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Prevalence and Antibiogram of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Various Clinical Samples in a Tertiary Care Hospital of West Bengal

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Abstract **Original Research Article**

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most important causes of health care acquired infection in terms of morbidity, mortality, length of hospital stay and economic burden on hospitals. Considering the paucity of data on its prevalence in West Bengal, the present study has been envisaged to assess the prevalence of MRSA among hospitalized patients. The study was conducted to assess prevalence of MRSA among hospitalized patients and to compare antibiogram of MRSA and Methicillin susceptible Staphylococcus aureus (MSSA).Staphylococcus aureus was identified using standard methods from various clinical samples collected over a period three months. Then, methicillin-resistant strains were identified by using screening technique i.e. cefoxitin disc (30µg) diffusion testing method. Finally, data were collected, and the prevalence was estimated.Out of the total of 1045 samples, Staphylococcus aureus was identified from 98 samples. Of the 98 samples, 67 (68.37%) were found to be methicillin resistant. MRSA strains were found to be more resistant to other antibiotics than MSSA strains.MRSA is posing threat in health-care institutions. Minimizing the emergence and spread of this organism is the need of the hour. A regular surveillance of hospital-associated infections is mandatory.

Keywords: Methicillin resistant, antibiotic, prevalence, Staphylococcus, infection.

INTRODUCTION

Staphylococcus aureus, a notorious pathogen, is responsible for causing a variety of infections, which include minor skin diseases to life-threatening infections [1]. It not only colonizes healthy individuals but also causes severe infection in hospitalized patients particularly. *Staphylococcus aureus* infections are susceptible to ß-lactam and related group of antibiotics but the emergence of *Methicillin-resistant S. aureus* (MRSA) has implicated in serious infections and nosocomial outbreaks including bacteremia, endocarditis, urinary tract infections and surgical site infections [2].

MRSA are prevalent worldwide and able to cause increased morbidity and mortality in the hospital settings. Methicillin was first introduced in medicine in the 1960s to treat the penicillin-resistant *S. aureus*, but MRSA emerged shortly. Methicillin resistance seen in *S. aureus* has several mechanisms out of which production of an altered penicillin-binding protein (PBP) with low affinity for β-lactam antibiotics is the most important. Their effects are determined by several structural genes (*mecA*)[3,4]. Other known mechanism of methicillin resistance is the hyperproduction of penicillinase enzyme [4, 5].

Infected and colonized patients in hospitals mediate the spread of MRSA strains, and health care workers remain the main source of transmission [6]. The possible predisposing factors that increase the chance of emergence and dissemination of MRSA are prolonged and repeated hospitalization, indiscriminate use of antibiotics, lack of awareness, intravenous drug abuse, and presence of indwelling medical devices [7].

MRSA strains, being multidrug-resistant, are difficult to eradicate and glycopeptides are the drugs of choice [1]. Although, resistance has been reported to these drugs from various parts of the country [8, 9]. The knowledge of prevalence of MRSA and their antimicrobial-susceptibility pattern is a must for proper treatment of these infections. The present study was aimed at to know the prevalence of MRSA in our tertiary care hospital of West Bengal.

MATERIALS & METHODS

The present study was conducted in the Department of Microbiology, Burdwan Medical

College, Purba Bardhaman, India, over 3 months from September 2018 to November 2018. After collection of various samples, first, direct smear was prepared and stained with gram stain and then the samples were cultured on various culture media, like Blood agar and MacConkey's agar. And after 24hrs of incubation, colony morphology was noted and from the colony; gram stain was performed again. Then, only samples showing gram positive cocci arranged in irregular grape-like clusters were taken into account. After that catalase test and slide and tube coagulase tests were performed to identify *Staphylococcus aureus*.

Antibiotics Susceptibility Testing

The antibiotic susceptibility pattern of all the *S. aureus* strains were assessed by modified Kirby–Bauer disc diffusion method on Mueller– Hinton agar against the following antibiotics: amoxicillin clavulanic acid (20/10 µg), azithromycin (15 µg), ciprofloxacin (5 µg), cefoxitin (30 µg), erythromycin (15 µg), linezolid (30 µg), vancomycin (30 µg) and ceftriaxone (30 µg). After incubation of 24 h at 37°C, the zone diameters measured around each disc were interpreted on the basis of guidelines published by the Clinical and Laboratory Standards Institute (CLSI)[10].

