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Original Research Article

Efficacy of combined phenotypic methods for methicillin-resistant *Staphylococcus aureus* detection and antibiotic susceptibility

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ABSTRACT

Background: The main aim of our study is to demonstrate comparative evaluation of oxacillin disc diffusion (ODD), oxacillin screen agar (OSA), CHROM agar (CA) with cefoxitin disc diffusion (CDD) method for the detection of methicillin-resistant Staphylococcus aureus obtained from various clinical samples.
Methods: This prospective study was conducted to detect methicillin resistance among staphylococcus aureus (MRSA) by four phenotypic methods isolated from various clinical samples received in the Department of microbiology MMIMSR, Mullana.
Results: The data was statistically analyzed, compiled in form of tables, graphs, percentage and test of significance will also be done wherever necessary (using Microsoft Excel, 2008 version) CDD+ODD+OSA+CA proved to be 100% followed by ODD+OSA+CA and CDD+OSA+CA 82.07% and CDD+ODD+OSA 80.1%.
Conclusions: Combined phenotypic methods are better in evaluating and studying MRSA infections in hospitals as compared to tests done in isolation for proper diagnosis and timely treatment of infections.

Keywords: MRSA, Phenotypic methods, Antibiotic sensitivity, Resistance

INTRODUCTION

The rising prevalence of MRSA has emerged to be a serious concern for clinicians and a threat to public health. In 2012, rates exceed 20% in all World health organization (WHO) regions and are above 80% in some regions.[1] India, being endemic the incidences reported to be varying from 6.9% to 81% .[2]

It is a versatile potential microbe which has got a peculiar genetic milieu that enhances their virulence to cause varied particular clinical syndromes.[3]

Polymerase chain reaction (PCR) is a quick and precise means of identification and obviating the need of isolation of bacterial colonies on solid media, however the cost and workload of a PCR exceed the demands of many clinical laboratories especially in developing country like India.[4] Major drawback of oxacillin and cefoxitin disc is the requirement of neutral pH, incubation temperature of 33 ºC-35 ºC, Mueller-Hinton agar or broth infused with 2-4% NaCl and 24-hour incubation time.[5] For clinicians preliminary direction for antibiotic selection is more important than the detection.

A wide range of phenotypic methods are accessible in clinical laboratories. The strains possessing mec gene (classic resistance) are resistant to penicillinase-resistant penicillins (PRPs), such as methicillin, oxacillin and naficillin. These are either homogeneous or

heterogeneous in their expression of resistance. In homogeneous expression, virtually all cells express resistance when tested by standard in vitro tests. However in heterogeneous expression, some cells appear susceptible and others appear resistant.[6]

Recently, performance of Cefoxitin disc is considered to be a gold standard. Cefoxitin is a cephamycin type antibiotic and has been described as an inducer of of the PBP2a-encoding mecAgene.[7] The uniqueness of cefoxitin is it is easy for interpretation and more sensitive for the detection of mecA-mediated resistance. The test can be an alternative to PCR for detection of MRSA in resource limited settings.[8]

Oxacillin agar screening test is a nutritious and selective medium containing peptones for growth, a high salt concentration and Lithium chloride to suppress non-staphylococcal growth.[9] Alike oxacillin disc it too could not detect low expression of resistance or borderline resistant strains, heteroresitant mecA-positive strains.[10]

A novel chromogenic medium named Chrom agar has been introduced recently which easily detects even low level resistant strains as mauve colour colonies after 24 hours of incubation with improved sensitivity and specificity.[11]

CHROM agar, a revolutionary product that is proving to be a major breakthrough decreasing workload of laboratory technologist, cost effective and rapid hence more helpful in infection control.[12]

METHODS

Study approach

The present study was conducted to detect Methicillin resistance among staphylococcus aureus by four phenotypic methods isolated from various clinical samples received in department of microbiology MMIMSR, Mullana for a period of two and half years from May 2015 to January 2018 after a proper ethical clearance from the concerned committee. Proper statistical tools were used and applied to obtain the results from the given data.

Study design

A prospective study was carried out in the department of microbiology, MMIMSR, mullana.

