

Risk factors associated with MRSA

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is associated with increased morbidity and mortality compared with infections caused by methicillin-sensitive *Staphylococcus aureus* (MSSA). Treatment of MRSA infection is complicated by the fact that these organisms are resistant to multiple antimicrobial agents, so treatment options are limited. The aim of the present study is determine risk factors association with MRSA as compared with MSSA and to compare the minimum inhibitory concentrations (MICs) of vancomycin, teicoplanin, linezolid and erythromycin to MRSA and MSSA.

Methods: A nine-month prospective study was carried out. *Staphylococcus aureus* strains with clinical correlation, isolated from hospitalised patients, were included in the study. MIC of vancomycin, teicoplanin, linezolid and erythromycin was determined by E-test (HIMEDIA). Risk factors such as immunosuppression, previous hospitalisation, surgical procedure done, invasive devices and antibiotic therapy were determined by a pre-set protocol.

Results: A total of 62 *S. aureus* strains were included in the study. Some 40% of *S. aureus* strains were methicillin resistant. The risk factors, invasive devices, previous hospitalisation and comorbid illness were found to be significantly associated with MRSA. Borderline significant association was observed with immunosuppression and antibiotic therapy. Erythromycin resistance was observed in 56% of MRSA, while no resistance was observed in MSSA. Teicoplanin MIC₅₀ values and mean MIC were found to be lowest in vitro among vancomycin and linezolid against both MRSA and MSSA. The efficacy of teicoplanin, in terms of clinical and microbiological cure, has not been proven to be superior to vancomycin, but it has a better toxicity profile and has demonstrated a reduced risk of adverse events.

Conclusions: Minimising risk factors and attention to alternative antibiotics and infection control practices may ease the problem of management of infections with MRSA.

Keywords: MRSA, MSSA, vancomycin

Introduction

The overall MRSA prevalence in India has been reported as 42% in 2008 and 40% in 2009. MRSA is now endemic in India.¹

Recognised risk factors for MRSA acquisition have included previous hospitalisation, admission to an intensive care unit, prolonged hospital stay, proximity to another patient with MRSA, older age, invasive procedures, presence of wounds or skin lesions, and prior antimicrobial therapy.² Serious endemic and epidemic MRSA infections occur globally as infected and colonised patients in hospitals mediate the dissemination of these isolates and hospital staff assist further transmission.³

For decades, glycopeptides (vancomycin or teicoplanin) have been the mainstay for the treatment of serious MRSA infections. The efficacy of teicoplanin, in terms of clinical and microbiological cure, has not been proven to be superior to vancomycin, but it has a better toxicity profile and has demonstrated a reduced risk of adverse events.⁴ The recently developed antimicrobial drug linezolid, the first of new class of antibiotics, the oxazolidinones, is available. Its spectrum includes medically important gram-positive bacteria such as methicillin-susceptible *Staphylococcus aureus* (MSSA) and MRSA.⁵

The optimal dosing of vancomycin and teicoplanin is contentious, such that clinical failures with isolates displaying elevated minimum inhibitory concentrations (MICs) are widely reported.

However, recently, MIC creep for vancomycin among MRSA isolates has raised serious concern because patients infected by these MRSA isolates are less responsive to vancomycin.⁴

Consideration of the MIC is important to study MIC creep, determination of optimal dosing, and evaluation of the efficacy of drugs in vitro. In MSSA infection, injudicious and infrequent use of antibiotics has resulted in emergence of the strains with higher MIC.

The present study aimed to determine the risk factor association with MRSA infection as compared with MSSA and to assess and compare MICs of clinically significant isolates of MRSA and MSSA, against vancomycin, teicoplanin, linezolid and erythromycin.

Methods

A prospective study was conducted at the Department of Microbiology, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, New Delhi for nine months.

Case definition

Clinical samples from hospitalised patients suspected of having infection were collected and processed as per standard bacteriological techniques. A presumptive identification of *S. aureus* was made on the basis of colony characteristics, gram staining, and catalase and slide coagulase tests. Confirmation was done by tube coagulase test.⁶

Non-duplicate strains of *Staphylococcus aureus* with clinical correlation (clinically significant), were included in the study.

S. aureus isolates in the study fulfilled the following criteria:

- Isolation from clinical samples like pus, blood, urine, catheters, sputum, cerebrospinal fluid, and other body fluids had to be ensured.
- An isolate was considered clinically significant if it was isolated from a sterile body site (e.g. blood culture or cerebrospinal fluid, joint aspirate or pleural fluid) or from a non-sterile body site (e.g. wound, skin, urine or sputum), in the presence of symptoms or signs of infection.