Detection Method for MRSA

Cefoxitin disc (30 µg) diffusion testing method was used to screen MRSA from all of the isolates as per CLSI guideline. From each strain, a suspension equivalent to 0.5 McFarland was prepared. After that, a swab was dipped in it and streaked on the surface of a Mueller–Hilton agar and Cefoxitin disc (30 µg) was placed onto it and incubated for 24 h at 35°C. The isolate was considered as MRSA if the zone of inhibition was ≤21 mm in diameter.

RESULTS

In this present study, a total of 1045 samples were collected in the Microbiology department, Burdwan Medical College, over a period of three months from September 2018 to November 2018. Of these, *Staphylococcus aureus* was isolated in ninety-eight (98) samples. Sixty-seven (68.37%) of these 98 samples were found to be methicillin resistant indicating prevalence of MRSA was as high as 68.37% in this institution (Figure 1).

In this study, MRSA were isolated mostly from skin and soft tissue samples (38.80%) followed by blood cultures (37.31%) and urine (23.88%) (Figure 2).

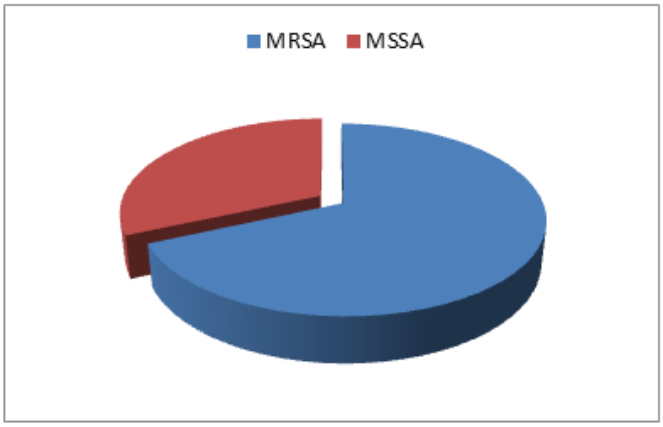

Fig-1: Percentage distribution of MRSA (n=67) & MSSA (n=31) amongst *Staphylococcus aureus* isolates. (n=98)

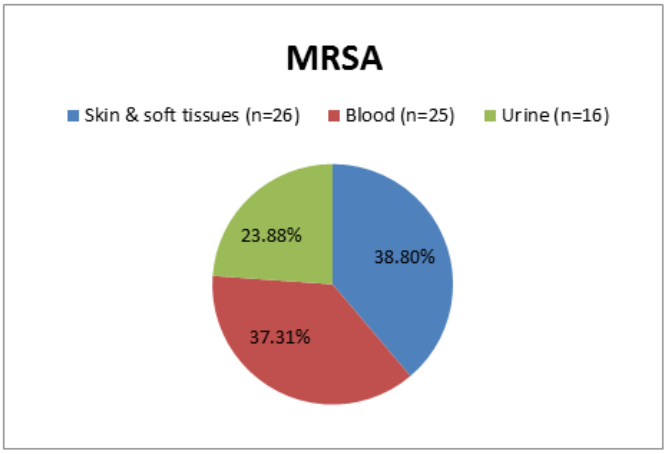

Fig-2: Percentage distribution of MRSA according to different sites. (n=67)

MRSA strains were found to be more resistant to other antibiotics than MSSA strains. We observed that MRSA strains were more resistant than MSSA strains particularly for coamoxyclav (94.64% & 58.82%), ciprofloxacin (63.33% & 52.94%) and ceftriaxone (71.42% & 33.33%) but not for azithromycin and erythromycin. We also observed that, 4.91% of MRSA isolates were resistant to vancomycin and 8.48% of MRSA isolates were resistant to linezolid (Figure 3).

