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Original Research Article

Linezolid susceptibility in MRSA isolates: insights into resistance and concordance in phenotypic detection methods

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ABSTRACT

Background: Methicillin-resistant *staphylococcus aureus* (MRSA) poses persistent threat, affecting both healthcare environment and communities, with substantial impact on infection rates, morbidity, mortality, and healthcare costs. Vancomycin, a longstanding cornerstone in MRSA treatment, but with the emergence of vancomycin resistant MRSA (VRSA), necessitating alternative antimicrobial solutions. Linezolid, stands out as a promising candidate. It has unique advantages such as an absence of renal toxicity and improved lung parenchymal diffusion compared to vancomycin, making it an appealing choice, especially for healthcare-acquired pneumonia by MRSA.
Methods: This cross-sectional study investigated linezolid susceptibility in 158 MRSA isolates using both disk diffusion and agar dilution method.
Results: Results indicated that the majority of isolates exhibited linezolid susceptibility, with 53.16% showing a minimum inhibitory concentration (MIC) of ≤2 µg/ml. However, two MRSA isolates, constituting 1.27% of the sample, displayed a MIC of 8 µg/ml, named them as a linezolid-resistant MRSA (LRSA). These findings align with previous research, mirroring resistance rates observed in different regions. Notably, vigilance against linezolid resistance is crucial, particularly due to its status as a last-resort MRSA treatment.
Conclusions: Remarkably, a 100% concordance was found between the disk diffusion and MIC methods for detecting linezolid resistance in MRSA, suggesting that the disk diffusion method may be practical choice for laboratories with heavy workloads. However, adherence to CLSI guidelines is essential, and cases of resistance by disk diffusion should be confirmed using MIC methods. Emergence of linezolid-resistant MRSA is a worrisome development, necessitating ongoing surveillance and vigilance.

Keywords: Linezolid, Linezolid resistant MRSA, Minimum inhibitory concentration, MRSA

INTRODUCTION

Methicillin-resistant *staphylococcus aureus* (MRSA) remains a pervasive threat, impacting both healthcare settings and communities, and playing pivotal role in infection rates, which in turn led to increased morbidity, mortality, and healthcare costs.[1]

Vancomycin has long been reserved for treatment of infections with MRSA. However, with the emergence of vancomycin resistant MRSA (VRSA), the status of vancomycin as gold standard to treat MRSA has been challenged. Therefore, in order to treat MRSA infections, certain novel antimicrobial medicines are introduced, in which linezolid has obvious advantages. It is the first antimicrobial of oxazolidinone group available since 2000. It is the only antibiotic available as an oral formulation for resistant Staphylococcus infection.[2] Unlike glycopeptides, linezolid achieves high levels in the epithelial lining fluid of the lungs, making it a promising candidate for treatment of patients with health care acquired pneumonia by MRSA.[3]

It acts by inhibiting bacterial protein synthesis through binding to specifically in the peptidyl transferase centre at site A in domain V of the 23S rRNA in the 50S subunit of the bacterial ribosome.[4,5]

Resistance to linezolid can occur through various mechanisms including point mutations in the 23S gene typically arising from spontaneous mutations and leading to slow transmissible resistance. A novel mechanism of linezolid resistance, involving the acquisition of the CFR-gene, which is capable of rapid spread and confers resistance to multiple antibiotics. Deletion or mutations in ribosomal protein L-3 and additional mutations in the 23S rRNA genes, as well as substitutions in the ribosomal protein L-4, have also been reported in laboratory derived linezolid resistant *S aureus* strain.[6,7] Although linezolid resistance is rare, it has been described as often associated with long-term treatments.[8]

CLSI recommended, both disk diffusion and MIC method for susceptibility testing of linezolid, but organism with resistant result by disk diffusion should be confirmed by MIC method.[9]

We have conducted this study to compare the result of disk diffusion and MIC method for detection of linezolid resistance in MRSA.

METHODS

The present 'cross-sectional study' was conducted in the Department of Microbiology, Government Medical College and Hospital, Nagpur from November 2020 to December 2022.

Samples were collected from patients suffering from different infections. Various specimens like pus/wound swab, blood, urine, sputum, tracheal aspirate, broncho-alveolar lavage, endotracheal tube secretion, ascitic fluid, pleural fluid, cerebrospinal fluid, synovial fluid, corneal scraping, etc received at the laboratory were included in the study. The quality control and rejection criteria for the inappropriate specimen were followed as per the standard guidelines.[10,11] *S. aureus* strains ATCC 25923, ATCC 51299, and ATCC 43300 were used as quality control strains for disk diffusion, MIC testing, and MRSA testing, respectively.