Isolates were stored at -70°C in a suitable cryostorage system until testing was performed. Further detection of methicillin resistance was done on the significant isolates of *S. aureus*.

Detection of methicillin resistance

Screening for MRSA was done by oxacillin screen agar (Mueller Hinton agar with 4% NaCL and 6 $\mu\text{g/ml}$ of oxacillin).⁷

MIC testing

The MICs of vancomycin, linezolid, teicoplanin and erythromycin were determined by HiComb™ MIC Strip (Himedia Laboratories, Mumbai, India) according to the manufacturers' instructions. This system provides a set of 16 different concentrations in gradient: vancomycin (Part A: 240–0.01 μg and Part B: 4–0.001 μg), teicoplanin (Part A: 240–0.01 μg and Part B: 1–0.001 μg), linezolid (Part A: 240–0.01 μg and Part B: 8–0.001 μg) and erythromycin (Part A: 240–0.01 μg and Part B: 4–0.001 μg), which can be used to deduce a functionally accurate minimum inhibitory concentration in microgram levels. MIC susceptibility breakpoints for vancomycin, teicoplanin, linezolid and erythromycin for *S. aureus* were taken as $\leq 2 \mu\text{g/ml}$, $\leq 8 \mu\text{g/ml}$, $\leq 4 \mu\text{g/ml}$ and $\leq 0.5 \mu\text{g/ml}$ respectively as per CLSI.⁷ For erythromycin resistance the MIC breakpoint was taken as $\geq 8 \mu\text{g/ml}$.

Quality control strains

S. aureus ATCC 29213 was taken as control strain for MSSA. *S. aureus* ATCC 43300 was taken as control strain for MRSA.

Table 1: Comparative analysis of MICs of MRSA and MSSA to vancomycin, teicoplanin, linezolid and erythromycin

Antimicrobials	MIC range ($\mu\text{g/ml}$)	Mean MIC ($\mu\text{g/ml}$)	Susceptibility (%)
	MSSA		
Vancomycin	0.001–1	0.465	100
Teicoplanin	0.001–0.5	0.261	100
Linezolid	0.001–1	0.707	100
Erythromycin	0.001–0.5	0.34	100
	MRSA		
Vancomycin	0.1–2	0.812	100
Teicoplanin	0.001–0.5	0.294	100
Linezolid	0.001–1	0.983	100
Erythromycin	0.001–> 240	0.31 for sensitive strains 174 for resistant	44

Risk-factor analysis

The patients from whom clinically significant isolates of *S. aureus* were isolated were enrolled for risk-factor analysis.

Details of prior hospitalisation and antibiotic usage within 12 months, immunosuppression, history of surgical procedure done, comorbid illness and presence of any invasive device were taken in a pre-set pro-forma. Invasive devices were considered anything foreign that entered the body and had an externalised segment (i.e. urinary catheter, central vascular access, suprapubic urinary catheter, tracheostomy tube, endotracheal tube).

Statistical analysis

Descriptive statistics were used to evaluate the MIC data and included the mean, range, MIC₅₀ and MIC₉₀. Statistical analysis was carried out using the software package IBM SPSS® v.23.0 (IBM Corp, Armonk, NY, USA). The odds ratio was calculated up to the 95% confidence interval. A *p*-value of < 0.05 was considered significant.

Result

A total of 62 strains of *Staphylococcus aureus* were isolated, of which 37 (59.6%) were MSSA while 25 (40.32%) were MRSA. It was found that 51.6% of MRSA isolates were from pus, followed by invasive devices (17.7%), 12.9% from body fluids, 8% from urine and 4.8% each from blood and sputum.

Risk-factor association with MRSA as compared with MSSA was done. Previous hospitalisation, invasive devices and co-morbid illness were found to be more significantly associated with MRSA than MSSA. It was found that there was a 4.12 higher chance of MRSA if patients have co-morbid conditions (OR 4.12, 95% CI 0.13–0.45, $p = 0.001$); with the use of invasive devices there were 3.5 times higher odds of being infected with MRSA (OR 3.45, 95% CI 0.16–0.52, $p = 0.001$) and 2.0 times higher chances with previous hospitalisation of having MRSA (OR 1.92, 95% CI 0.029–0.96, $p = 0.03$), when compared with MSSA. Borderline significance ($p = 0.05$) was observed with antibiotic therapy and immunosuppression association with MRSA as compared with MSSA, while no significance was observed with surgical procedures.