Study population

Study was conducted on 200 staphylococcus aureus isolates from various clinical samples received in the department of microbiology.

Inclusion criteria

Staphylococus aureus isolates from various clinical samples was the inclusion criteria.

Exclusion criteria

Organisms other than *S. aureus was the exclusion criteria*.

Phenotypic methods of detection of MRSA strains are:

Oxacillin disc diffusion method

All strains were tested with 1 µg oxacillin discs on Mueller-Hinton agar plates. For each strain, a bacterial suspension was adjusted to 0.5 McFarland. Zone size was interpreted according to CLSI criteria: susceptible, 13 mm; intermediate, 11-12 mm and resistant 10 mm.[8]

Cefoxitin disc diffusion method

All the isolates were subjected to cefoxitin disc diffusion test using a 30 microgram disc. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture was done on MHA plate. Plates were incubated at 35 °C for 18 h and zone diameters measured. Zone size will be interpretated according to CLSI (2015) criteria ≤21 mm mecA positive (methicillin resistant). ≥22 mm mecA negative (methicillin sensitive).

Oxacillin screen agar test

A bacterial inoculum of each strain was made and turbidity adjusted to 0.5 McFarland. One drop of this suspension will be inoculated on Mueller-Hinton agar containing 4% NaCl and 6 mg oxacillin ml. Any strains showing growth on the plate containing oxacillin were considered to be resistant to methicillin.[21]

CHROMagar

CHROMagar is a new chromogenic medium for the identification of MRSA. For each strain, a bacterial suspension adjusted to 0.5 McFarland will be used. Subsequently, a swab was dipped in the suspension and streaked onto a CHROMagar plate. The growth of any green colony was considered to be positive, indicating MRSA.

Antimicrobial susceptibility testing

Antibiotic susceptibility test was done by the Kirby Bauer Disc diffusion method on Muller Hinton agar and interpretation was made according to CLSI (2015) guidelines. S. aureus ATCC 25923 were used as controls for the antibiotic susceptibility test.

MRSA strains were subjected to the following antimicrobial agents-cotrimoxazole (25 µg),

erythromycin (15 µg), clindamycin (10 µg), ciprofloxacin (30 µg), netilmicin (30 µg), amikacin (10 µg), linezolid (30 µg), vancomycin (30 µg) and dalfopristin/quinpristin (15 µg), tigycycline (15 µg), ceftaroline (30 µg) were be tested.

RESULTS

Table 2 demonstrates predominance of male 72 (67.92% in comparison to female 34 (32.07%).

Table 1: Rate of MRSA from staphylococcus aureus isolates.

Total no. of sample	No. of MRSA isolates	No. of MSSA isolates
200	106 (53%)	94 (47%)

Table 2: Demographic profile of patients with MRSA strains.

Parameters		No. of positive patients
		N (%)
Gender	Male	72 (67.92)
	Female	34 (32.07)
Age	<20	20 (18.86)
	20-40	52 (49.05)
	40-60	22 (20.75)
	>60	12 (11.32)
Residential status	Rural	58 (54.71)
	Urban	48 (45.28)

Table 3: MRSA distribution in various clinical specimens.

Nature of specimen	No. of *S. aureus* isolates	No. of MRSA
		N (%)
Pus	80	55 (68.75)
Sputum	45	25 (55.55)
Urine	20	13 (65)
Blood	40	11 (27.50)
Pleural fluid	15	2 (13.33)
Total	200	106

Table 3 demonstrates maximum number of MRSA was found in pus (68.75%) followed by urine (65%) then sputum (55.55), blood (27.50%) and pleural fluid (13.33%).

Table 4 illustrate sensitivity of MRSA strains-highest to vancomycin (96.2%) followed by other drugs.

Table 6 revealed effect of various phenotypic methods in combination CDD+ODD+OSA+CA proved to be 100% followed by ODD+OSA+CA and CDD+OSA+CA 82.07%and CDD+ODD+OSA 80.1%.

Table 4: Pattern of antibiotic sensitivity in MRSA isolates.

