Antimicrobial Susceptibility Profile of Methicillin Resistant *Staphylococcus Aureus* (MRSA) Isolates in a Tertiary Care Hospital, Mysuru, India

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How to cite this article: Eeshita Dhar, Tejashree Anantharaj Urs, Krishna Karthik Manthravadi. Antimicrobial Susceptibility Profile of Methicillin Resistant Staphylococcus Aureus (MRSA) Isolates in a Tertiary Care Hospital, Mysuru, India. Indian Journal of Public Health Research and Development 2023;14(2).

Abstract

Background: MRSA strains are the most common causes of community and hospital acquired infections. Antimicrobial medications have become a significant issue in the healthcare sector as a result of the increased death rate from Staphylococcus aureus infections, the appearance of methicillin-resistant and other multi-drug resistant strains. The study's goals and objectives were determining the prevalence of MRSA and assessing the antibiotic efficacy of MRSA isolates from various clinical samples.

Materials and Methods: A total of 395 MRSA were isolated from various clinical specimens and identified by using standard microbiological techniques at tertiary care hospital of Mysore, South India. Methicillin resistance was determined by standard Kirby-Bauer disc diffusion test using cefoxitin 30µg disk.

Antimicrobial resistance patterns were determined by automated Vitek2 system.

Results: A total of 246 (62.27%) isolates were identified as MRSA out of 395 S. aureus isolates collected from various clinical samples such as pus samples (86.17%), blood (3.65%), Et swabs (3.65%), ear swabs (2.84%), sputum (1.6%), urine (0.81%), and other sterile body fluids (1.21%). All MRSA isolates were susceptible (100%) to vancomycin, linezolid and daptomycin followed by other antibiotics like rifampicin (99%), tigecycline (96.74%), tetracycline (95.93%), teicoplanin (95.5%), gentamicin (73.17%). Most of the MRSA isolates were resistant to oxacillin (97.15%), trimethoprim/sulfamethoxazole (95.12%), Levofloxacin (93.49%), ciprofloxacin (92.68%), clindamycin (87.80%), erythromycin (63.41%).

Conclusion: In our study, we found MRSA isolates were susceptible to most active and reliable routinely used antibiotics. Good infection control procedures like thorough hand washing, identifying and treating MRSA carriers, and prudent use of antimicrobial medicines are advised to prevent the formation of drug-resistant isolates. In addition to the Vitek 2 approach, we may conclude that cefoxitin disc diffusion is an essential test to diagnose MRSA.

Keywords: Antibiotic resistance, Methicillin resistant Staphylococcus aureus, Cefoxitin.

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Introduction

MRSA has become one of the most significant multi-resistant pathogen worldwide, in hospitals and community, shortly after the introduction of methicillin in 1961.[1] A wide range of antibiotics are used to treat staphylococcal infection including penicillin, cephalosporin, macrolide, fluoroquinolone and glycopeptide group of antibiotics.[2]

In early nineties, the major treatment available to combat this organism was penicillin. Over the past 50 years, S. aureus has undergone genetic modification that has resulted in antibiotic-resistant strains. Resistance to these drugs occurs because of the acquisition of genes that encode drug inactivating enzymes, initially known as penicillinase and now called β-lactamase that hydrolyse the penicillin.

Resistance to oxacillin is mediated by penicillin-binding protein, in short PBP2a, with low affinity to β-lactams, encoded primarily by the mecA gene and are clinically resistant to all available β-lactams. [3] Isolates containing the PBP2a-mediated resistance mechanism are clinically resistant to all available β-Lactams. PBP2a is capable of substituting the biosynthetic functions of the normal PBPs even in the presence of the β-lactams and preventing cell lysis. [4,5]

MRSA strains associated with community infection are usually resistant to beta-lactams but susceptible to other antimicrobial classes, where, hospital associated MRSA strains are usually resistant to all classes of antibiotics.[6] These methicillin resistance staphylococci possesses a great challenge to the clinicians as these microbes are also resistant to other higher β-lactam group of antibiotics and have a tremendous impact on the morbidity, mortality and cost of hospitalization.

Therefore it is important to restrict the spread of MRSA.[7]

To control the spread of MRSA in the human population requires the identification of MRSA isolates. S. aureus strains harboring mecA gene shows higher MIC to oxacillin than those strains carrying other mec-gene (mecC or mecB gene). Susceptibility testing studies have shown that some MRSA typically tests as cefoxitin resistant but oxacillin susceptible.[3] As per CLSI, standardized methods need incubation at temperatures not greater than 35^0C and require reading to be obtained after a full 24hr of incubation for MIC tests and after 16–18hr for cefoxitin-based tests. [8, 9] Disc diffusion method requires 16-18 hrs incubation which is a disadvantage, where Vitek can classify the isolate within 8hrs and also combines an oxacillin screen and MICs to detect MRSA or MSSA. [10]

Materials and Methods

This study was conducted on 395 S. aureus isolates collected in the department of microbiology from various clinical samples that were received for routine diagnosis of culture and sensitivity. Clinical samples that were included in the study were pus, blood, endotracheal aspirates, ear swabs, sputum, urine, and other sterile body fluids, from all age groups and both the sexes.

