

Antimicrobial Susceptibility Profile of Methicillin-resistant *Staphylococcus aureus* at a Tertiary Care Centre

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Abstract

Background: *Staphylococcus aureus* is a known cause of an array of infections ranging from minor skin and soft tissue infections to chronic bone infections and overwhelming septicemia and endocarditis. Methicillin resistant *Staphylococcus aureus* (MRSA) is problematic, as the therapeutic outcome of MRSA infections is much worse than those caused by methicillin sensitive strains.

Aim: This study was conducted with an aim to determine the prevalence of MRSA among clinical isolates of *Staphylococcus aureus* and also determine the antimicrobial susceptibility pattern of MRSA isolates.

Methods: This cross sectional study was carried out from November 2011 to October 2013 in which 510 non-repetitive clinical isolates of *S. aureus* were identified by conventional phenotypic methods. MRSA isolates were identified using cefoxitin (30 µg) disc diffusion method. Complete antimicrobial susceptibility profile was also determined by Kirby Bauer disc diffusion method. Susceptibility testing to vancomycin was done by E-test.

Results: The prevalence of MRSA among *S. aureus* isolates was found to be 20.2%. None of the isolates were sensitive to penicillin. However, all the isolates were found to be sensitive to vancomycin, teicoplanin and linezolid. Varying levels of resistance was seen to other antibiotics.

Conclusion: In this study, the prevalence of MRSA isolates was 20.2% which is much less as compared to other studies both in India as well as Western world. This study recommends routine screening of MRSA by cefoxitin disc and also determining the complete antimicrobial susceptibility profile of these isolates to treat these infections effectively.

Keywords: Methicillin resistant *Staphylococcus aureus* (MRSA); Cefoxitin; E-test; Clinical and Laboratory Standards Institute (CLSI)

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Introduction

Staphylococcus aureus has been renowned as an important cause of human disease for more than 100 years [1]. Alexander Ogston first isolated *Staphylococcus aureus* from a surgical abscess in 1880 and described the role of *S. aureus* in localized infection and septicemia, including the use of animal models for infection [2,3]. *Staphylococcus aureus* is recognized as a cause of a wide range of infections, ranging from minor skin infections and chronic bone infections to devastating septicemia and endocarditis [4]. Significant events in the evolution of *S. aureus* have included the development

of methicillin resistance, now a problem for many hospitals around the world. The recent emergence of community strains of *S. aureus* that are not only methicillin resistant but also harbor genes associated with increased virulence has become a therapeutic challenge [5,6]. Methicillin-resistant *Staphylococcus aureus* (MRSA) alone (which probably accounts for less than one-third of all *S. aureus* infections) caused more deaths in the United States in 2005 than human immunodeficiency virus infection. It also caused more invasive infections than other important bacterial pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis* [7,8].

MRSA is now endemic in India. Its incidence varies from 25% in Western India to 50% in South India [9,10]. Early detection of MRSA and its susceptibility profile becomes important from treatment point of view because it leaves us with very few treatment options such as glycopeptides and linezolid.

This study was conducted with an aim to determine the prevalence and antimicrobial susceptibility pattern of MRSA isolates at a tertiary care centre in order to implement effective control measures and control their spread.

Materials and Methods

This cross sectional study was carried out from November 2011 to October 2013. A total of 510 non-repetitive clinical isolates of *Staphylococcus aureus* were isolated from various clinical specimens received in microbiology laboratory of a tertiary care centre. These isolates were identified by conventional phenotypic methods such as colony morphology, Gram's stain, catalase test, slide and tube coagulase test, growth on mannitol salt agar, DNase production, gelatinase production and phosphatase production.

Identification of MRSA

All the isolates of *Staphylococcus aureus* isolates were subjected to Cefoxitin disc diffusion testing using a 30 µg cefoxitin disc. The results were interpreted according to CLSI guidelines 2013. An inhibition zone diameter of ≤ 21 mm was reported as methicillin resistant and ≥ 22 mm was reported as methicillin sensitive.

