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Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Multidrug Resistance among them in a Tertiary Care Rural Hospital

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A B S T R A C T

Infections caused by *Staphylococcus aureus* have a poorer prognosis when the infecting strain is Methicillin-resistant *Staphylococcus aureus* (MRSA) additionally MRSA strains are important for their resistance to many other commonly used antibiotics. The objectives of this study were to estimate the prevalence of MRSA strains in clinical specimens and to investigate their sensitivity pattern. A total of 480 various samples received in the department of Microbiology at MIMER Medical college and hospital, Talegaon were included in the study. The MRSA strains were identified by using Oxacillin resistance screening agar and Cefoxitin (30µg) disc diffusion. Their sensitivity pattern was investigated using disc diffusion and minimum inhibitory concentration (MIC) by E test. Prevalence of MRSA in our study is 19.37%. There is significant difference between rates of isolation of MRSA from various clinical specimens. All MRSA strains tested against vancomycin and linezolid showed 100% sensitivity. Highest resistance among MRSA was found for amoxicillin–clavulanic acid (74.19%). There is a need to reduce the burden of infections caused MRSA. Continuous efforts should be made to prevent the spread and the emergence of resistance by early detection and using the proper infection control measures.

Introduction

In the genus *Staphylococcus, Staphylococcus aureus (S. aureus)* is the most important pathogenic organism and it is also one of the most common versatile nosocomial pathogen.[1] It is responsible for most common causes of blood stream infection (BSI), skin and wound infections, osteomyelitis, endocarditis, and nosocomial infections, especially pneumonia, surgical site infections, and continues to be a major cause of community-acquired infections.[2,3]

It has overcome most of the therapeutic agents that have been developed over the years. The important notable example was the methicillin resistant *Staphylococcus aureus* (MRSA) which was reported just one year after the launch of methicillin.[1] S. aureus strains expressing the mecA determinant are termed as MRSA.[4] The worst feature of MRSA is simultaneous drug resistance to many antibiotics and chronic carrier stage among the healthcare workers[1]. Accurate and prompt detection of Methicillin Resistance Staphylococcus aureus (MRSA) in patient's

sample is of utmost importance in managing these infections and preventing their spread.

Keeping in mind the havoc, MRSA can create, we decided to estimate the prevalence of MRSA in our hospital; and susceptibility pattern of MRSA towards various antibiotics.

Materials and Methods

Study was conducted in the department of microbiology at MIMER Medical college and hospital, Talegaon. It was a cross-sectional type of study. Study period was from January 2014 to June 2015. All samples received in the microbiology laboratory from various clinical departments of the hospital were processed for isolation of S. aureus and detection of MRSA. Total 480 samples of S. aureus were obtained during study period. Samples included were blood, fluid, stool, pus, sputum, throat swab, urine, miscellaneous samples (it includes, post operative wound swab, high vaginal swab, urinary catheter tips, endotracheal tubes and umbilical catheter tips etc.) Processing of S. aureus isolates were done by using standard methods.

MRSA were detected by using, two methods

Oxacillin resistance screening agar and Cefoxitin disc diffusion.

Antibiotic susceptibility testing was done by Kirby Bauer's disc diffusion method. For determining vancomycin susceptibility, feasible, cost-effective and more reliable E test method was used.

Results and Discussion

Detection of *mecA* gene or its product, penicillin binding protein (PBP2a), is considered the gold standard for MRSA confirmation. [5] As suggested by CLSI guidelines; cefoxitin-based methods predict the presence of *mec*A-mediated resistance; their use is preferred to tests using oxacillin because they are better predictors of the presence of *mec*A than are oxacillin-based methods, including the oxacillin salt-agar screening plate.[6] Therefore, as depicted in table 1, we have considered prevalence of MRSA based on cefoxitin disc diffusion method i.e. 19.37%.

The findings of isolation of MRSA from various clinical specimens are depicted in table 2. In our study, we found significant difference between rate of isolation of MRSA from various clinical specimens (*p* value <0.05). Highest rate of isolation of MRSA from miscellaneous group of samples, i.e. 29.71%. Second highest isolation of MRSA was obtained from blood i.e. 24.73% followed by pus (17.77%).

