Prevalence of MRSA and its Antibiotic Susceptibility Pattern in a Tertiary Care Center,Karimnagar

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Abstract

Background & Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) is a well-recognized public health problem throughout the world.This study was aimed to isolate the Staphylococcus aureus from various clinical specimens, and to study the prevalence of methicillin resistance among Staphylococcus aureus and to evaluate phenotypic and genotypic methods in detection of MRSA and to determine its Aantibiotic susceptibility pattern in MSSA and MRSA isolates. The study was undertaken during November 2018to October 2019.various clinical samples received from the surgical and medical departments are included in the study.

Methods: Total 218 staphylococcus aureus strains were isolated from various clinical samples S. aureus was identified by characteristic growth on blood agar, MacConkey agar, Gram staining and various biochemical tests, e.g. catalase test, free and bound coagulase test, and anaerobic mannitol fermentation[1]. Among them 48(22.01%) were MRSA positive 170(77.9%) were MSSA

Conclusion: Early identification of MRSA is an important step toward timely implementation of appropriate treatment. The prevalence of resistance is bound to increase with increased irrational use of antibiotics. Robust antimicrobial stewardship and strengthened infection control measures are required to prevent spread and reduce emergence of resistance.

Keywords*:MRSA, MSSA, Vancomycin, Teicoplanin, Antimicrobial Susceptibility.*

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) strains or multidrug-resistant S. aureus, initially described in 1960s, emerged in the last decade as a cause of nosocomial infections responsible for rapidly progressive, potential fatal diseases including life-threatening pneumonia, necrotizingfasciitis, endocarditis, osteomyelitis, severe sepsis, andtoxinoses such as toxic shock syndrome[1].

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MRSA is now a common cause of serious hospital-acquired infections. MRSA can be clinically grouped as community-associated (CA-MRSA) or hospital/ healthcare-associated (HA-MRSA).[2]At present, healthcare-associated methicillin-resistant S.aureus (HA-MRSA) is associated with significant mortality and morbidity (longer hospital stays) and imposes a serious economic burden on scarce healthcare resources worldwide[1].

In India, high rates of MRSA have been reported in clinical isolates from various studies, with rates as high as 54.8% (ranging between 32% and 80% among the S. aureus pool)[3]. Methicillin resistance is due to the production ofpenicillin binding protein (PBP) PBP2a which has less affinity to beta lactam drugs and it is mediated by mecAgenes. In India reported MRSA

incidence varies from29.10% to 54.9%.[5]

Materials and Methods

The study was conducted during the period of november 2018 to october 2019. a total of 218 strains of S. aureus, isolated consecutively from various clinical samples in the Department of Microbiology, Chalmeda Anand Rao Institute of Medical Sciences & Hospital, Karimnagar, Telangana,India, was evaluated. The clinical samples from which the strains were isolated were wound swab, urine,catheter tip,blood,sputumand stool. S. aureus was identified by characteristic growth on Blood agar, MacConkey agar, Gram staining and various biochemical tests, e.g. catalase test, free and bound coagulase test, and anaerobic mannitol fermentation[4]

Screening of MRSA was done using cefoxitin disc (30μg) as per CLSI recommended disc diffusion method. 0.5 McFarland standard inoculum of Staphylococcus aureus were inoculated on Muller hinton agar and Cefoxitin disc was placed on it and plates were incubated at 35°c for 18 hrs. Zone diameter of ≤21 mm was considered as methicillin resistance and ≥22 mm was reported as sensitive.[5]

Determination of Minimum Inhibitory Concentrations (MICs) of OxacillinMICs of oxacillin (Table 1) for all isolates of Staphylococcus aureus were determined by broth microdilution method as described by Andrews and CLSI M07-A9 guidelines. The results were interpreted according to CLSI guidelines. The serial dilutions of oxacillin concentrations were made from 0.0125 μg/mL to 128 μg/mL[6]

Detection of *mecA*gene: Extraction of DNA was performed by CTAB (Cetyltrimethylammonium bromide) method mecA gene was detected by usingThe primer mecAF (5′-actgctactcaccctcaaac-3′) and the reverse primer mecAR (5′-ctggtgaagttgtaatctgg-3′) supplied by takara emerald master mix kit(7). Antimicrobial susceptibility testing.:The Kirby Bauer disc diffusion method was used routinely to detect the sensitivity of all S. aureus isolates and interpretations were made according to CLSI guidelines. All discs were supplied by Hi-Media. For MRSA, Cotrimoxazole (25 μg), Erythromycin (15 μg), Clindamycin (10 μg),Chloramphenicol (30μg),Gentamicin (10 μgm), Ciprofloxacin (30 μg),Amikacin (10 μg), Linezolid (30 μg), Vancomycin (30 μg) and were tested. For MSSA, the same antibiotics as for MRSA were used, as well as ampicillin (10 μg), and Amoxicillin/Clavulanic acid (30 μg).