All isolates were found to be sensitive to vancomycin, linezolid and teicoplanin. Erythromycin resistance was observed in 56% of MRSA, while no resistance was observed in MSSA (Table 1).

Mean MICs of vancomycin, teicoplanin and linezolid were found to be high for MRSA as compared with MSSA. For MRSA the mean teicoplanin MIC was found to be consistently lower (0.294 $\mu\text{g/ml}$) than that for vancomycin (0.812 $\mu\text{g/ml}$) and linezolid (0.983 $\mu\text{g/ml}$).

MIC₅₀ (inhibiting 50% of the strains) and MIC₉₀ (inhibiting 90% of the strains) of vancomycin, teicoplanin and linezolid were compared for MRSA and MSSA (Figure 1). Higher MIC₉₀ values of vancomycin were found against MRSA than against MSSA and were the highest amongst teicoplanin and linezolid.

Teicoplanin MIC₅₀ for MSSA was found to be lower than MRSA. Also, MIC₅₀ for linezolid was found to be lower for MSSA than MRSA (0.5 $\mu\text{g/ml}$).

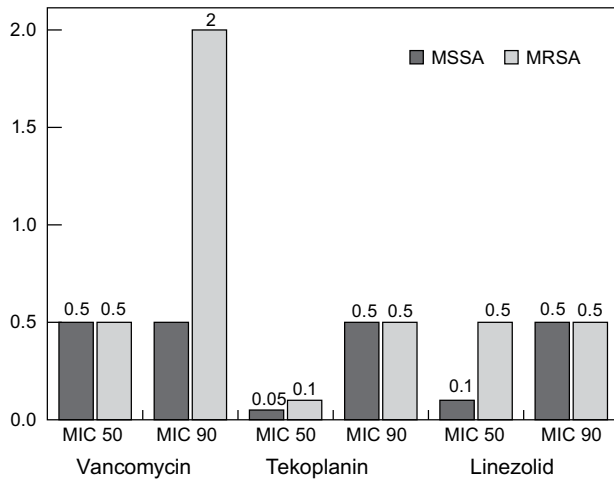


Figure 1: MIC comparison of MIC₅₀ (µg/ml) and MIC₉₀ (µg/ml) of vancomycin, linezolid and teicoplanin against MRSA and MSSA.

Discussion

Infection caused by MRSA increases the length of hospital stay and it is also responsible for raising health-care expenses, morbidity and mortality.

In the present study, MRSA isolation was found to be 40.32%. As high as 51.6% isolates are from pus, which is higher than the observation made by Mehta, who in his study on control of MRSA in a tertiary care centre had reported an isolation rate of 33% from pus and wound swabs.⁸

Previous hospitalisation has been found to be more significantly associated with MRSA infection than with MSSA in the present study. The study conducted by Mehta *et al.* found that patients staying for more than 15 days in the hospital had increased risk of infection.¹⁰

Co-morbid conditions have been found to be significantly associated with MRSA while borderline significance has been observed with immunosuppression. In a study by Srinivasan *et al.*, the presence of underlying diseases led to an earlier chance of MRSA being isolated than for those who had a history of prior antibiotic use.³ In total, 21.73% of MSSA and 20% of MRSA were found to be associated with surgical procedures. No significant association has been found with surgical procedures. The maximum number of MRSA were recovered from postoperative surgical site infections in a study conducted by Krishna *et al.*⁹

In the present study, borderline significance is found to be more associated with prior antibiotic therapy in the case of MRSA than MSSA; 60% of the patients who developed infection by MRSA had consumed antibiotics either as outpatients or as inpatients either continuously or intermittently. Ciprofloxacin usage has already been known to be associated with selection of MRSA.¹⁰ Srinivasan *et al.* in their study found 44% of the patients who developed infection by MRSA had consumed antibiotics either as outpatients or as inpatients for more than one to two weeks either continuously or intermittently. Prior antibiotic treatment primes the organism to develop resistance.³

In the present study, all isolates have been found to be sensitive to vancomycin, linezolid and teicoplanin. However, mean MICs of vancomycin, teicoplanin and linezolid were found to be higher for MRSA as compared with MSSA. A study by Chitnis *et al.*

reported MIC for vancomycin and teicoplanin among MRSA ≤ 3 µg/ml and MIC for linezolid in the range of 0.25–1 µg/ml for MRSA.¹¹ The range of MICs for vancomycin, teicoplanin and linezolid for MRSA and MSSA is lower in our study as compared with a study in Taiwan while the mean MIC of linezolid for MRSA and MSSA is consistent with a study in the UK by Gemmella.¹³