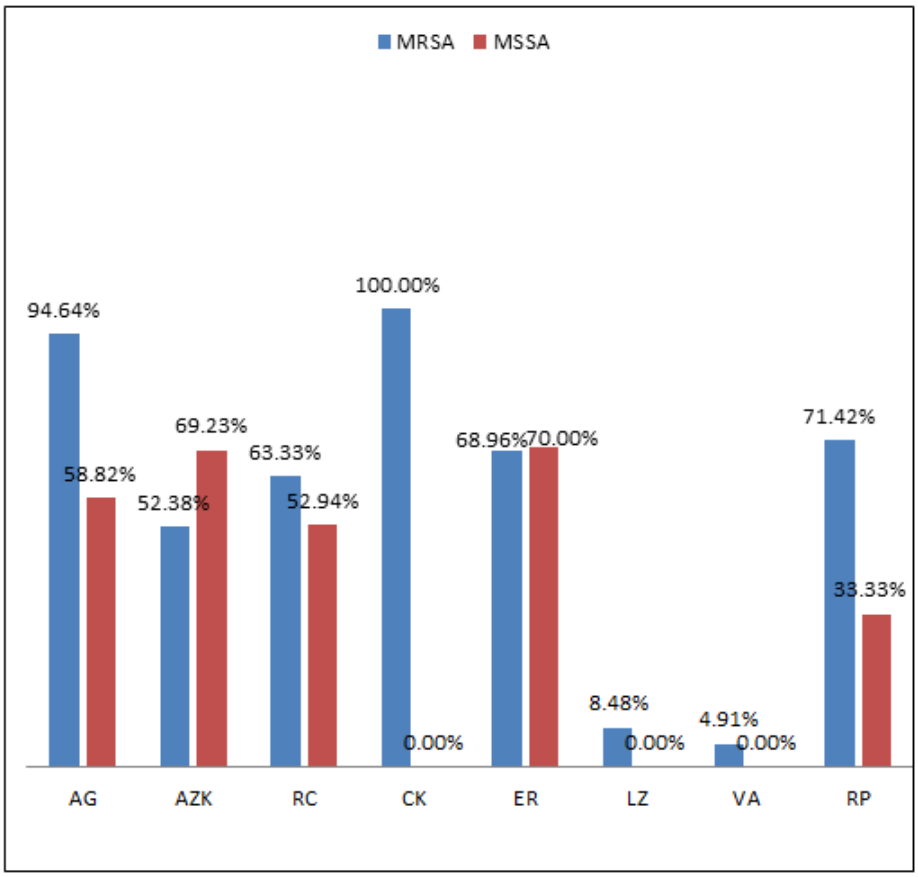

Fig-3: Comparison of resistant pattern of MRSA (n=67) & MSSA (n=31) with various antibiotics
Key: S=Sensitive, R=Resistant, AG=Amoxyclav, AZK=Azithromycin, RC=Ciprofloxacin, CK=Cefoxitin, ER=Erythromycin, LZ=Linezolid, VA=Vancomycin, RP=Ceftriaxone.

DISCUSSION

In the present study we observed that the prevalence of MRSA was as high as 68.37%. It has been seen that in Indian studies, the incidence of MRSA shows a large variation, from 6.9% to 81%. Some studies have reported comparable prevalence: 54.8% in Uttar Pradesh [7] 52.9% in Assam [11], 80.89% in Indore [12], and 19.56% in Nagpur [13]. According to a different study carried out by Tsering *et al*.[14] the prevalence rate was 38.14%, and in the study of Joshi *et al*.[15] and Arora *et al*.[16] the prevalence rates were 41% and 46%, respectively. This variation could be due to several factors like efficacy of infection control practices, healthcare facilities and antibiotic usage that vary from hospital to hospital.