As shown in graph no. I, we observed significant difference ($p<0.05$) in rate of isolation of MRSA from different clinical specialties also i.e. most of the MRSA isolates belonged to surgical units [includes surgery (46.57%), orthopedics (33.33%), obstetrics & gynecology (21.87%), ENT (4%)] rather than medical units [includes ICU (31.37%) NICU (28.57%), medicine (14.70%)].

Table 3 depicts antibiotic sensitivity pattern of all MRSA isolates. It showed that all MRSA were sensitive to linezolid followed by amikacin [sensitive (45.16%) and moderately sensitive (7.52%)] while highest resistance was found for amoxicillin-clavulanic acid (74.19%) followed by azithromycin and clarithromycin i.e 68.81% to be followed by cotrimazole and ciprofloxacin i.e. 66.66%. One of the important finding in our study is that, for every antimicrobial agent, percentage of sensitivity was considerably high among total *S. aureus* strains than the percentage of

sensitivity of MRSA strain. e.g. amoxacilin-clavulanic acid sensitive *S.aureus* were 51.25% while sensitive MRSA were 25.80 % and for cotrimoxazole, sensitive *S.aureus* strains were 41.66% while sensitive MRSA strains were 21.50%. In our study, all the MRSA strains which were tested against vancomycin susceptibility by E test showed 100% sensitivity. But out of total 93 MRSA cases 37 (39.78%) were having MIC 1 mcg/ml and 32(34.40%) MRSA strain was sensitive within the range 2 mcg/ml. This suggests that though all these MRSA strains were within the susceptibility range.

Table.1 Prevalence of MRSA

Method	No. of MRSA isolates	Percentage
Oxacillin screening agar method	92	19.16%
Cefoxitin disc diffusion	93	19.37 %

Table.2 Sample wise distribution (MRSA isolates)

Type of Sample	No. of S. aureus isolates n1= 480	MRSA isolates n2= 93	MRSA Percentage
Miscellaneous	138	41	(29.71%)
Pus	135	24	(17.77%)
Blood	93	23	(24.73%)
Urine	70	4	(5.71%)
Sputum/ Throat swab	32	1	(3.12%)
Fluid	6	0	0
Stool	6	0	0

Chi-square value = 28.02 *p* value = 0.00009314 i.e. $p<0.05$

There is significant difference between rate of isolation of MRSA from various clinical specimens (*p* value <0.05).

n1=Total no. of S.aureus isolated from various clinical samples =480

n2=Total no. of MRSA isolated from various clinical samples =93

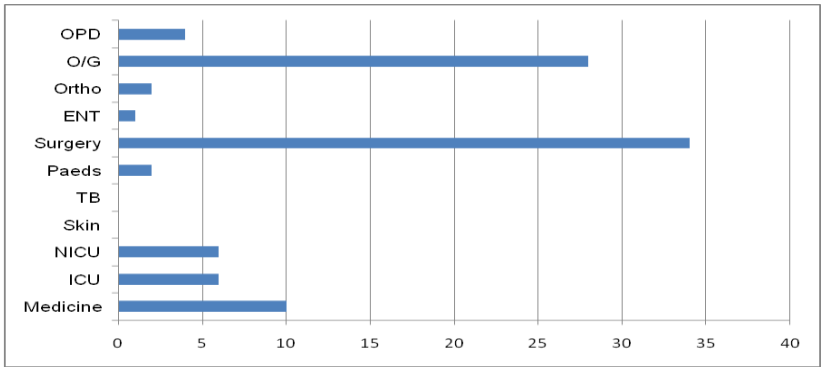
Percentage of MRSA=$\frac{\text{No. of MRSA isolated from one type of clinical samples}}{\text{No. S. aureus isolated from the same type of clinical samples}} \times 100$

