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A Study of Antimicrobial Resistance Pattern of Methicillin Resistance Staphylococcus Aureus in Hospitalized Patients.

Mudda Rajesh[1], M. F. Aleemullah[2], A. Karthik[3]

[1]Assistant Professor, Department of Microbiology, Madha Medical College, Kovur, Chennai.
[2]Associate Professor, Department of Microbiology, Madha Medical College, Kovur, Chennai.
[3]Tutor, Department of Microbiology, Madha Medical College, Kovur, Chennai.

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ABSTRACT

Background: Many hospitals struggle with increasing amounts of MRSA, which are "multi-resistant" against all β-lactam antibiotics. Often, applicable antibiotics for treatment are only glycopeptides like Vancomycin and Teicoplanin. We are here tried to study the prevalence rate of MRSA among clinical S.aureus isolates by Kirby Bauer Disk Diffusion method, agar dilution method using cefoxitin disc and its antibiotic resistance pattern. **Methods:** All clinical samples (751) collected from February 2017 to April 2018 were processed at Microbiology department and Staphylococcus aureus isolates were identified as per the standard bacteriological techniques, MRSA detected using cefoxitin disc diffusion and agar dilution techniques according to CLSI guidelines. **Results:** Out of 751 clinical indicated samples 110 (14.6%) staphylococcus aureus organisms were isolated, among these 56 (50.9%) were detected as Methicillin resistant Staphylococcus aureus (MRSA). All 56 MRSA isolates diagnosed by cefoxitin disc diffusion test were inhibited by >4mg/L i.e., they were between 8 and 256 mg/L. Almost 80.3% of isolates had an MIC between 128 and 256 mg/L. **Conclusion:** Outbreaks of MRSA must be effectively controlled with immediate screening of all health care personnel in that area. Both cefoxitin disc testing and 4 mg/L cefoxitin in agar appear to be very accurate and easy to perform methods for detecting MRSA isolates.

Keywords: Antibiotic resistance, MRSA.

INTRODUCTION

Staphylococci-related infections are one of the most common causes of nosocomial (hospital-acquired) infections, yet they are increasingly difficult to treat due to the rate at which bacteria acquire antibiotic resistance. Staphylococci rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems.

Healthcare-associated Methicillin-resistant S. aureus (MRSA) is a major cause of nosocomial infections worldwide, with significant attributable morbidity and mortality in addition to pronounce healthcare costs.[1] Many hospitals struggle with increasing amounts of MRSA, which are "multi-resistant" against all β-lactam antibiotics. Often, applicable antibiotics for treatment are only glycopeptides like Vancomycin and Teicoplanin. The development of MRSA results when the strain of methicillin-susceptible S.aureus (MSSA) acquires a large genetic element, known as Staphylococcus cassette chromosome mec-SCCmec.[2,3] MRSA became a leading cause of nosocomial infections worldwide and later became endemic in United States hospitals.[4]

The transmission of MRSA within and between healthcare facilities has been documented very well using molecular typing techniques. Outbreaks involving clonal spread within single facilities has been frequently reported.[5] Various studies have been conducted on the detection of mecA gene and its comparison with Oxacillin resistance on disc diffusion and MIC. Antimicrobial resistance in health care-associated pathogens is a growing concern for health care and for public health.

We are here tried to study the prevalence rate of MRSA among clinical S.aureus isolates by Kirby Bauer Disk Diffusion method, agar dilution method using cefoxitin disc and its antibiotic resistance pattern.

Name & Address of Corresponding Author
Dr. Mudda Rajesh,
Assistant Professor,
Department of Microbiology,
Madha Medical College,
Kovur, Chennai.

MATERIALS AND METHODS

The study of Prevalence of Methicillin resistant Staphylococcus aureus (MRSA) from various clinical specimens was conducted in the microbiology department of Madha Medical College over a period of one year two months from February 2017 to April 2018.

Various clinical specimens viz. Pus and wound swabs, blood, urine, CSF, ascitic and pleural fluids,

sputum and other respiratory samples, intravascular devices and catheters etc from patients of different clinical specialties were included in this study.