Samples were processed and identified by standard microbiological methods and also by Vitek 2 system. In brief, the clinical samples were inoculated on to Blood agar and Mac Conkey agar for isolation of the pathogens. Those samples yielded the growth of Staphylococcus aureus that were identified by standard procedures like catalase test, coagulase test and Vitek 2 ID were further included for the study. Resistance patterns of the isolates were documented from Vitek 2 system.

Methicillin resistance was detected by disc diffusion method (Kirby Bauer) using cefoxitin(30µg) and oxacillin(1µg) disk. S. aureus isolates were lawn cultured onto Muller Hinton agar and the plates were incubated at 37°c for overnight. Strain with zones of inhibition ≤21mm on MHA around cefoxitin

30µg (HiMedia) disk and ≤ 10mm around Oxacillin disk was considered as MRSA, as per CLSI guidelines 2021.

Results

Among 395 S. aureus isolates collected from various clinical specimens such as pus samples (n=212;86.17%) followed by blood (n=9;3.65%), Et swabs (n=9;3.65%), ear swabs (n=7;2.84%), sputum (n=4;1.6%), urine (n=2;0.81%), and other sterile body fluids (n=3;1.21%), 246 (62.27%) isolates were

identified as methicillin resistant by cefoxitin disc diffusion method. Disk diffusion method showed only 187(76.16%) were resistant to both cefoxitin and oxacillin disk, out of 246 (62.27%) MRSA isolates. 36 (14.63%) isolates were oxacillin sensitive and 23 (9.34%) isolates were oxacillin intermediate sensitive (Table 1).

All the 246 MRSA isolates detected by cefoxitin disc diffusion method were resistant to oxacillin (97.15%), Trimethoprim/sulfamethoxazole (95.12%), Levofloxacin (93.49%), ciprofloxacin (92.68%), clindamycin (87.80%), erythromycin (63.41%) and gentamicin (21.54%) respectively by Vitek 2 system. All MRSA isolates were susceptible (100%) to vancomycin, linezolid and daptomycin followed by other antibiotics such as rifampicin (99%), tigecycline (96.74%), tetracycline (95.93%), teicoplanin (95.5%), gentamycin (73.17%). (Table 2)

Table 1: Showing Cefoxitin and Oxacillin susceptibility testing of MRSA isolates.

Method	Resistant	Intermediate	Sensitive
Cefoxitin disc (30mg)	246 (62.27%)	-	149 (37.72%)
Oxacillin disc (1mg)	187 (76.16%)	23 (9.34%)	36 (14.63%)
Vitek 2 System	395	-	-

Table 2: Antimicrobial susceptibility profile of MRSA isolates by Vitek 2 system.

Antibiotics	Resistant	Intermediate	Sensitive
Ciprofloxacin	228 (92.68%)	3 (1.21%)	15 (6.09)
Clindamycin	216 (87.80%)	-	30 (12.2%)
Erythromycin	156 (63.41%)	16 (6.5%)	74 (30%)
Oxacillin	239 (97.15%)	-	7 (2.85%)
Levofloxacin	230 (93.49%)	-	16 (6.51%)
Linezolid	-	-	246 (100%)
Rifampicin	2 (1%)	-	244 (99%)
Teicoplanin	11 (4.9%)	-	235 (95.5%)
Tetracycline	10 (4.7%)	-	236 (95.93%)
Tigecycline	8(3.25%)	-	238(96.74%)
Trimethoprim/ Sulfamethoxazole	234 (95.12%)	-	12 (4.88%)
Vancomycin	-	-	246 (100%)
Gentamicin	53 (21.54%)	13 (5.29%)	180 (73.17%)
Daptomycin	-	-	246 (100%)

Discussion

Staphylococcus aureus is currently a significant health concern for the general public. The current study was confined to prevalence and antibiogram of MRSA isolates. In this study, total 395 samples were detected as MRSA by Vitek 2 system, out of which 62.27% (n=246) isolates were resistant to cefoxitin. Total of 76.16% (n=187) isolates were resistant by both cefoxitin and oxacillin by disk diffusion method, which is a great variation with vitek2 system result. A similar study by Sandrine Roisin et al., found the overall sensitivities for oxacillin resistance detection were 97.5% for the Vitek 2 automated system, and 99.6% for 30µg cefoxitin disks diffusion method.[11]

Our study showed 76.16% (n=187) MRSA isolates were resistant to cefoxitin and oxacillin both and 23.9% (n=59) of the MRSA isolates were cefoxitin resistant but oxacillin sensitive, similar result reported by Liu J-L et al., where 95.49% MRSA isolates were resistant to both cefoxitin disk diffusion and oxacillin broth micro-dilution method and 2.91% were cefoxitin resistant but oxacillin sensitive.[12]

