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RESEARCH ARTICLE

Incidence of Methicillin Resistant *Staphylococcus aureus* [MRSA] in Pus Samples and Associated Risk Factors in a Tertiary Care Hospital

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Abstract

Background: *Staphylococcus aureus* is a human pathogen with a remarkable propensity for development of antibiotic resistance. The aim of our study is to correlate the phenotypic and genotypic methods and to study the associated risk factors for methicillin resistant *Staphylococcus aureus* [MRSA] in pus samples. **Material and Methods**: A total of 200 clinical pus samples were collected. Routine antibiotic susceptibility testing was performed including oxacillin disk [5µg]. Oxacillin screen agar plates with 4% NaCl and 4 µg /ml, 6 µg /ml, 8 µg /ml of oxacillin were inoculated and interpreted as per standard guidelines. Cefoxitin disk diffusion test was performed using 30µg disc and zone sizes were measured. PCR for *mec* A gene was performed. Associated risk factors like sex, diabetic or not, hospital acquired or community acquired and diabetes was also considered. **Results:** Out of 200 clinical pus samples 80 strains were isolated as *Staphylococcus aureus.* Among them 28 were found to be MRSA by cefoxitin disc [30-µg] test and 29 by *mec* A gene. Cefoxitin results with 96.55% and 96.22% sensitivity and specificity respectively. Cefoxitin is less specific and sensitive to PCR, gold standard for detection of MRSA but is more sensitive to oxacillin agar screen [8µg].

INTRODUCTION

Staphylococcus aureus causes a variety of suppurative infections and toxinoses in humans. It causes superficial skin lesions as well as more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections, and deep-seated infections such as osteomyelitis and endocarditis[9]. *S. aureus* is a major cause of nosocomial infection of surgical wounds and infections associated with indwelling medical devices. Methicillin resistant *Staphylococcus aureus* [MRSA] has become established outside the hospital environment and is now appearing in community populations without identifiable risk factors[4]. MRSA is one of the major pathogen associated with community acquired serious nosocomial infection because these strain generally are multi drug resistance which limit possibilities of treatment[4].

The *mec* A gene is highly conserved in staphylococcal strains and thus is a useful marker of methicillin/oxacillin resistance[5]. Penicillin binding protein [PBP2 a] of *mec* A gene is presently considered to be the gold standard for detection of MRSA. The aim of our study was to screen for MRSA from clinical pus samples, comparison of phenotypic and genotypic methods and to study the associated risk factors.

MATERIAL AND METHODS

A total of 200 clinical pus samples were collected from October 2014 to April 2015 from Hind Institute of Medical Science, Barabanki district, Uttar Pradesh with a study of associated risk factors like sex, diabetic or non-diabetic, hospital acquired or community acquired. Criteria for hospital acquired category was considered as sample

collected from patients after 72 hours of admission in any wards and community acquired are the ones received from the outpatient department [OPD]. Confirmations of the strains were done using standard tests like catalase, slide and tube coagulase, DNase and growth on mannitol salt agar. Routine antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method for antibiotics: Vancomycin [30µg], Amikacin [30µg], Oxacillin [5µg], Ciprofloxacin [5µg], Erythromycin [15µg], Amoxycillin [30µg], Tetracycline [30µg] and Clindamycin [10µg]. Quality control strain – Methicillin sensitive *S.aureus* [MSSA ATCC 25923]; Methicillin resistant *S.aureus* [MRSA ATCC 43300] were used as negative and positive control respectively.

i. Oxacillin screen agar

Mueller Hinton agar [MHA] plates containing 4% NaCl with 4µg/ml, 6µg/ml and 8µg/ml of oxacillin were prepared. Plates were inoculated with 10 ml of 0.5 Mc Farland suspension of the isolate by streaking in one quadrant and incubated at 35^0C for 24 h. Plates were observed carefully up to 24 hours for any growth and was considered to be oxacillin resistant.

ii. **Cefoxitin disc diffusion test**

A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture done on MHA plate, cefoxitin disc [30µg] were put and plates were incubated at 37^0C for 18 hr. and zone of inhibition were measured. An inhibition zone diameter of ≤19 mm was reported as oxacillin resistant and ≥ 20 mm was considered as oxacillin sensitive.