Antimicrobials	MRSA strains (n=106)	MSSA strains (n=94)
	N (%)	N (%)
Cotrimoxazole	78 (73.58)	69 (73.40)
Clindamycin	59 (55.66)	82 (87.23)
Ciprofloxacin	29 (27.35)	68 (72.34)
Amikacin	81 (76.41)	78 (82.97)
Netilmicin	85 (80.18)	90 (95.77)
Cephalexin	14 (13.20)	38 (40.42)
Amoxicillin/clav	10 (9.43)	15 (15.95)
Vancomycin	102 (96.22)	94 (100)
Linezolid	95 (89.62)	93 (98.93)
Dalfopristin	99 (93.39)	-
Ampicillin	5 (4.71)	27 (28.72)
Erythromycin	17 (16.03)	75 (79.78)
Tigecycline	88 (83.01)	-
Ceftaroline	98 (92.45)	-

Table 5: Detection of MRSA by various phenotypic methods.

Total no. MRSA isolates	Disc based methods		Agar based methods	
	Cefoxitin disc diffusion	Oxacillin disc diffusion	Oxacillin screen agar	CHROM agar
	N (%)	N (%)	N (%)	N (%)
106	103 (97.16)	99 (93.39)	85 (80.18)	89 (83.96)

Table 6: effect of various phenotypic methods in combination for detection of MRSA.

Phenotypic methods	No of MRSA isolates
	N (%)
CDD+ODD+OSA+CA	106 (100)
CDD+ODD+OSA	85 (80.18)
ODD+OSA+CA	87 (82.07)
CDD+OSA+CA	87 (82.07)

DISCUSSION

The current study was conducted in the department of microbiology on 200 strains of staphylococus aureus isolates commencing various clinical samples received from IPD and OPD patients of MMIMSR, Mullana, Ambala for the detection of Methicillin Resistance among staph aureus isolates by using four phenotypic methods-cefoxitin disc diffusion method, oxacillin disc diffusion method, oxacillin screen agar and CHROM agar.

Staphylococcus aureus frequently colonizes the human skin and is present in the nose. Nowadays in hospital as well as community it may get replaced by MRSA. Contaminated hands, medical equipment and surfaces in places such as hospitals, clinics, or nursing homes allow the spread of MRSA from colonized or infected patients. In the community, anything that allows for skin-to-skin contact can spread MRSA. Leading to various infection and for the diagnosis it should be isolated from the site of infection.

In present study maximum number of MRSA was found in pus (68.75%) followed by urine (65%) then sputum (55.55), blood (27.50%) and pleural fluid (13.33%). which was in accordance with Jones et al which showed maximum number in pus samples which is 51.22%, urine 28.15% followed by sputum 15.85%.[13] While a study carried out by Tsering et al showed maximum number in sputum 56.5%,urine 45.83% followed by pus 27.05%.[14] In a study carried out Sasirekha et al showed pus 71.89% followed by sputum 8.49%, urine 7.5%.[15] Though there was a variation in samplewise distribution but common feature was pus, urine andsputum were the samples which showed MRSA.

The present study demonstrates sensitivity of MRSA isolates to vacomycin (96.2%) followed by dalfopristine (93.39%), ceftaroline (92.45%), linezolid (89.62%), tigecycline (83.01%), netilmicin (80%), Amikacin (76.41%), cotrimoxazole (73.58%), clindamycin (55.66%), ciprofloxacin (27.35%), erythromycin (16.03%), cephalexin (13.20%), amoxiclav (9.4%), and ampicillin (4.71%).Which was in accordance with the study carried out by Vasuki et al showed all MRSA isolates were susceptible to vancomycin, (100%), linezolid, (100%), tigecycline (100%), cotrimazole (80%), amikacin (73.3%), ciprofloxacin (26.7%), erythromycin (17.8%), coamoxiclav (15.6%) and by Tandra et al 17 showed vancomycin (100%), Netilmicin (81.7%), Amikacin (76%), clindamycin (31.7%), ciproflaxin (30.8%), cotrimazole (24%), erythromycin (5.8%), Ampicillin (0%) by Balamurli et al vancomycin (100%), linezolid (90.9%), amikacin (69.6%), clindamycin (58.3%) respectively and by Renushri et al showed vancomycin (100%), linezolid (100%), netilmicin (100%), clindamycin (75%), cotrimoxazole (35.7%), erythromycin (35.7%), ciprofloxacin(28.6%).[17-19] Newer drugs has also included in present study like Ceftaroline (92.4%) accordance with Gaikwad et al (93.3%) sensitive. Dalfopristin showed (93.39%) sensitivity which was in accordance with Dardi et al (94.44%).in addition cephalexin (13.20%) which was in accordance with Raess et al 13.6%.[20-22]