Table.3 Antibiotic sensitivity pattern of all MRSA isolates

Antibiotic	No. of Sensitive isolates	No. of Moderately Sensitive isolates	No. of Resistant isolates
Vancomycin	93 (100%)	0	0
Linezolid	93(100%)	0	0
Amoxicillin –Clavulanic acid	24(25.80%)	0	69(74.19%)
Co-trimoxazole	20 (21.50%)	11 (11.82%)	62 (66.66%)
Clindamycin	32 (34.40%)	12 (12.90%)	49 (52.68%)
Amikacin	42 (45.16%)	7 (7.52%)	44 (47.31%)
Azithromycin	23 (24.73%)	6 (6.45%)	64(68.81%)
Ciprofloxacin	20 (21.50%)	11 (11.82%)	62(66.66%)
Clarithromycin	25 (26.88%)	4 (4.30%)	64(68.81%)

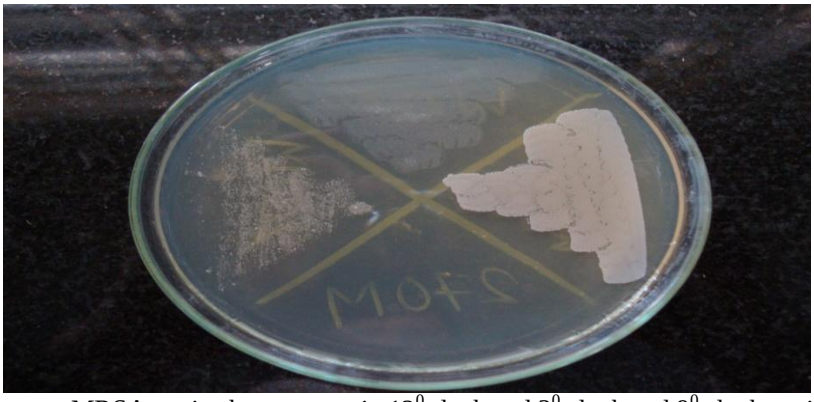
[n= No. of Methicillin resistant *S. aureus* (MRSA) isolates = 93]

Graph.1 Clinical Specialty wise distribution of MRSA isolates



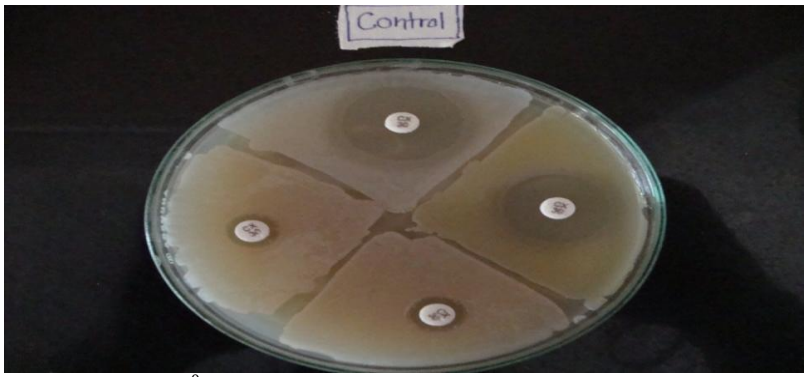
Chi-square value = 65.19 *p* value = 0.000 i.e. $p<0.05$
Isolation of MRSA sample was significantly different among different clinical specialties.($p<0.05$)

Image.1 Oxacillin resistance screening agar



- MRSA strains have grown in 12^0 clock and 3^0 clock and 9^0 clock position quadrants
- *S.aureus* strain at 6^0 clock is MSSA strain

Image.2 Cefoxitin disc diffusion testing for MRSA



- Strain at 12^0 clock position is a control strain
- S. aureus strain at 3^0 clock position is an MSSA strain
- While strains at 6^0 clock and 9^0 clock position are MRSA strain

At the time of the introduction of penicillin in the early 1940s, *S. aureus* was uniformly susceptible to this drug. However, widespread resistance to penicillin developed during the 1950s, followed in the 1970s by increasing resistance to the new semisynthetic penicillinase-resistant antimicrobial agents (i.e., methicillin, oxacillin, nafcillin, dicloxacillin). By 1990s, resistance to the penicillinase-resistant penicillins spread throughout the world, compromising the use of these drugs for empiric therapy for staphylococcal infections. This has led to increased reliance on vancomycin for treatment of documented methicillin-resistant *S. aureus* (MRSA) infections, as well as for empiric therapy of infections in populations where the prevalence of MRSA is high.[7]