iii. Detection of mec A gene

Bacterial genomic DNA was isolated by CTAB method[14]. PCR was performed to amplify 533 bp of mec A gene from genomic DNA as described previously[14]. PCR was performed using forward primer corresponding to nucleotides 1282-1303 [5'- *AAAATCGATGGTAAAGGTTGGC*] and the reverse primer was complementary to nucleotides 1793-1814 [*5'-AGTTCTGCAGTACCGGATTTGC*]. The reactions were carried out in a final volume of 50µl containing 50 ng of genomic DNA, 500mM KCl, 100mM Tris-HCl, 0.5 mM each dNTP, 2.5 mM $MgCl_2$, 0.15 µM of each primer and 2U of Taq DNA polymerase. DNA amplification was carried out in an automated thermocycler [MJ Research PTC-200]. PCR was performed at 95°C for 5 min followed by 40 cycles at 94°C for 30 sec, annealing at 54°C for 30 sec and extension at 72°C for 90 sec, followed by an additional cycle of 5 min at 72°C to complete partial polymerizations. Amplified products were analyzed using horizontal 1·5% agarose gel electrophoresis.

RESULTS

- Among 80 *S.aureus* strains isolated, hospital acquired infection were 71.25% [57/80] and community acquired 28.75% [23/80], diabetic were 32.5% [26/80] and non diabetic 67.5% [54/80], males 66.25% [53/80] and females 33.75% [27/80]
- 25 isolates were found to be MRSA and 55 were MSSA by oxacillin [5µg] disc diffusion test. 26 were MRSA and 54 were MSSA by oxacillin [6µg] agar screen test and 29 were found to be positive for MRSA by mec A gene PCR. The sensitivity and specificity of three phenotypic tests as compared with genotypic test are given in table II.

DISCUSSION

The accurate and early determination of methicillin resistance is of key importance in the prognosis of infections caused by *S.aureus*. In the present study the sensitivity and specificity was 100% for *mec* A gene which is consistent with a previously reported study of Anand et al., 2009[7]. In our study hospital acquired-MRSA was 68.96% while community acquired-MRSA was 31.03%. Prevalence of MRSA in diabetic was 34.48% and in non-diabetic 65.51%, while in males 62.06% and females 37.93%. The major reservoir of staphylococci in hospitals are colonized/infected hospital patients and colonized hospital workers, with carriers at risk for developing endogenous infection or transmitting infection to health care workers and patients while transient hand carriage of the organism on the hands of health care workers account for the major mechanism for patient to patient transmission. Low prevalence of MRSA colonization in an adult outpatient population indicated that MRSA carriers most likely acquired the organism through contact with healthcare facilities rather than in the community.

Borderline strains that contain *mec* A are extremely heterogeneous methicillin resistant strains that produce PBP2a. These strains have resistant subpopulation of cells, although these might be quite small, that can grow at a high concentration of oxacillin. Borderline strains that do not contain *mec* A can be differenciated phenotypically from extremely heterogeneous *mec* A positive strains.

The result of oxacillin disk diffusion test and PCR sensitivity is 86.20% and 100%, while specificity is 69.86% and 100% respectively. Previous studies of Kircher et al., [2004]; Sakoulas et al., [2001]; Velasco et al., [2005] have reported the sensitivity and specificity of the Oxacillin agar screen test to be 94.3%–100% and 83%–100 %, respectively[8,15,19]. According to Ferreira et al., [2003], the sensitivity and specificity of the Oxacillin agar screen test were 99.7% and 100 %, respectively, at a concentration of 4 µg ml^{-1} and 75.7% and 100 % respectively, at a concentration of 6µgml^{-1}. In our study sensitivity and specificity of Oxacillin agar screen test was 89.65% and 80.95% respectively at 6µgml^{-1} concentration. Previous studies of Flayhart et al, [2005]; Skov et al, [2003]; Swenson & Tenover, [2005]; Velasco et al., [2005], have shown, the sensitivity and specificity of the cefoxitin disc diffusion test for *S. aureus* to be 95–100% and 98–100%, respectively while in our study the sensitivity and specificity for cefoxitin disk diffusion test was 96.55% and 96.22% at 30µg[6, 16, 19].