Proper selection of the antibiotics based on antibiotic susceptibility test is used for effective treatment and prevention of resistance in MRSA and MSSA. The existing study demonstrates MSSA sensitive isolates in the order of vancomycin (98.94%), linezolid (98.93%), netilmicin (95.77%), clindamycin (87.23%), amikacin (82.79%), erythromycin (79.78%), cotrimoxazole (73.40%), ciprofloxacin (72.34%), cephalexin (40.42%), ampicillin (28.72%), amoxiclav (15.95%) which was in accordance with Anjali et al which showed MSSA sensitive isolates in order of linzolid (100%), vancomycin (100%), clindamycin (85%), erythromycin (80%), cotrimoxazole (67%) and ciprofloxacin (61%) and Azher et al showed linzolid (100%), vancomycin (100%), cotrimoxazole (90.2%), clindamycin (84.10%), erythromycin (81.70%), ciprofloxacin (75.60%), and ampicillin (34.10%) and Lahari et al showed clindamycin (90.56%), erythromycin (75%), amikacin (61.1%), ciprofloxacin (48.33%), cotrimoxazole (38.89%) and cephalexin (25%) and Nitish et al showed linzolid (99.03%), netilmicin (96.10%) and amoxyclav (19.30%) and Shilpa et al showed linezolid (100%), vancomycin (100%), amikacin (91.10%), cephalexin (63.70%), ciprofloxacin (60%) and erythromycin (59.50%).[23-27] The antibiotic sensitivity pattern varies from time, place as well even within localized communities (Table 8).

In the present study, the combination of all four methods showed 100% positivity followed by decrease in the rate, as less number of tests were evaluated. The good results were obtained when all four methods were performed together.

Therefore, it was concluded that, all laboratories should include these tests to evaluate the better positivity rate. As per the present study, CDD+ODD+OSA+CA proved to be 100% followed by ODD+OSA+CA and CDD+OSA+CA 82.07% and CDD+ODD+OSA80.1%,

The present study demonstrates predominance of males 72 (67.92% in comparison to females 34 (32.07%) which is in accordance with Bhatt et al which showed 66.99% males and 33.00% females, Veni et al showed males 70% and females 30% and Khyati et al showed males 71.23% and females 28.76%.[28-30] This is because female have XX genotype which makes them less prone to infections and makes their immunity more strong than males.

The present study demonstrates predominance of MRSA in age group of 20-40 years (49.05%) which was in accordance with Hannath et al which showed 52% in age group of 18- 45 years, Sasirekha et al showed 26.19% in 20-30 years age group, Ankur et al 51% in age group of 20-40.[31-33]

The present study showed increased incidence in rural 58 (54.71%) population as compared to urban population 48 (45.28%) which is in accordance with Srinivas et al which showed urban population of 53.46% and rural as 58.80%.[34] The increased, incidence of rural population is because there are less health care facilities, treated usually by quakes. The limitations of the study are small sample size.

CONCLUSION

MRSA is responsible for variety of serious infections which are difficult to treat. Newly acquired methicillin-resistant strains and weakened immune system of patients makes hospital set up more prone to it consequently leads to morbidity. The antibiogram of MRSA is important to select suitable empirical antibiotic treatment in patients. In current study maximum sensitivity was vancomycin (96.2%) dalfopristin (93.39%), ceftaroline (92.45%) and minimum with ampicillin (4.7%). From this study it was concluded that, combined phenotypic methods for the detection of methicillin resistance was found to be more efficacious than a single method used for detection of such resistant strains.

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