Specimens were processed by standard microbiological techniques.[10,12] MRSA isolates were screened by standard Kirby-Bauer disc diffusion test using 30 µg cefoxitin disk (Hi-Media, Mumbai, Maharashtra). Only the first isolate of a MRSA encountered was included in case there were repeat samples from the same patient. The study excluded the repeat isolates and colonizer isolates of *S. aureus*.

A total of 158 MRSA isolates from various clinical specimens were subjected to disk diffusion method with antibiotic disks (Hi-Media, Mumbai, Maharashtra) to determine the susceptibility against clindamycin, erythromycin, gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole (TMP-SMX), ciprofloxacin, ofloxacin, linezolid, amikacin and nitrofurantoin. In addition, MICs of vancomycin, teicoplanin and linezolid were determined by agar dilution method and that of daptomycin was determined by broth microdilution using cation-supplemented Mueller-Hinton broth (Oxoid), as recommended by the CLSI guidelines.[9]

For statistical analysis, data was entered into Microsoft excel data sheet and was analysed using SPSS 22 version software. Categorical data was represented in the form of frequencies and proportions. Kappa statistics was applied to interpret the agreement between two phenotypic methods.

RESULTS

Observed MIC values of linezolid for MRSA isolates are shown in Table 1. The result of linezolid resistance by disk diffusion method and agar dilution method were compared in Table 2. In addition, distribution of MRSA isolates based on susceptibility to different antimicrobials is given in Table 3.

Table 1: MIC of linezolid in MRSA (n= 158).

Linezolid MIC (µg/ml)	MRSA (%)
1	3 (1.89)
2	81 (51.27)
4	72 (45.57)
8	2 (1.27)
16	Nil
32	Nil
Total	158 (100)

Note: Interpretative categories and MIC breakpoints for linezolid. Sensitive- ≤4 µg/ml, Resistant- ≥8 µg/ml

Table 2: Comparison of disk diffusion and MIC method (agar dilution) for detection of linezolid resistance in MRSA.

Disk diffusion	MIC		Total
	S	R	
S	156	0	156
R	0	2	2
Total	156	2	158

Note: S- Sensitive, R- Resistant. Kappa statistical value =1

Table 3: Distribution of MRSA isolates (n=158) based on resistance to different antibiotics.

Antibiotic group	Antibiotic	No. of resistant strains (%)
A	Cefoxitin	158 (100)

Continued.

Antibiotic group	Antibiotic	No. of resistant strains (%)
	Erythromycin	105 (66.46)
	Clindamycin	54 (34.18)
	Co-trimoxazole (TMP-SMX)	65 (40.50)
B	Doxycycline	14 (8.86)
	Daptomycin	0 (0%)
	Vancomycin	0 (0%)
C	Ciprofloxacin	135 (85.44)
	Gentamicin	36 (22.78)
	Chloramphenicol	79 (50%)
U	*Nitrofurantoin	0 (0%)
O	Amikacin	56 (36.08%)
	Ofloxacin	95 (60.13%)
Inv.	Teicoplanin	0 (0%)

*Nitrofurantoin is tested against only 3 MRSA in urine. Note- Erythromycin and clindamycin susceptibility results are not routinely reported on organisms isolated from the urinary tract and CSF. But, in the present study, they have been included to study their susceptibility pattern on the isolates.

DISCUSSION

The advantage of linezolid is absence of renal toxicity and better pulmonary parenchymal diffusion as compared to vancomycin.[13]

In the present study, linezolid susceptibility was detected by agar dilution method. Maximum number of isolates (53.16%) showed MIC of ≤2 µg/ml followed by isolates with MIC 4 µg/ml (45.57%). Two (1.27%) MRSA isolates had MIC of 8 µg/ml were termed as LRSA (Table 1).