In a large-sample study by Nicole M. Broekema et al., also reported cefoxitin sensitivity and specificity compared to those of oxacillin were 97.3% and 100%, respectively.[13] Cefoxitin disc diffusion results are easier to interpret than oxacillin results due to the frequent hazy oxacillin zones, which are commonly misinterpreted as evidence of oxacillin susceptibility. Cefoxitin is more sensitive for the detection of resistance as it is a better inducer of PBP-2a encoding mec-A gene. In the present study total 62.27% (n=246) MRSA isolates were detected by both disc diffusion and Vitek2 system, which is similar to the study of V. Vasuki et al., Sapkota et al., and Singh et al., where prevalence of MRSA was 54.2% (n=45), 70.6% (n=94), and 53.6% (n=180) respectively. [14,15] MRSA isolates processed by Vitek 2 system were 100% susceptible to Vancomycin, linezolid and daptomycin in the present study which is very much similar to the study of V. Vasuki et al., where all the MRSA isolates were susceptible to vancomycin, teicoplanin, tigecycline and linezolid. [14] Among other antibiotics rifampicin (99%), tigecycline (96.74%), tetracycline (95.93%), teicoplanin (95.5%), gentamicin (73.17%) were also active against MRSA isolates. A number of earlier reports from the Indian subcontinent and foreign groups also can be compared to the current study where susceptible to tetracycline reported as 86.2% by Brown et al., in 2007, 87.2% by Adhikari et al., in 2017, 79.3% by Raut et al., in 2017, and 92% by Sanjana et al., in 2010.[16-19] Gentamicin susceptibility reported as 58.15% by Rajaduraipandi et al., in 2006, 73.3% by Khanal et al. in 2018 and 69% by Sanjana et al., in 2010, in their studies.[19-21]

The rapid evolution of antibiotic resistance in S. aureus is of considerable concern. In the current study all the 246 MRSA isolates detected by Vitek 2 system were resistant to oxacillin (Ox97.15%), trimethoprim/sulfamethoxazole (95.12%), Levofloxacin (L-93.49%), ciprofloxacin (Cip92.68%), clindamycin (Cd-87.80%), erythromycin (E-63.41%), which indicate the emergence of multiple drug resistant S. aureus strains. Antibiotic-resistant pattern of all the MRSA isolates according to vitek 2 method can be compared to a various national and international studies. The high resistant pattern of oxacillin (97.15%) can be compared to Khadri et al., Suzanne et al., and Bala et al., (100%).[22-24] In contrast, resistant pattern of ciprofloxacin (92.68%), clindamycin (87.80%), erythromycin (63.41%) can be comparable to the studies of Anupurba et al (CIP-84.1%, E-80.1%), Subedi et al (CIP-94.4%, E-83.4%,), Kumari et al. (E–70.41%, CIP-67.35%), Sanjana et al. (CIP71.08%, E-58.06%), Arora et al (CIP-67.8%, E-61.7%), Bala et al. (CIP-98.68%, E58.63%), Mulla et al.(CD-68.4%, E-63.1%), Ahmed et al. (CD-81.4%, E–77.7%), Orret et al. (E86.7%, CD-75.3%, T-78.7%, CIP-59.1%), Khadri et al.(E-83%), and Onwubika et al.(E-100%), respectively.[23,25-32,] In the present study gentamicin resistant rate is 21.54%, similar pattern also found in the study of Suzanne et al., (10.2%), and Eyob Yohanesset al., (1.2%).[29, 33]

Conclusion

Methicillin resistant S. aureus is one of the most important pathogens, causing severe morbidity and fatal infections. Most active and reliable antibiotics are vancomycin, daptomycin, tigecycline, teicoplanin and linezolid etc. used for infections caused by the MRSA. In our investigation, increased susceptibility to routinely used antibiotics was discovered. The findings of our study's Vitek 2 system and cefoxitin disc diffusion methods demonstrated a remarkable disagreement. The use of cefoxitin disc diffusion as a superior approach to oxacillin disc diffusion for MRSA detection, however, is also in conflict with this. Our findings show that some MRSA strains were incorrectly categorised as susceptible by the disc diffusion approach or resistant by the vitek 2 system. It emphasizes the value of utilizing automated microbial identification and sensitivity techniques that give the microbiologist greater information. Therefore, given that cefoxitin disc diffusion has already been indicated as a surrogate test, we can draw the conclusion that in addition to the Vitek 2 method, it is also a crucial test to diagnose MRSA. In our study, it was also observed that several MRSA isolates had high levels of resistance to oxacillin, trimethoprim/sulfamethoxazole, Levofloxacin, ciprofloxacin, clindamycin, and erythromycin. Hence, good infection control procedures like thorough hand washing, identifying and treating MRSA carriers, and prudent use of antimicrobial medicines are advised to prevent the formation of drug-resistant isolates.

Acknowledgements: The authors would like

to acknowledge the management of JSSAHER for permitting us to conduct this Research.

Conflict Of Interest: Authors declare no conflict of interest

Source Of Funding: Self

Ethical Clearance: This current study is approved by the Institutional ethics committee of JSS Medical College and Hospital, JSSAHER, Mysore.

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