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PHENOTYPIC DETECTION OF METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS* ISOLATES FROM VARIOUS CLINICAL SPECIMENS IN A TERTIARY CARE INSTITUTE IN NORTH WEST REGION OF RAJASTHAN

Medical Microbiology

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ABSTRACT

Introduction: The drug resistant phenomenon is being worldwide concern especially in the last 20 years. Among the most threatening antibiotic-resistant pathogens known are strains of methicillin-resistant S. aureus (MRSA), they are resistant to ß-lactams and other cell-wall-active agents. Methicillin-resistant Staphylococcus aureus (MRSA) is a major nosocomial pathogen worldwide, which has emerged over the past 30 years as a leading cause of both nosocomial and community-acquired infections. Accurate and rapid identification of MRSA in clinical specimens is essential for timely decision on effective antimicrobial chemotherapy. The present study tries to describe an accurate and quick detection for of clinically relevant antibiotic resistance gene of Staphylococcus aureus using phenotypic technique.

Material and methods: The current study is a hospital-based cross sectional observational study. Duration of the study was 1 year and August 2018 to Jan 2021. An informed written consent was obtained from all the subjects. A total of 254 Staphylococcus aureus strains have been taken for study from various clinical specimens like pus, blood, sputum, throat swab, ear swab, high vaginal swab, CSF, urine, pleural fluid, semen, bile, corneal swab, etc. clinically significant MRSA isolates were collected in Department of Microbiology at our tertiary care hospital,Bikaner.

Results: It is observed that the total of 254 was found to be most sensitive and specific test for MRSA detection in phenotypic method. Although in our study, Sensitivity of all four methods-Disc diffusion, Oxacillin Screen agar and E Strip were found most sensitive method for the detection of MRSA..(Table-3). Total 254 Staphylococcus aureus isolates were tested for antibiogram, in the present study Staphylococcus aureus has shown high sensitivity towards Vancomycin 99.61% , Amplicillin sulbactum 99.21%, and Teicoplanin 96.85% followed by Linazolid 82.28%, Doxycycline74.41%, Tetracycline 71.65%, Cefuroxime 60.63%, Ciprofloxacin 54.72%, Augmentin -53.54% and Gentamycin 44.48%, Rest of other primary antibiotics showed low sensitivity such as Penicillin 14.57%, Erythromycin 16.93% , Clindamycin 17.72, Azthromycin 21.26%, and Cotrimoxazole 24.01%. revealed.

Conclusion: On the basis of our findings, phenotypic methods (cefoxitin disc diffusion and oxacillin broth micro dilution) could be used for routine diagnosis of MRSA; however cefoxitin disc diffusion might be preferred over MIC method considering time and labour factor.

KEYWORDS

Methicillin Resistance, Staphylococcus Aureus, Phenotypic Method

INTRODUCTION

Staphylococcus aureus is one of the major resistant pathogens in clinical practice. The reservoir of methicillin-resistant S. aureus (MRSA) is infected and colonized patients, and the major mode of transmission from patient to patient is through the contaminated hands of healthcare workers.[1] MRSA was first detected in Britain in 1961 and is now "quite common" in hospitals.[2] MRSA is defined as a strain of S. Aureus that is resistant to a large group of antibiotics called β-lactams, that includes penicillins and cephalosporins.

MRSA is a major nosocomial pathogen worldwide which has emerged over the past 30 years as a leading cause of both nosocomial and community-acquired infections.[3,4] Methicillin resistance is caused by the presence of mecA gene, which encodes an additional 78 kDa low-affinity penicillin binding protein (PBP)-2a or PBP2' which has a low affinity for β-lactam antibiotics.[5] There has been a steady increase in the prevalence of MRSA all over the world.[6] In a meta-analysis of 31 studies, Cosgrove et al. reported that MRSA bacteremia is associated with increased mortality as compared with methicillin-sensitive S.aureus (MSSA) bacteremia.[7] The treatment of MRSA infections is entirely different from that of MSSA infections. Accurate and rapid detection of MRSA permits timely implementation of effective antimicrobial therapy, preventive infection control strategies like immediate patient isolation (and occasional ward closure), screening of the patient contacts and staff, and appropriate disinfection measures which in turn reduce the costs.[8,9] An additional concern is the emergence of vancomycin-intermediate S. aureus (VISA) and, more recently, vancomycin-resistant S. aureus (VRSA),[10,11] one of the main reasons assumed to be due to the injudicious use of vancomycin for wrongly diagnosed MRSA (false-positive MRSA).

