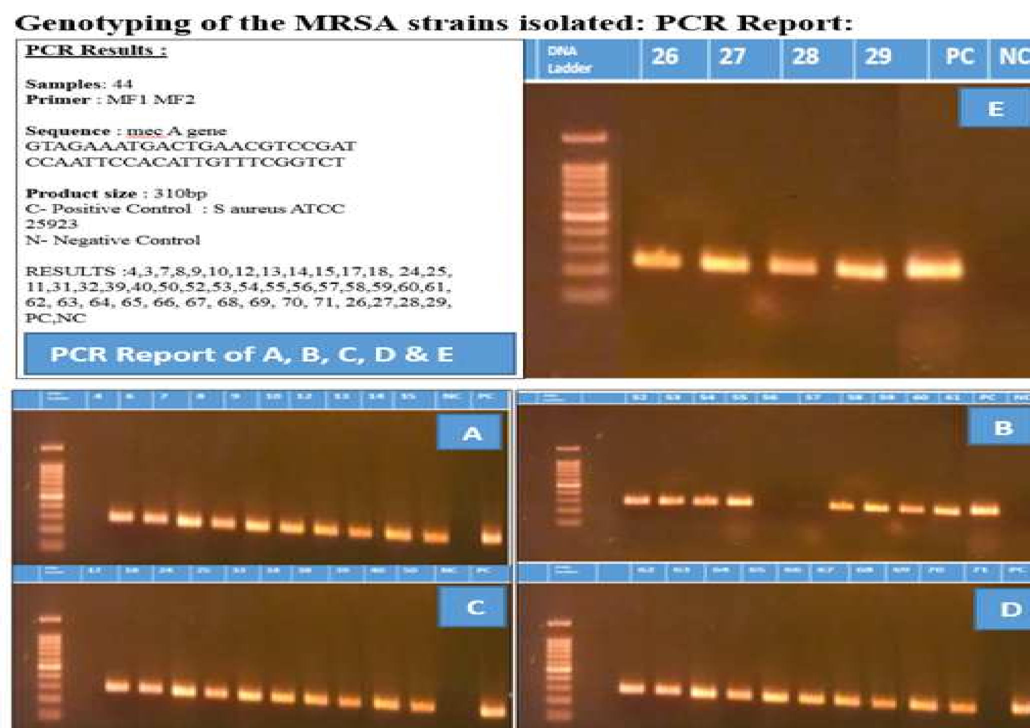
Figure I: Shows the different tests used in characterization of Methicillin resistant Staphylococcus aureus

Methicillin Resistant Staphylococcus aureus on Oxacillin Screen Agar

Mannitol Salt Agar with *S. aureus*

ODD & CxDD tests for MRSA

Figure II: Shows the Genotyping results of the MRSA Isolates in the study



Discussion

MRSA are being recognised as important human pathogens causing significant morbidity & mortality in hospitals and community; and are difficult to eradicate because they are multi-drug resistant. With reference to world-wide resistance among *S. aureus* strains, early detection of reduced susceptibility to β-lactam antibiotics is important for clinicians. CLSI recommends use of cefoxitin as preferred method for testing *S. aureus* as surrogate marker for detecting oxacillin resistance. Detection of *mecA* gene or its product PBP2a is considered the gold standard for MRSA confirmation [11]. The prevalence of MRSA in our hospital was found to be 26.5%. Similar isolation rates were also found in studies byKumari N et al &Pramodhini Set al [14, 15].Higher prevalence rates ranging from 40 – 60% were found in some studies [16, 17]. This variation might be because of changes in antibiotic usage & infection control practices in different hospitals.

In the present study, maximum isolation of MRSA was from Surgery & Paediatrics departments (31.8%) each, followed by Ortho (13.6%), OBG (9%) & Medicine (4.5%) which correlates with pus, blood & other samples. Similar results were reported by Kumari N et al &Pramodhini S et al., which can be explained by the fact that *Staphylococcus* will be present as part of the commensal flora of the skin [14, 15].

CxDD was found to be highly sensitive & specific (100%) while sensitivity & specificity of ODD was 94% & 80%. The results of disc diffusion methods showed that CxDD is a better alternative for MRSA detection. Similar results were quoted in several other studies [3, 8, 18, 15, 21].

Sensitivity & specificity of OSA were 100% & 99%. Similar finding were reported by Pramodhini S et al, Kumar S et al, Murakami K et al & Manju Pillai et al [15, 18, 21, 8].

In the present study, isolated MRSA strains were 100% sensitive to linezolid, vancomycin & teicoplanin. The isolates were highly resistant to cefoxitin, ampicillin & cephalexin (95 – 100%). The isolates showed varying resistance to other antibiotics like erythromycin (68.2%), clindamycin (52%) & cotrimoxazole (48%).

AST report in the present study was coherent with that in the study by Anupurba et al [17].

Our study showed sensitivity & specificity of genotyping by PCR for MRSA to be 100% which was in concordance with other studies like James John et al, Kumar S et al, Manju Pillai et al, Swenson JM et al & Fernandes CJ et al [4, 18, 8, 19, 20].

Conclusion

Rapid and accurate identification of MRSA is required for therapeutic and epidemiological reasons; to immediately start appropriate antimicrobial therapy & to avoid the spread of these strains. Phenotypic methods are still preferred for species identification. But for the reliable detection of MRSA an algorithm should include a combination of tests; and apply a genotypic method for confirmation of resistant isolates showing discordant results. The hospital infection control policy & guidelines should be strictly implemented so as to enable clinicians to deliver better and proper health care to the patients. Results of cefoxitin disc diffusion test is in concordance with the genotyping results for *mecA* gene. So this test can be an alternative to PCR for detection of MRSA in resource constraint settings.

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