Erythromycin resistance was observed in 56% of MRSA, while no resistance was observed in MSSA. The MIC range for erythromycin for resistant strains of MRSA has been found to be 174 µg/ml, thereby ruling out its role in the treatment of MRSA and reinforcing the resistance of MRSA to multiple antibiotics. In a study done by Rajadurai *et al.*, 63.2% of MRSA were found to be resistant to gentamycin, cotrimoxazole, cephalixin, erythromycin and cephotaxim.¹⁴

MRSA with reduced susceptibility to vancomycin was first reported in 1997 in Japan.¹⁵ Resistant strains responsible for serious infections underscore the need for the development of alternative antimicrobial agents to vancomycin.

Although intermediate and high-level resistance to vancomycin has been reported,² no vancomycin intermediate sensitive *Staphylococcus aureus* (VISA) or vancomycin resistant *Staphylococcus aureus* was found in the present study. The present study showed a mean MIC value for MRSA against vancomycin to be 0.81 µg/ml, which is higher than the 0.46 µg/ml for MSSA. The highest MIC₉₀ values were observed with MRSA against vancomycin. An MIC of 2 µg/ml has been noted in three strains of MRSA isolated from blood, pus and central line tip respectively. This is of concern as the level is at the higher margin of the susceptible level. This may be a prelude to developing tolerance or frank resistance. This conforms to the study by Kishore *et al.*, where it was reported that the number of strains with higher ranges of MICs of vancomycin was greater as compared with those that had higher ranges of MICs for linezolid.⁵ Among MRSA strains for which vancomycin MICs are elevated (1–2 µg/ml or 2–4 µg/ml), failure of vancomycin therapy or reduction in its efficacy has been widely reported.^{7,8} For complicated infections (bacteraemia, endocarditis, osteomyelitis, meningitis and hospital-acquired pneumonia) and for infections caused by strains with MICs of > 1 µg/ml, trough levels of 15 to 20 µg/ml are recommended.¹⁶ MIC creep and correlation of MIC with the clinical condition of the patients needs further evaluation.

Linezolid MIC₉₀ is found to be lower in vitro against MRSA than vancomycin and similar to teicoplanin. Similarly, low MIC values for linezolid were also reported by other investigators.^{5,16} However, being bacteriostatic the drug has to be given for a longer duration, which may counterbalance its cost efficacy. Although most patients tolerate linezolid well, clinicians must be aware of potential adverse reactions, some of which are serious (anaemia, thrombocytopenia) and can be permanent (e.g. peripheral neuropathy, optic neuritis). Linezolid should be considered as an alternative to vancomycin for complicated surgical site infections (SSI), and necrotising infections, including skin lesions, fasciitis and pneumonia.^{17–19}

Teicoplanin MIC₅₀ was found to be lowest in vitro among vancomycin and linezolid against both MRSA and MSSA. For MRSA the mean MIC for teicoplanin was consistently lower (0.294 µg/ml) than that for vancomycin (0.812 µg/ml) and linezolid (0.983 µg/ml). Similar findings have been reported by Lowmen.⁴

Considering the controversy surrounding the optimal dosing of teicoplanin, it is evident that MIC is important in dose optimisation. Given the lower rate of adverse events with teicoplanin, the use of teicoplanin for the treatment of infections caused by MRSA should be considered.²⁰

Conclusion

MIC evaluation is important in dose optimisation and detection of intermediate and resistant strains of *S. aureus* to teicoplanin, linezolid and vancomycin.

The present study suggests teicoplanin and linezolid as alternatives to vancomycin in cases with higher MICs > 2 µg/ml and MIC1 –2 µg/ml associated with clinical failure, for the treatment of MRSA infection. Recent consensus guidelines also recommend that clinicians consider using alternative agents for MRSA infection when the vancomycin MIC is greater than 1 µg/ml, especially if there is evidence of clinical failure with regard to vancomycin treatment.^{16,21} In situations that limit vancomycin use, consideration of patient-specific parameters, cost and relevant clinical data demonstrating drug safety and efficacy should be employed for the selection of the appropriate alternative agent.

Attention should be paid to minimising the risk factors which pertain to limiting the use of invasive devices, antibiotic usage and hospitalisation only as and when indicated.

The key to MRSA control is early treatment of MRSA infections and the following of good infection control practices. As only limited drugs are available for the treatment of MRSA, irrational use of antibiotics should be avoided and a rational antibiotic policy must be adopted.

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