In India also there have been reports of increasing isolation rates of MRSA. Literature shows that MRSA incidence was as low as 6.9 per cent in 1988 and reached to 24 and 32.8 per cent in Vellore and Lucknow in 1994, respectively and was of the same order in Mumbai, Delhi and Banglore in 1996 and in Rohtak and Mangalore in 1999.[11] However, in some of the centres, it was as high as 80 per cent and in India the isolation cases varied from 20-40 per cent.[12]

Laboratory diagnosis and susceptibility testing are crucial steps in treating, controlling, and preventing MRSA infections. Discrepancies in detection have an adverse effect on patient management, thereby highlighting the importance of accuracy in detection. Hence, methods used to detect MRSA in clinical samples should have high sensitivity and specificity and, most importantly, the result should be available within a short time. Various methods have evolved for rapid detection of methicillin-resistant staphylococci, but the optimal method for the detection remains controversial. The most commonly used method in the laboratories is culture and antibiotic sensitivity test (AST) [oxacillin disk diffusion (ODD)]. Other methods available for diagnosing MRSA include mannitol salt agar (MSA) with oxacillin (agar screening method), minimum inhibitory concentration (MIC) tests, agar dilution tests etc., All these tests are the conventional phenotypic methods of MRSA identification.[12,13]

MATERIALAND METHODS

It is a hospital-based cross sectional observational study. Duration of the study was 1 year and August 2018 to Jan 2021. The study was started after getting the ethical clearance from the Institutional Research Committee of the institution. An informed written consent was obtained from all the subjects.

A total of 254 Staphylococcus aureus strains have been taken for study from various clinical specimens like pus, blood, sputum, throat swab, ear swab, high vaginal swab, CSF, urine, pleural fluid, semen, bile, corneal swab, etc. clinically significant MRSA isolates were collected in Department of Microbiology at our tertiary care hospital, between August 2018 to Jan 2021

STUDY PROTOCOL

LABORATORY DIAGNOSIS:

The samples received in the laboratory were processed in the following manner. The following criteria are undertaken for the selection of the patients in the study.

INCLUSION CRITERIA:

All strains (non-repetitive) were collected from various specimens of patients attending outpatient departments and admitted in wards at P.B.M. hospital and associate group of hospitals. Specimens included are urine, blood, pus, wound swab, sputum and other respiratory tract specimen, high vaginal swab, and CSF etc.

Medical and demographic data of the patients were collected using a questionnaire. Data recorded were as follows: demographic characteristics (age, gender); underlying diseases ; presence of intravascular or urinary catheters; prolong hospitalization(>one week); history of intensive care unit (ICU) stay; nursing home residency; being on mechanical ventilation; prolong antibiotic use(> three week); and recent surgery (within one month); post operative wound infections; sepsis; poor nutritional status; haemodialysis etc.

EXCLUSION CRITERIA

Three different organisms with no predominating organism and repeated isolate from same patient were excluded from study.

DETECTION OF METHICILLIN RESISTANCE BY PHENOTYPIC METHODS

In the present study, Methicillin resistance in S.aureus detected by phenotypic methods namely Cefoxitin disc diffusion method, Oxacillin resistant screen agar method and E- test for Cefoxitin MIC .

1.The Cefoxitin Disc Diffusion Test:

Testing for Methicillin resistance was done by using 30µg Cefoxitin disc by Kirby Bauer disc diffusion method. 0.5 McFarland standard suspension of the 254 isolate was made and lawn culture was done on MHA plates, and incubating at 37o C for 18-24 hours and zone diameter was measured. The results were interpreted as per CLSI standards with appropriate ATCC controls.

Cefoxitin	SUSCEPTIBLE	RESISTANT
(30µg)	≥22mm	≤21mm

2. The Oxacillin Resistant Screen Agar Test

All methicillin resistant strains were also confirmed by oxacillin screen agar test26. Mueller Hinton agar plate with 4% NaCl and 6% oxacillin are prepared. 0.5 Mc Farland suspension of isolate is made and streaked on the one quadrant of plate. The plate in incubated at 350C for 24 hour. Plates were observed carefully in transmitted light for any growth. Any growth after 24 hour is considered methicillin resistant.