All clinical samples (751) collected were processed and Staphylococcus aureus isolates were identified as per the standard bacteriological techniques. All clinical samples after receiving to Microbiology department, immediately inoculated in basal media such as Nutrient agar, Macconkey agar, Blood agar, Brain Heart Infusion Broth and selective media including mannitol salt agar DNAse agar. All Media plates after inoculation were incubated for 24 hours at 370C. Sterile precautions have taken while processing samples.

After 24 hours, Staphylococcus aureus was identified from samples by colony characteristics such as golden yellow appearance on nutrient agar, pale lactose fementer colonies on Macconkey agar and yellow color colonies on mannitol salt agar indicates mannitol fermented, whereas clearing around the colony in DNAse agar indicates production of thermostable nuclease enzyme. Characteristic features such as DNAse agar clearing, mannitol fermentation, tube coagulase positive, urease utilization, catalase positive were demonstrated to confirm as Staphylococcus aureus

Antibiotic susceptibility testing (Modified Kirby – Bauer's Method) was done to all staphylococus aureus isolates and MRSA was screened using 30 µg cefoxitin disc on Mueller Hinton agar, incubated at 370C for16-18 hours according to CLSI guidelines. Isolates with Zone of Inhibition of cefoxitin disc ≤21 mm were considered as mecA positive (MRSA) and ≥22mm were considered as mecA negative (MSSA). Cefoxitin agar dilution was performed using oxoid Mueller hinton agar, incubated at 370C for 16-20 hours. Staphylococcus aureus isolates inhibited at MIC concentration of >4µg/mL were considered as mecA gene positive (MRSA) and isolates requiring ≤4µg/mL of cefoxitin were grouped under mecA gene negative (MSSA).[6] Data entered into excel sheet, analyzed and tabulated.

RESULTS

Table 1: Distribution of Staphylococcus aureus in various clinical specimens.

Clinical specimen	Total no. of clinical specimens	Staphylococcus aureus	Percentage
Pus/ wound swab	316	66	20.8
Urine	264	32	12.1
Blood	41	2	4.8
Respiratory samples	27	3	11.1
Fluids (CSF, Ascitic fluid, Pleural fluid)	39	1	2.5
Miscellaneous *	64	6	9.3
Total	751	110	14.6

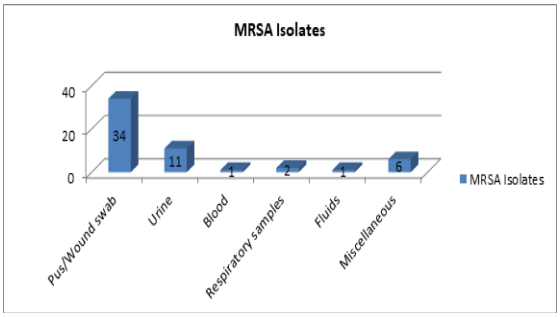

Figure 1: shows the distribution of MRSA among Staphylococcal isolates from different clinical specimens.

Table 2: Antibiotic susceptibility pattern of MRSA isolates.

Antibiotics	Symbol	Disc potency	Total MRSA isolates	No. of sensitive strains (%)	No. of resistant strains (%)
Penicillin G	P	10 Units	56	5 (8.9%)	51 (91%)
Amikacin	AK	30µg	56	35 (62.5%)	21 (37.5%)
Ciprofloxacin	CIP	5 µg	56	31 (55.3%)	25 (44.6%)
Cefoxitin	CX	30 µg	56	0 (100%)	56(100%)
Rifampicin	RIF	5 µg	56	48 (85.7%)	11 (19.6%)
Teicoplanin	TEI	30 µg	56	56 (100%)	0%
Vancomycin	VA	30 µg	56	56 (100%)	0%
Clindamycin	CD	2 µg	56	42 (75%)	14 (25%)
Linezolid	LZ	30 µg	56	48 (85.7%)	11 (19.6%)

Staphylococcus aureus was isolated from various clinical samples such as Pus/Swab, urine, blood, respiratory samples, fluids and others. Out of 751 clinical indicated samples 110 (14.6%) staphylococcus aureus organisms were isolated. Staphylococcus aureus was predominantly isolated from pus/swab samples i.e, 20.8% followed by urine (12.1%) and respiratory samples (11.1%) [Table 1].