We conclude that the accuracy of the cefoxitin disc diffusion test was better than that of the oxacillin disc diffusion test and oxacillin agar screen test for the detection of MRSA. It also does not require special testing conditions such as a lower incubation temperature [35^0C] and NaCl supplementation in the testing media, as required by the oxacillin disc diffusion test. Cefoxitin is considered to be a better predictor than oxacillin for the detection of hetero resistance because it is a stronger inducer of penicillin-binding protein 2a [PBP2a]. There is an increase prevalence of MRSA and multi drug resistance in staphylococci and vancomycin is the only drug of choice. Resistance pattern seems to be an alarming threat to antimicrobial therapy. Health care providers are also at risk of developing carrier state, which further increase the rate of nosocomial infection in hospitalized patients. MRSA could be prevented by identifying and screening for MRSA carriers inside high-risk wards as it is an important clinical problem in hospitals. It should not be ignored as it can seriously disrupt the efficient delivery of healthcare services in the hospital. Preventing colonization and infection remains the most effective way to control the spread of MRSA and simple measures such as patient isolation, cohorting doctors and nurses working with patients. Strict enforcement of hand washing and early discharge of infected patients will go a long way towards reducing the spread of this pathogen in the hospital. Follow-up of discharged patients to measure MRSA cultures and sensitivities is more important than ever. Hence, the present study provides incidence of MRSA, as 85% of MRSA infections are healthcare associated and MRSA accounts for 60 % of all staphylococcal infections.

CONCLUSION

We conclude that the accuracy of the cefoxitin disc diffusion [30µg] test was better than that of the oxacillin disc diffusion [5µg] test and oxacillin agar screen test [4 µg /ml, 6 µg /ml, 8 µg /ml] for the detection of MRSA where PCR the gold standard method is not available in majority of labs, more expensive and is not feasible technically for all the samples.

Table I Prevalence of ***S.aureus***, MRSA and MSSA in the community

	Hospital acquired	**Community acquired**	**Diabetic**	**Non-Diabetic**	**Male**	**Female**	**Total**
No. of *S.aureus* isolated	57 [71.25%]	23 [28.95%]	26 [32.5%]	54 [67.5%]	53 [66.25%]	27 [33.75%]	**80**
No. of MRSA isolated	20 [68.96%]	9 [31.03%]	10 [34.48%]	19 [65.51%]	18 [62.06%]	11 [37.93%]	**29**
No. of MSSA isolated	37 [72.54%]	14 [27.45%]	15 [29.41%]	36 [70.58%]	35 [68.27%]	16 [31.37%]	**51**

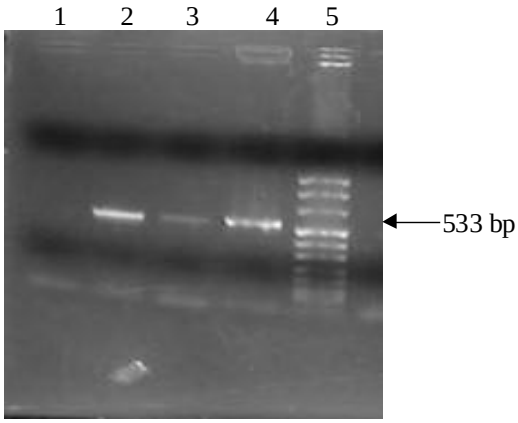
Table II Comparison of phenotypic with genotypic method of detection of MRSA.

TEST METHODS	MRSA	SENSITIVITY [%]	SPECIFICITY [%]
Oxacillin disc diffusion [5µg]	25	86.20%	69.86%
Oxacillin agar screen [4 µg]	25	86.20%	80.95%
Oxacillin agar screen [6 µg]	26	89.65%	80.95%
Oxacillin agar screen [8 µg]	27	93.10%	96.22%
Cefoxitin disc diffusion [30µg]	28	96.55%	96.22%
PCR for *mec* A gene	29	100%	100%

Table III Comparison of mec-A +ve and mec-A –ve strains

Strain	No. of isolates	ODD [5µg]	OAS [4µg]	OAS [6µg]	OAS [8µg]	Cefoxitin [30µg]
Mec A +ve	2	R	R	R	S	S
	4	S	R	R	R	R
	1	R	R	R	S	R
Mec A-ve	10	R	R	R	S	S
	2	S	R	R	R	R
	12	R	S	S	S	S

Figure I Representative gel picture of mec A gene



Lane 1: MSSA ATCC 29213
Lane 2: MRSA ATCC 43300
Lane 3,4 : Clinical samples
Lane 5: 19-1114bp DNA marker

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