3. Cefoxitin Mic E-Test

Oxacillin MIC was done with E-Strip (Hi-media,Mumbai, India) using 0.5 McFarland inoculum according to manufacturer's instruction. MHA plates supplemented with 2% NaCl were used.

STATISTICALANALYSIS

Detailed data of all subjects of case and control group were collected. After complete evaluation all findings were expressed in terms of Mean ± SD. Comparison between case group and control group was done using paired "t" test. All statistical analysis was done using SPSS software version 20.

RESULTS

A total of 254 isolates of Staphylococcus aureus were isolated from specimens of respiratory tract infection, wound infection, urinarytract infections, septicaemia, postoperative wound infections,e arinfections etc.,satisfying both inclusion and exclusion criteria. The isolated S.aureus were subjected to disk diffusion test with cefoxitin (30µg) and oxacillin sreeen agar to detect the methicillin resistance and the resulting MRSA is further confirmed by E strip and Molecular mec. The demographic profile of the study population was also analysed. Out of 254 S.aureus isolates, 113 strains (44.49%) were found to be methicillin resistant and 141 strains (55.51%) were sensitive to methicillin.(Table-1). Total 254 clinical isolated Staphyloccous aureus, maximum isolates (43.31%) were found in pus samples, followed by blood samples 26.77%. Among pus samples 20.08% were MSSA and 23.23% were MRSA while in Blood Sample 17.72% were MSSA and 9.05% were MRSA. apart from staphyloccous aureus were found in urine 22.05%, sputum 3.15% CSF & Vaginal swab 1.97% each and 0.79% in pleural fluid.(Table-2) Results of table 3 was found to be most sensitive and specific test for MRSA detection in phenotypic method. Although in our study, Sensitivity of all four methods-Disc diffusion, Oxacillin Screen agar and E Strip were found most sensitive method for the detection of MRSA..(Table-3). Total 254 Staphylococcus aureus isolates were tested for antibiogram, in the present study Staphylococcus aureus has shown high sensitivity towards Vancomycin 99.61%, Amplicillin sulbactum 99.21%, and Teicoplanin 96.85% followed by Linazolid 82.28%, Doxycycline 74.41%, Tetracycline 71.65%, Cefuroxime 60.63%, Ciprofloxacin 54.72%, Augmentin -53.54% and Gentamycin 44.48%, Rest of other primary antibiotics showed low sensitivity such as Penicillin 14.57%, Erythromycin 16.93%, Clindamycin 17.72, Azthromycin 21.26%, and Cotrimoxazole 24.01%.(Table-4)

OBSERVATION TABLES

Table-1 Prevalence of MSSA and MRSA among Staphylococcus aureus isolates

Staphylococcusaureus isolate	Number of cases (N=254)	Percentage (100%)
Methicillin sensitive S.aureus	141	55.51
Methicillin resistant S.aureus	113	44.49

Table-2 Distribution of MSSA and MRSA in clinical samples

Specimen	MSSA		MRSA		Total
	Number	Percentage (%)	Number	Percentage (%)	
Pus	51	20.08	59	23.23	110 (43.31%)
Blood	45	17.72	23	9.05	68 (26.77%)
Urine	35	13.78	21	8.27	56 (22.05%)
Sputum	5	1.97	3	1.18	8 (3.15%)
Pleural Fluid	1	0.39	1	0.39	2 (0.79%)
CSF	3	1.18	2	0.79	5 (1.97%)
Vaginal Swab	1	0.39	4	1.57	5 (1.97%)
Total	141	55.5	113	44.5	254 (100%)

Table-3 Performance parameters of all Methicillin resistance methods tested for drug resistance in *Staphylococcus aureus* isolated

Test	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Accuracy (%)
Disc Diffusion	94.12%	99.26%	99.12%	95.04%	96.85%
Oxacillin Screen agar	94.12%	99.26%	99.12%	95.04%	96.85%
E Strip	98.32%	100%	100%	98.54%	99.21%