Antibiotic susceptibility testing

All MRSA isolates were tested for their susceptibility to various antibiotics by Kirby Bauer disc diffusion method (Table 1). Since Kirby Bauer disc diffusion method is not recommended for susceptibility testing of *Staphylococcus aureus* to vancomycin, determination of minimum inhibitory concentration (MIC) has to be done either by E-test or agar dilution method. In this study, we used vancomycin E-strips (bio-Merieux) to determine the MIC of vancomycin (Figure 1). MIC ≤ 2 µg/ml was interpreted as sensitive according to CLSI guidelines.

Results

Out of 510 isolates of *Staphylococcus aureus*, 103 isolates were found to be methicillin-resistant *Staphylococcus aureus* (MRSA). The prevalence of MRSA was 20.2%.

Table 1: Antibiotics tested against MRSA isolates by Kirby Bauer disc diffusion method.

S. no.	Antibiotic	Concentration
1.	Penicillin	10 units
2.	Erythromycin	15 µg
3.	Clindamycin	2 µg
4.	Co-trimoxazole	1.25/23.75 µg
5.	Ciprofloxacin	5 µg
6.	Levofloxacin	5 µg
7.	Amikacin	30 µg
8.	Teicoplanin	30 µg
9.	Linezolid	30 µg

The various clinical samples from which MRSA were isolated are shown in Figure 2. The most common clinical samples were pus (70) followed by blood (12), central line tip (06) and urine (04).

The age of the patients from whom MRSA were obtained ranged from 1.5 months to 79 years of age. It was noticed that amongst the 103 isolates, 69 were from male patients and 34 were from female patients. Male to female ratio was 2:1. Maximum numbers of isolates were from age group 21 to 30 years comprising 35% of the total followed by age group 31 to 40 years (23.3%). Table 2 shows the age and sex wise breakup of the various isolates of MRSA.

Antimicrobial susceptibility testing of all MRSA isolates was done by Kirby Bauer disc diffusion method. Antibiogram of the isolates is shown in Figure 3. All the 103 MRSA isolates were found to be resistant to penicillin. In contrast, all the isolates were found to be sensitive to both teicoplanin as well as linezolid. The sensitivity

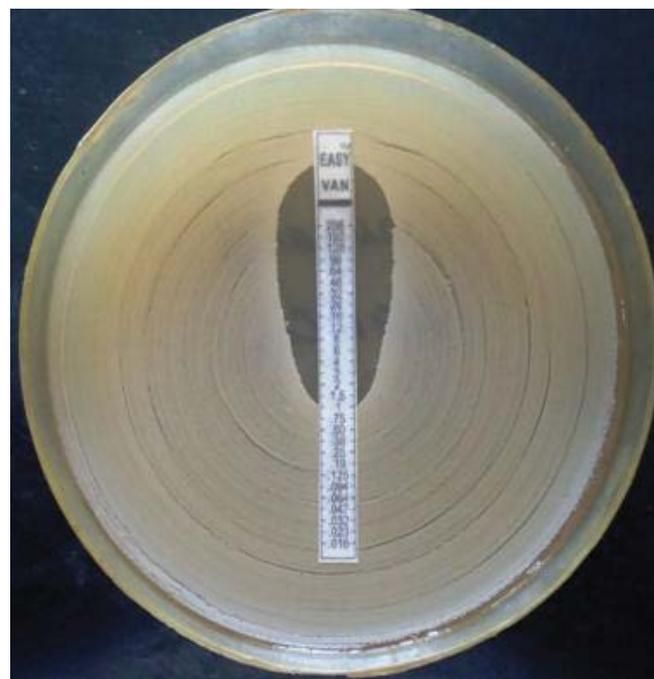


Figure 1 E-test for determination of MIC of vancomycin against *Staphylococcus aureus* strain.