Our research findings show similarities to studies conducted by Khanam et al in Bangladesh and Mamtora et al in Mumbai, where they observed 2.6%, and 2% of MRSA strains, respectively, exhibiting resistance to linezolid.[2,14] Additionally, Morales et al from Spain, identified 12 patients in the intensive care unit and 3 patients in other wards infected with a MRSA that displayed resistance to linezolid (MIC range- 16 mg/l to 32 mg/l).[6]

Some studies have reported higher rates of linezolid resistant MRSA. For example, Wali et al from Pakistan reported, among 85 MRSA isolates, 35% were linezolid resistant. MIC level of 128 µg/ml was observed among 3.5% of the LRSA isolates. Similarly, MIC level of 64 µg/ml, 32 µg/ml, 16 µg/ml and 8 µg/ml were noted for 3.5%, 4.7%, 8.2% and 15.3% isolates respectively.[15] Singh et al in Southern Rajasthan, Mandal et al in Bihar and Hussain et al in Delhi found 12%, 1.85%, and 7.55% linezolid resistant MRSA respectively in their studies.[16-18]

In contrast to our study, some studies reported 100% sensitivity to linezolid in MRSA isolates.[16,19-21]

The emergence of linezolid resistant MRSA in our study may be attributed to empirical and prolonged linezolid therapies. Linezolid is a valuable oral antibiotic for a MRSA treatment, particularly for outpatient care. However, it's noteworthy that up to a quarter of patients prescribed with oral linezolid are generally non-compliant with the treatment regimen. In our institution, all cases of linezolid resistant MRSA with available clinical data indicated prior exposure to linezolid.

In the present study, 156 MRSA strains were found to be sensitive to linezolid by disk diffusion method which were also sensitive by the MIC method (agar dilution). Two MRSA strains were linezolid resistant by both disk diffusion and MIC method (Table 2). Complete concordance has been observed between disk diffusion and MIC method for detection of linezolid resistance in MRSA. Kappa statistical value is calculated as 1 which signifies perfect agreement between two methods. Similar findings were also reported by Kakhandki et al in Karnataka and Thool et al in Central India who observed concordance between disk diffusion and MIC method of linezolid susceptibility in MRSA.[22,23] In a study by Azhar et al in Pakistan, linezolid resistance detected by disk diffusion test and MIC method was 48.1% and 46.3% respectively. Thus, they also found the values to be very close for the disk diffusion method and MIC method (E-test).[24]

In the present study, among the MRSA isolates, maximum isolates showed resistance to the antimicrobials of quinolone class (for ciprofloxacin 85.44% and for ofloxacin 60.13%) followed by macrolide class (erythromycin 66.46%) and folate pathway antagonist class (40.50% for cotrimoxazole). While resistance against chloramphenicol, amikacin, clindamycin, gentamicin, and doxycycline was i.e. 50%, 36.08%, 34.18%, 22.78%, and 8.86% respectively. None of the isolate was resistant to nitrofurantoin, vancomycin, teicoplanin, and daptomycin (Table 3).

These findings are comparable to the study done by Mamtora et al[14] who also reported MRSA isolates showing high resistance towards quinolone class (ciprofloxacin- 89.8%), macrolide class (erythromycin-76%) and folate pathway antagonist class (cotrimoxazole-60.8%). Other workers also reported high resistance in MRSA isolates for various antimicrobials.[25,26]

The higher resistance among MRSA for quinolones (ciprofloxacin, ofloxacin), macrolide (erythromycin) and sulfonamide (cotrimoxazole) might be due to the evident fact that, MRSA possesses multi-drug resistance genotype, including quinolones, macrolides and folate pathway antagonist class.[27] Further, as these drugs are relatively cheaper and easily available over-the-counter in India, there have been found indiscriminate and empirical use of these drugs.

The present study is not without its constraints, primarily attributed to a relatively small sample size, which may impact the generalizability of our findings. It is important to note that we did not undertake a direct comparison of the MIC of linezolid with automated methods, introducing a potential source of variability. Additionally, the absence of genotyping analysis for MRSA isolates constitutes a limitation, as it prevents a more nuanced exploration of strain-specific characteristics that could have influenced our results. These limitations should be taken into consideration when interpreting the implications of our study.

CONCLUSION

As 100% concordance found between disk diffusion and MIC method in detection of linezolid resistance in MRSA, one can choose disk diffusion method in laboratory with heavy workload since determination of MIC is tedious and cumbersome. CLSI states that *S. aureus* showing resistance by disk diffusion should be confirmed using MIC method. The detection of few linezolid-resistant MRSA isolates is worrying as linezolid is one of the last resorts for MRSA. One has to keep strict vigilance and surveillance for linezolid resistance.

Interestingly, most of the multidrug resistant MRSA isolates were found susceptible to gentamicin, clindamycin and doxycycline. So, considerations of conventional antibiotics are also necessary with newer antibiotics during such treatment.

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