Results

Among 7224 samples processed during the study, 218 showed culture positivity for S.aureus. Out of 218 S. aureus, 48 isolates were MRSA by cefoxitin disc diffusion method, confirmed by oxacillin broth dilution MIC method and mecA gene was detected by PCR in all the isolates (Table1)31(64.5%) of MRSA isolates were from male patients and 17(35.41%) from female patients, Highest number of staphylococcus aureus were recovered from pus samples136(62.38%) followed by urine 35(16.05%) ,catheter tip21(9.6%),blood11(5.04%), sputum7(3.2%), BAL 5(2.2%), stool 3(1.3%)Most of the MRSA isolateswere resistant to commonly prescribed antibiotics,All the isolates of MRSA(48) andMSSA (170) exhibited 100% sensitivity to Tigecycline.MRSA isolates exhibited highest resistance compared to MSSA isolates.MRSA isolates exhibit 100% resistance to Ampicillin followed by Ciprofloxacin(79.16%), Erythromycin(77.8%), Chloramphenicol(75%), Gentamicin (58.3%). Of the 35 isolates from urine tested for Nitrofurantoin and Nalidixic acid MSSA and MRSA exhibited 20(57.14%) and (42.85%) respectively.

Table 1: Comparison of the phenotypic and genotypic methods for detection of MRSA.

Cefoxitin disc diffusion	oxacillin broth microdilution	Polymerase chain reaction
Strains of S. aureus having zone of inhibition of ≤21 mm to cefoxitin disc (30 μg) (48)	Strains of S. aureus having oxacillin MIC of ≥4 μg/mL (48)	Strains of S.aureus harboringmecA gene (48)

Table 2: Specimen wise distribution of staphylococcus aureus

Specimen	Sample number	Percentage
Wound swab	136	62.38%
Urine	35	16.05%
Catheter tip	21	9.6%
Blood	11	5.04%
Sputum	7	3.2%
BAL	5	2.2%
Stool	3	1.3%

Table 3:Antibiotic susceptibility results of *Staphylococcus aureus* Nov 2018- Oct 2019

Antibiotic	MSSA(170) No of smplesResistant(%		MRSA(48) No of samples Resistant(%)	
Erythromycin	45	26.47	37	77.08
Clindamycin	24	14.11	22	45.83
Chloramphenicol	126	74.11	36	75
Gentamicin	30	17.64	28	58.33
Co-trimaxazole	46	27.05	26	54.16
Ciprofloxacin	79	46.47	38	79.16
Vancomycin		0	3	6.25
Linezolid	0	0	2	4.16
Amoxyclav	74	43.52	34	70.83
Teicoplanin	0	0	1	2.08
Tigecycline	0	0	0	0
ampicillin	163	95.83	48	100

Discussion

This study was designed to determine the occurrence of MRSA in S. aureus isolates from various clinical samples The overall MRSA prevalence in our study was 48 samples (22.01%) our study correlated with other studies according to sader HS et al the prevalence of MRSA was 22.5%[7]. According to Mehta et al, verma et al ,velasco et al the prevalence of MRSA may varies between 20 and 40%.[8,9,10,]. In India, high rates of MRSA have been reported in clinical isolates from various studies, with rates as high as 54.8% (ranging between 32% and 80% among the S. aureus pool)[4].

In this study staphylococcus aureus was predominantly isolated from pus sample(62.38%) which is inaccordance with other studies[11,12,13,]followed by urine(16.05%),catheter tip(9.6%), Blood (5.04%), sputum (3.2%), BAL(2.2%), and stool (1.3%)Similar higher rate of isolation of S.aureus from pus were reported by Rashmi et al. &Jeydev Pandya et al.[14,15] The predominance in pus could be due to exposure of wound to microorganism in the environment and S.aureus present on skin as commensal makes the wound more prone for infection· MRSA is considered in opportunistic human pathogen with demonstrated capacity to aquire more resistance to nearly overall antibiotics used to treat it.Vancomycin is the drug of choice for treatment of MRSA cases in our study we reported 2(4.1%) vancomycin resistance similar findings(3.92%) are reported by jyothi kumari et al from manipal[16]

In our study we reported 1(2.08%)in contrast to Harcharan Singh et al fom rajasthan reported 12% of linezolid resistance[17] In contrast to Shariq et al from pakistan, Priya Datta et al from chandighar reported 100% sensitivity to linezolid[18,19]All MRSA isolates showed high level of resistance to commonly prescribed antibiotics than MSSA in we observed 100% to ampicillin and ciprofloxacin(79.1%) Similar findigs were observed by avinash et al who observed 100% resistance to ampicillin and 81.3% restant to amoxyclav[20]

Conclusion

This study reveals Cefoxitin disc diffusion method gives promising results compared with Oxacillin agar dilution method and mecA gene detection by PCR. Tigecycline, Teicoplanin and Linezolid found to be one of the drug of choice for MRSA infection these are effective antibiotics and thus should not be misused and should preserved for future decades in preventing the development of drug resistance. Hence it is necessary to establish an antimicrobial susceptibility surveillance system and to improve current infection control programs in health care institutes to prevent the spread of resistant microorganisms.

Conflicts of Interest: NIL

Source of funding: Self

Ethical Clearance: Clearance taken from 'ChalmedaAnand Rao Institute of Medical sciences ethical Committee'.

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