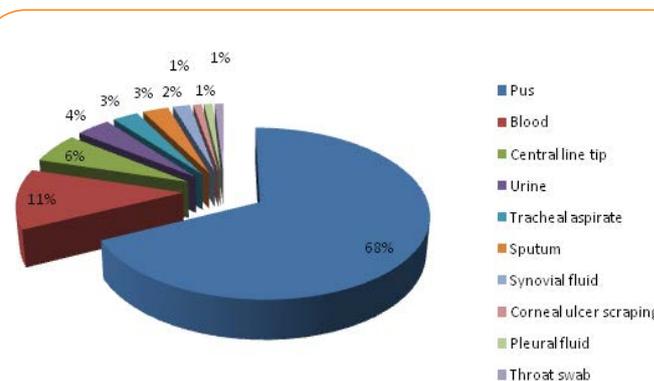


Figure 2 Distribution of MRSA isolates from various clinical samples.

to erythromycin, clindamycin, ciprofloxacin, levofloxacin, cotrimoxazole and amikacin was found to be 24%, 37%, 20%, 27%, 37% and 44% respectively.

MIC of vancomycin was determined by E-test and all the isolates of MRSA were found to be sensitive to vancomycin.

Discussion

Staphylococcus aureus has long been recognized as a major human pathogen responsible for a wide range of infections, ranging from mild skin infections to wound infections and bacteremia. Although the introduction of antibiotics over the last 50 years has reduced the mortality rate from these infections, the bacteria have developed resistance mechanisms to all the available antimicrobial agents.

The introduction of penicillin offered an opportunity to successfully treat serious staphylococcal infections. However, in 1944, penicillinase (β -lactamase) producing *S. aureus* was described which were resistant to penicillin. This enzyme was responsible for the clinical failures that appeared soon after the introduction of penicillin [12]. During the early 1950s, a series of semi-synthetic penicillins were developed that were stable to destruction by bacterial β -lactamases. Methicillin was one such compound which was introduced into clinical practice in 1959. Soon after its introduction, the first methicillin-resistant *S. aureus* (MRSA) was detected and the first clinical failure of methicillin for the treatment of *S. aureus* described [11,13]. Methicillin resistance in *S. aureus* isolates is mediated by an exogenous gene, *mecA* [12,14]. This gene, *mecA* encodes for a particular penicillin-binding protein (PBP) called PBP2A, which has a low affinity for methicillin and most other β -lactam drugs which is responsible for the intrinsic resistance of MRSA to almost all β -lactams [15].

Since its discovery, the prevalence of MRSA progressively increased thereafter. One survey of the National Nosocomial Infections Surveillance (NNIS) reported that the hospital prevalence rate of MRSA increased from 2.1% in 1975 to 35% in 1991 [16]. It is now as high up to 60% in certain centres in the United States, but great geographic variations exist worldwide [17]. The prevalence of MRSA in a study from Chennai was reported as 40-50% [18]. Similarly, the overall prevalence reported in Indian network for surveillance of antimicrobial resistance (INSAR) group study was 42% in 2008 and 40% in 2009 [19]. However, in the present study, the prevalence of MRSA was found to be 20.2% which is much less as compared to other studies, both in India as well as Western world.

Though MRSA does not show predilection for any particular age or sex, no age is exempt from these infections. In the present study, it was found that maximum number of isolates came from the age group of 20-40 years, comprising 61.5% of the total isolates (Table 2). Higher incidence of MRSA infections among males has also been reported by Chua T et al. which correlate with our findings [20].

The antimicrobial susceptibility pattern of all the 103 MRSA isolates was determined by Kirby Bauer disc diffusion method and has been shown in Figure 3. It was found that all the isolates were resistant to penicillin. Approximately 75-80% isolates were found to be resistant to ciprofloxacin and levofloxacin which is a cause for concern. About 63% isolates were found to be resistant to cotrimoxazole and the resistance to erythromycin and clindamycin ranged from 63-76%. Similar findings were also observed in the INSAR group study. It was also seen that all the isolates were sensitive to teicoplanin and linezolid.

Disk diffusion sensitivity testing by standard 30 μ g vancomycin frequently misclassifies intermediately susceptible isolates as fully susceptible. Presently, MIC determinations by broth or agar dilution or by E test are the gold standard for determining vancomycin susceptibility [21]. In this study, MIC determination by E-test was used to detect vancomycin susceptibility and fortunately 100% of the isolates were found to be vancomycin susceptible.