Table-4 Antibiogram of Staphylococcus aureus isolates among present study

Antibiotics	Sensitivity (n=254)	Sensitivity Percentage (%)
Penicillin	37	14.57
Azthromycin	54	21.26
Erythromycin	43	16.93
Clindamycin	45	17.72
Augmentin	136	53.54
Gentamycin	113	44.49
Ciprofloxacin	139	54.72
Cefuroxime	154	60.63
Tetracycline	182	71.65
Doxycycline	189	74.41
Linazolid	209	82.28
Vancomycin	253	99.61
Teicoplanin	246	96.85
Ampicillin sulbactum	252	99.21
Cotrimoxazole	61	24.01

DISCUSSION

Recently increasing rate of methicillin resistant strains of S. aureus has

been posed a great difficulty in choosing suitable antibiotics for the treatment of infection caused by this microorganism [14]. In India about 52% isolates of S. aureus were resistant to methicillin [15]. Timely and correct identification of MRSA is very important issue for prognosis and treatment infections caused by S. aureus [16].There are multiple laboratory methods for detection of MRSA, however many of these methods have not sufficiently sensitivity and specificity. This may be due to the persons carrying the test and material, methods and heterogeneous resistance clinical isolates of S. aureus [17,18].

In present study, majority of the MSSA isolates (21.98%) were between 21-30 years of age followed by 20.57% among 41-50 years of age. On the contrary, majority of the MRSA maximum numbers were isolated from the age group41-50 years (23.89%) followed by 21.24% in age group of 21-30 yrs. Similar results were observed by Awadh R et al170who reported 46.6% methicillin resistant Staphylococcus aureus isolated from old age patients.[19]Total 254 clinical isolated Staphyloccous aureus, maximum isolates (43.31%) were found in pus samples, followed by blood samples 26.77%. Among pus samples 20.08% were MSSA and 23.23% were MRSA while in Blood Sample 17.72% were MSSA and 9.05% were MRSA. apart from staphyloccous aureus were found in urine 22.05%, sputum 3.15% CSF &Vaginal swab 1.97% each and 0.79% in pleural fluid. This is comparable to the study by Terry Ali et al where majority of the MRSA isolates were from pus (21.4%). The study from PIMS, found a high prevalence from post operative surgical infections (80%). [20-23]

In the current study Showing phenotypic detection Disc diffusion sensitivity 94.12%, specificity 99.26%, Oxacillin screen agar sensitivity 94.12%, specificity 99.26%, which was similar to cefoxitin disc diffusion, E Strip sensitivity 98.32%, specificity 100% were found most sensitive phenotypic method for the detection of MRSA.In the study of mohammed reza, MIC strip test showed the sensitivity and specificity about 91.6% and 100%, respectively. In the study of Rahbaret al., sensitivity and specificity were both 100%. Five isolates in our study showed discordant results for MIC of Cefoxitin. This can be probably explained by the fact that not all S. aureus isolates express their mecA gene. In our study, the sensitivity and specificity of cefoxitin disc diffusion test were 94.12% and 99.26%, respectively. In the study of Farahani et al. the sensitivity and specificity of the cefoxitin disc diffusion method was 100 and 73.6%, respectively. In previous study that performed by Pillaiet al., the sensitivity and specificity were reported 93.5% and 83.5%, respectively.[24,25]

CONCLUSIONS

On the basis of our findings, phenotypic methods (cefoxitin disc diffusion and oxacillin broth micro dilution) could be used for routine diagnosis of MRSA; however cefoxitin disc diffusion might be preferred over MIC method considering time and labour factor. There is a global epidemic of methicillin resistant Staphylococcus aureus (MRSA) giving is to increased morbidity and mortality. It is now responsible for around 30% or more of all serious infections. There is an increased chance to cause cross infection and MRSA also has the ability to colonize individuals for months and years. The further research also required for detection of methicillin resistant Staphylococcus aureus and diagnosis along with treatment modalities.

DISCLOSURE STATEMENT

This study is investigator initiated. The authors declare that they have no competing/conflict of interests in relation to this article.

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CONSENT FOR PUBLICATION

- Not applicable.
- Ethics approval and consent to participate.
- The study protocol was approved by the medical ethics committee of the Rajasthan University of Health Sciences, Jaipur, Rajasthan (State), India.

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