*Biopsy specimens, Granulation tissue, Bone pieces, Cervical and high vaginal swabs, fine needle aspirates, Drained tips, Foley's catheter tips, Ear swabs etc.

Out of 110 Staphylococcus aureus isolated from various clinical samples, 56 (50.9%) were detected as Methicillin resistant Staphylococcus aureus (MRSA) using cefoxitin disc diffusion according to CLSI guidelines. 66.6% MRSA isolates observed in respiratory samples, 51.5% were from pus/swab, 34.3% were from urine samples. 50% of Staphylococcus aureus isolates from blood was

MRSA. All isolates (100%) from fluids, miscellaneous samples were MRSA [Figure 1].

Out of the 56 MRSA isolates, 31 were from male patients (i.e., 55.3%) and 25 isolated were from female patients (i.e., 44.6%). The male to female ratio was not statically significant.

Age – wise distribution of the patients with MRSA infections showed 96.4% of isolates were from the age group of more than 10 years and only 3.5% were from patients <10 years of age. Majority of MRSA were isolated from >50 years of age group patients i.e., 33.9% followed by 21-30 years(25%), 31-40 years (14.2%) and 41-50 years age group(14.2%).

Antibiotic sensitivity pattern of MRSA isolates showed 8.9% were sensitive to penicillin, 55.3% were sensitive to ciprofloxacin, 62.5% were sensitive to amikacin, 75% were sensitive to clindamycin, 85.7% were sensitive to linezolid, rifampicin, 100% isolates were sensitive to teicoplanin and vancomycin [Table 2].

Cefoxitin agar dilution testing showed 1 Staphylococcus aureus isolate were inhibited at concentration of 8 mg/L. All 56 MRSA isolates diagnosed by cefoxitin disc diffusion test were inhibited by >4mg/L i.e., they were between 8 and 256 mg/L. Almost 80.3% of isolates had an MIC between 128 and 256 mg/L [Table 3].

Table 3: Cefoxitin Agar Dilution testing of all S.aureus isolates.

Concentration of Cefoxitin (mg/L)	Number of MRSA isolates Resistant	Number of MRSA isolates Sensitive
0.25	00	6
0.5	00	9
1	00	10
2	00	14
4	00	15
8	1	00
16	2	00
32	2	00
64	6	00
128	21	00
256	24	00

DISCUSSION

Tests that define resistance based on detection of mecA are newer alternatives to routine susceptibility testing for identification of MRSA. The mecA gene is highly conserved and the resistance associated with this gene and its PBP2a product is known to be clinically relevant. DNA detection method depends upon whether or not the mecA gene is present and not on phenotype thus completely avoiding the problems of heterogenous resistance. Therefore, detection of mecA by PCR remains the gold standard for detection of MRSA. However PCR is not available in most laboratory and test costs are relatively high.[7]

In this study, out of 751 clinical indicated samples 110 (14.6%) staphylococcus aureus organisms were isolated. Mehta et al in 1998 reported a high prevalence of Staphylococcus aureus (20.65%) from 26,261 clinical specimens in five year study.[8] The MRSA Surveillance Study Group in 1997 studied a total of 13,610 various clinical specimens across three centers in India and reported a prevalence of 5.42% of Staphylococcus aureus.[9] Pal and Ayyagiri reported a low prevalence of Staphylococcus aureus. They isolated 1770 isolates of Staphylococcus aureus from 91,367 different clinical specimens i.e. only 1.93% in the study conducted in 1991.[10]

Out of 110 Staphylococcus aureus isolated from various clinical samples, 56 (50.9%) were detected as Methicillin resistant Staphylococcus aureus (MRSA) as per this study. Distribution of MRSA strains differ from study to study from various parts of the world and depending on the researcher.