The degree of morbidity and mortality attributable to MRSA is difficult to assess. Many studies have compared the mortality of MRSA and MSSA bacteremia with conflicting results. A meta-analysis of 31 studies by Cosgrove and colleagues concluded that bacteremia as a result of MRSA is associated with an increased mortality compared with MSSA bacteremia with an odds ratio of 1.93 (95% CI, 1.54 \pm 2.42; $P < 0.01$) [11,22].

Vancomycin is considered inferior to β -lactams for the treatment of MSSA bacteremia and endocarditis [19,23]. Therefore, the first-generation cephalosporins are the drugs of choice for the treatment of MSSA infections in patients who are unable to tolerate anti-staphylococcal penicillins. De-escalation of vancomycin to β -lactams should be encouraged in all cases of MSSA. With MRSA isolates being widespread, it is imperative that treating physicians de-escalate to β -lactams once the culture sensitivity results reveal a MSSA isolate. Preservation of glycopeptides and linezolid for use only in MRSA cases should be encouraged [19].

Conclusion

The prevalence of MRSA infection, in the present study, was found to be 20.2%. As the antimicrobial susceptibility profile of MSSA and MRSA differs considerably, the identification of MRSA using cefoxitin disc diffusion testing must be done in all microbiology laboratories. Treatment of multi drug resistant MRSA is problematic because the choice of antibiotics in such cases is very limited. The activity of all available drugs must be tested against the isolate to establish which ones could be used to treat these infections, in order to control the dissemination of such multidrug resistant bacteria.

Conflict of Interest

None identified

Table 2 Age and sex wise distribution of patients from whom MRSA were isolated.

Age	Male	Female	Total	%age
< 10 yrs	03	02	05	4.8
11-20 yrs	05	02	07	6.9
21-30 yrs	24	12	36	35
31-40 yrs	16	08	24	23.3
41-50 yrs	11	05	16	15.5
51-60 yrs	04	02	06	5.8
> 60 yrs	06	03	09	8.7
Total	69	34	103	100

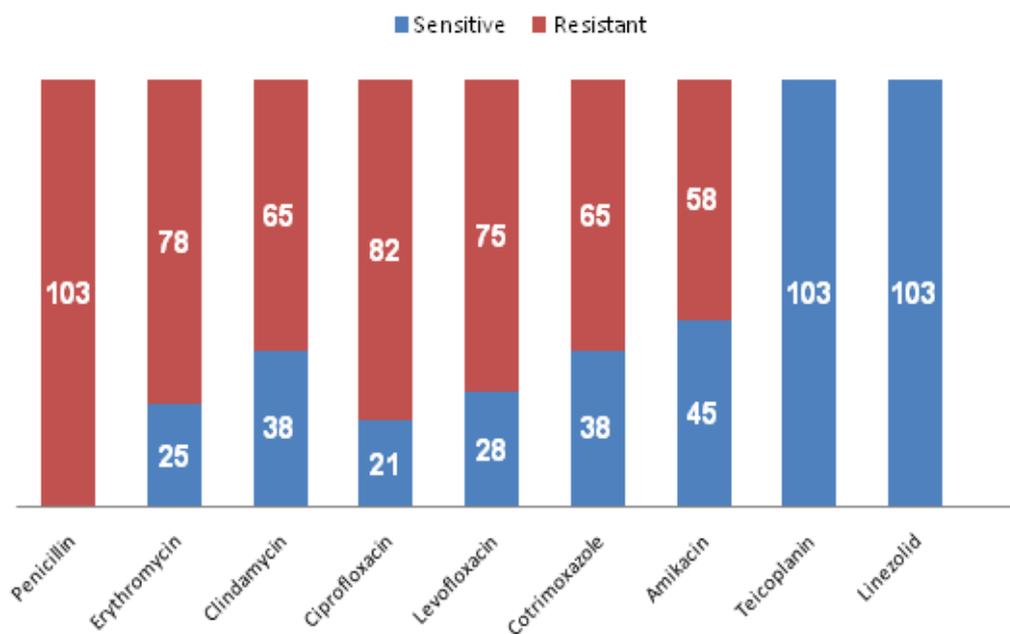


Figure 3 Antibiogram of MRSA isolates.

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