Detection of *mecA* gene or its product, penicillin binding protein (PBP2a), is considered the gold standard for MRSA confirmation.[8] A study conducted by KB Anand et al provides an evidence that cefoxitin can be used as an accurate surrogate marker in routine susceptibility testing at 37^0C for 18 to 24 hours. In addition, the results have shown 100% sensitivity and specificity as compared to *mecA* gene detection by PCR. Hence, it can be used as an alternative to the technically demanding PCR for the detection of MRSA.[9] Therefore, in our study we considered rate of MRSA prevalence based upon 'Cefoxitin disc diffusion method'. We reported 19.37% MRSA prevalence in our hospital.

In India, a study was conducted by Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group. This study was conducted in 15 Indian tertiary care centers during a two years period to determine the prevalence of MRSA and susceptibility pattern of *S. aureus* isolates in India.[10] According to this study, prevalence of MRSA was 42% in 2008 and in 2009 it is 40%.

In our study, we found significant difference between rate of isolation of MRSA from various clinical specimens (*p* value <0.05). Second highest isolation of MRSA was obtained from blood i.e. 24.73% followed by pus (17.77%).

In a study conducted by INSAR (Indian Network for Surveillance of Antimicrobial Resistance), observed highest rate of isolation of MRSA in 2008 and in 2009 was from 'other specimens' (46%) and blood(48%) respectively which is similar to our findings is similar to our findings.[10] While In a study conducted by Sharma P. et al the highest rate of isolation of MRSA was from pus samples (76.92%) followed by blood (16.66%) and sputum (3.8%).[11] Observations of above mentioned studies are somewhat different from our study.

We found significant difference (p<0.05) in rate of isolation of MRSA from different clinical specialties. In a study carried by S Srinivasan *et al* who observed that surgical units (postoperative wound infections) accounted for 80% of MRSA isolates when compared to 20% in medical units.[12] Results observed in the above mentioned studies are in concordance with the findings of our study i.e. most of the MRSA isolates belonged to surgical units [includes surgery (46.57%), orthopedics (33.33%), obstetrics & gynecology (21.87%), ENT(4%)] rather than medical units.

Another study conducted by JB Sarma *et al.*, showed, 100% of MRSA isolates were resistant to ciprofloxacin; 85% to clindamycin and 96% were resistant to co-trimoxazole and amikacin. In contrast, 100% of MSSA isolates were sensitive to these antimicrobials. [13] On the contrary, in our study we found 66.66% MRSA strains to be resistant to ciprofloxacin and cotrimoxazole, while 47.31% were resistant to amikacin.

According to S Srinivasan et al, linezolid appears to be one of the few available antimicrobial agents with proven activity against MRSA including strains with reduced susceptibility to glycopeptides.[12] They observed that 100% MRSA strains were within susceptibility range of linezolid by using both disc diffusion and MIC agar dilution method. This proves to be true for our study. As we have found that all MRSA strains were sensitive to linezolid, though we have used only Kirby-Bauer disc diffusion technique.

Clindamycin, was found to be another useful alternative in treating patients with MRSA. In a study by Shrinivasan *et a.,l* it was observed that all 50 isolates were sensitive to clindamycin.[12] It is some what contrary to our findings, where we have observed more of sensitivity among all *S.aureus* isolates [(sensitive (45.20%), moderately sensitive (16.66%)] and of sensitivity among MRSA [sensitive (34.40%), moderate sensitive (12.90%)].

In a study conducted by Rajduraipandi et al., out 250 MRSA strains all (100%) MRSA strains recorded sensitive to vancomycin, which was followed by 97.6% to linezolid.[75]Which were almost similar to our findings.

To conclude, MRSA prevalence in our hospital is 19.37%, which is although not alarming, does not allow for complacency. Enforcing strict infection control practices will help us to tackle the situation. All MRSA isolates showed 100% sensitivity to vancomycin but nearly 75% isolate were having higher end of MIC which is a matter of concern for us. Though MRSA strains showed complete susceptibility to linezolid, it should be used very cautiously as a reservoir drug for serious MRSA infections.

Conflicts of interest: None

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