Methicillin resistant strains accounted for less than 0.1% of all Staphylococcus aureus in the 1960s, when methicillin first became available for the treatment of penicillinase producing Staphylococcus aureus infections. Since then the prevalence of MRSA has continued to rise with each passing year.[11] Kono and Arakawa in 1995, reported a prevalence of 41.4% MRSA from Fuluoka, Japan.[12] Hsu et al reported a prevalence of 49% MRSA in 1998 from Chicago.[13] Hanumanthappa in 2003 has reported 43% resistance methicillin in a study conducted at Karnataka, India.[14] The MRSA Surveillance Study Group in 1997 reported 31.8% methicillin resistance in their study conducted across three centres in India.[9] In a study of Staphylococcus aureus strains submitted to the Indian National Reference Laboratory at Maulana Azad Medical College, New Delhi, it was found that prevalence of MRSA has increased from 9.83% in 1988 to 45.44% in 1992.[15]

As per this study, antibiotic sensitivity pattern of MRSA isolates showed 8.9% were sensitive to penicillin, 55.3% were sensitive to ciprofloxacin, 62.5% were sensitive to amikacin, 75% were sensitive to clindamycin, 85.7% were sensitive to linezolid, rifampicin, 100% isolates were sensitive to teicoplanin and vancomycin. Study by Mahmood K et al and D Majumder et al reported penicillin G resistant as 92% and 94.1% respectively.[16,17]

In a study by Maple et al, resistance to aminoglycosides was encountered in more than 90% of MRSA strains.[18] E Marais et al stated that 29% of MRSA isolates were Amikacin resistance.[19] Vidya Pai et al observed <30% of resistance for Amikacin,[20] whereas Samia Perwaiz et al observed 54% of resistance.[21] S Anupurba et al noted 60.5% of resistance to Amikacin.[22] High level of resistance pattern to Ciprofloxacin was observed by Simon W J et al and S Anupurba et al,[22,23] which is 91.4% and 84.1% respectively whereas Vidya Pai et al and D Majumder et al observed <30% and 22.8% of resistance pattern respectively.[17,20]

High level of resistance pattern to Clindamycin was observed by Simon W J et al (87.9%), Samia Perwaiz et al (90%), Faiz Idrees et al (79%).[21,23,24] Compared to our study, E Marais et al and Faiz idrees et al reported higher percentage of rifampicin resistance, which is 38% and 50% respectively.[19,24] In Mahmood K et al and Samia Perwaiz et al study resistance to Teicoplanin was 0%.[16,21] High level of resistance pattern to Teicoplanin was observed in Arakawa soichi et al study which is 31.2%.[25] Jorge cepeda et al observed 10.2% resistance.[26]

In the present study, Cefoxitin agar dilution testing showed 1 Staphylococcus aureus isolate were inhibited at concentration of 8 mg/L. All 56 MRSA isolates diagnosed by cefoxitin disc diffusion test were inhibited by >4mg/L i.e., they were between 8 and 256 mg/L. Almost 80.3% of isolates had an MIC between 128 and 256 mg/L.

Perez et al,[27] evaluated the agar dilution test of cefoxitin and oxacillin for detection of MRSA obtained with break points of 4 mg/L for oxacillin and 8 mg/L for cefoxitin, also suggested either cefoxitin or oxacillin can use for detection of MRSA.

Coban AY et al,[28] documented that sensitivity and specificity of cefoxitin disc diffusion method was detected as 100%, when comparing with both gold standard (PBP2a presence) method and oxacillin both microdilution and agar screening methods.

Clarence J Fernandes et al,[29] studied that all 575 strains of MSSA were inhibited by cefoxitin at concentrations of ≤ 4 mg/L. Among 95% of MRSA strains had an MIC between 128 and 256 mg/L. MRSA strains which are not multidrug resistant were ranged between 8 and 256 mg/L.

Both cefoxitin disc testing and 4 mg/L cefoxitin in agar appear to be very accurate and easy to perform methods for detecting MRSA isolates. Cefoxitin disc diffusion test is a reliable test to investigate MRSA in routine laboratories where it is cumbersome to perform agar dilution on daily basis and molecular tests are not available.

CONCLUSION

Antibiotic resistance pathogens constitute an important and growing threat to the public health. Many hospitals struggle with increasing amounts of MRSA, which are "multi-resistant" against all β-lactam antibiotics. The number of hospitals affected by these strains has increased significantly in the past five years. MRSA has a propensity to spread via transient hand carriage from person to person and colonize rapidly and therefore necessitates immediate infection control measures like hand washing, use of personal protective equipment, barrier nursing and strict implementation of hospital hygiene practices. Outbreaks of MRSA must be effectively controlled with immediate screening of all health care personnel in that area.

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