In this study, MRSA were isolated mostly from skin and soft tissue samples (38.80%) followed by blood cultures (37.31%) and urine (23.88%) which is comparable to the study by Tribedi *et al*.[17] in which isolated MRSA were mostly from skin and soft tissue samples (71%), followed by blood cultures (15%), miscellaneous (5.26%), and respiratory specimen(4.09%); and the study done by Shah *et al*.[18] in which MRSA isolated were in majority from pus samples (74.13%), followed by blood cultures (18.97%), miscellaneous (5.17%), and sputum (1.72%).

The association of multidrug resistance with MRSA adds to the problem and it is rightly said that the hospital dust is more dangerous than the roadside dust [16]. In our study, we noticed that MRSA strains were found to be more resistant to other antibiotics than MSSA strains. We observed that MRSA strains are more resistant than MSSA strains particularly for coamoxyclav (94.64% & 58.82%), ciprofloxacin (63.33% & 52.94%) and ceftriaxone (71.42% & 33.33%) but not for azithromycin and erythromycin. Similarly, in the study by Arora et al., MRSA strains were found to be more resistant to other antibiotics than MSSA strains. It was observed by them that significant difference (*P*-value < 0.05) was observed in case of

penicillin, erythromycin, cephalexin, ciprofloxacin, gentamicin and amikacin. However, they also noticed that the difference observed in case of norfloxacin, nitrofurantoin and linezolid was statistically insignificant (*P*-value > 0.05)[16]. Vidhani *et al*. also found that there was a marked difference between sensitivity pattern of MRSA and MSSA isolates [19]. Majumder *et al*. also reported that coexisting resistance to different antibiotics (except penicillin) with methicillin was significantly higher in comparison to methicillin-sensitive strains [20].

In our study, we noticed 4.91% of MRSA isolates were resistant to vancomycin and 8.48% of MRSA isolates were resistant to linezolid. However, in the study by Arora *et al*. all MRSA strains were sensitive to vancomycin which is in accordance with other studies [1, 6, 7]. Moreover, vancomycin-intermediate and vancomycin-resistant Staphylococcus aureus (VISA and VRSA) strains have been reported recently from various parts of the country [8, 9]. Emergence & spread of VRSA seen in our hospital is possibly due to exploitation of vancomycin and linezolid by clinicians.

To conclude, glycopeptides seems to be the only antimicrobial agents that may be used as the drug of choice to treat MRSA infections. The high prevalence of MRSA and glycopeptide use, both thought to be risk factors for vancomycin resistant *Staphylococcus aureus* (VRSA), make the widespread dissemination of these organisms an alarming and realistic possibility once it happens to emerge. So, glycopeptides must be kept reserved for life-threatening infections caused by MRSA.

The marked difference between antibiogram of MRSA and MSSA isolates calls for the routine testing of methicillin-resistance, which may preferably be done by using cefoxitin disc. The most effective way to prevent MRSA infections is by doing continuous surveillance of antibiotic resistance profiles of local *Staphylococcus aureus* isolates to formulate antibiotic policies and effective infection control practices.

Conclusion

From the overall observation, it was noticed that prevalence of MRSA is very high (68.37%) in this institution and 4.91% of MRSA isolates were resistant to vancomycin and 8.48% were resistant to linezolid. It was also noticed that, MRSA strains were more resistant to other antibiotics than MSSA strains. The high prevalence of MRSA and emerging vancomycin and linezolid resistance amongst these isolates pose a threat in this institution. To minimize the emergence and spread of MRSA is really an uphill task to be addressed urgently. Vancomycin use should be restricted to those cases where they are clearly indicated particularly in life-threatening infections. However, due to the increasing use of vancomycin, regular monitoring of vancomycin sensitivity by MIC for MRSA and routine testing of other new glycopeptides should be performed regularly. A regular surveillance of healthcare associated infection, monitoring of antibiotic sensitivity pattern of MRSA, strict antibiotic policy with stringent implementation of antimicrobial stewardship programme are mandatory to control the situation. Moreover, prevention and infection control strategies should be applied strictly in tertiary-care hospitals including general measures (hand hygiene, cleanliness, proper disinfection, use of contact precautions, and education and training of all health-care workers) and specific measures (patient isolation and cohorting, eradication of MRSA carriage, and surveillance and screening of patients and health-care workers).

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