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BRIEF REPORT

In-vitro susceptibility of linezolid against methicillin resistant *Staphylococcus aureus* at a tertiary care hospital in Pakistan

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ABSTRACT

Objective: Methicillin resistant *Staphylococcus aureus* is a major nosocomial pathogen causing significant morbidity and mortality. The aim of this study was to evaluate the in vitro activity of linezolid against methicillin-resistant *S. aureus*.

Methods: This was a descriptive study carried out at the Department of Microbiology, Army Medical College Rawalpindi from January to July 2010. The in vitro minimum inhibitory concentration of linezolid was determined against 74 strains of methicillin-resistant *S. aureus* by using the Epsilon- test (E-test) method (AB Biodisk, Sweden). Methicillin-resistant *S. aureus* was isolated from routine clinical specimens using standard microbiological procedures. Cefoxitin (30 µg) disk was used for detection of methicillin-resistant strains of *Staphylococcus aureus*.

Results: Seventy-four isolates of methicillin-resistant *S. aureus* were obtained from various clinical samples. The majority of samples were from the pus followed by nasobronchial lavage, urine and vaginal swabs. All the isolates were highly susceptible to linezolid with minimum inhibitory concentration range of 0.023 - 0.75 mg/dL having MIC50 0.25 and MIC90 0.5 mg/dL respectively.

Conclusion: Linezolid shows good in vitro activity against methicillin-resistant *Staphylococcus aureus*. *J Microbiol Infect Dis 2013;3(4): 203-206*

Keywords: Linezolid, methicillin resistance, minimum inhibitory concentration, *Staphylococcus aureus*, vancomycin.

Pakistan'da bir üçüncü basamak hastanede metisilin dirençli *Staphylococcus aureus*'a karşı in-vitro linezolid duyarlılığı

ÖZET

Amaç: Metisilin dirençli *Staphylococcus aureus* belirgin morbidite ve mortaliteye neden olan önemli bir nozokomiyal patojendir. Bu çalışmanın amacı, metisilin dirençli *S. aureus*'a karşı linezolidin in vitro etkinliğini değerlendirmektir.

Yöntemler: Army Medical College Rawalpindi Mikrobiyoloji Anabilim Dalında, Temmuz-Ocak 2010'da yürütülen tanımlayıcı bir çalışmadır. Linezolid için in vitro minimum inhibe edici konsantrasyon, metisiline dirençli 74 *S. aureus* türü için Epsilon- test (E-test) metodu (AB Biodisk, İsveç) kullanılarak belirlendi. Metisiline dirençli *S. aureus* standart mikrobiyolojik yöntemler kullanılarak rutin klinik örneklerden izole edilmiştir. Metisilin dirençli *S. aureus* suşlarının saptanması için sefoksitin (30 µg) disk kullanılmıştır.

Bulgular: Metisilin dirençli 74 *S. aureus* izolatı çeşitli klinik örneklerden elde edilmiştir. Numunelerin çoğunluğu nasobronchial lavaj, idrar ve vajinal sürüntülerden alınan püydü.

Tüm izolatların 0.023-0.75 mg/dL minimum inhibitör konsantrasyon aralığında sırasıyla MIC50 0.25 ve MIC90 0.5 mg/dL ile linezoilde son derece duyarlı idi.

Sonuç: Linezolid metisiline dirençli *S. aureus* karşı i in vitro iyi aktivite gösterir.

Anahtar kelimeler: Linezolid, metisilin direnci, minimum inhibitör konsantrasyon, *Staphylococcus aureus*, vankomisin

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INTRODUCTION

Over the last two decades, the increasing incidence of methicillin-resistant Staphylococci has caused significant clinical concern worldwide. Methicillin resistance in *S. aureus* (MRSA) is also associated with resistance to several commonly used antimicrobial agents such as the macrolides, lincosamides, quinolones, trimethoprim-sulfamethoxazole and aminoglycosides.[1] During the past 15 years, the appearance and world-wide spread of epidemic clones of MRSA have caused major therapeutic problems in many hospitals, as well as diversion of considerable resources to attempts at controlling their spread.[2] With the increasing incidence of multi-drug resistant Staphylococci and the emergence of resistance to glycopeptides in MRSA worldwide, therapeutic options are becoming increasingly limited.

Resistance to all the available antibiotics against Staphylococcus except vancomycin has been reported.[3] The undesirable side effects of vancomycin make it unsuitable in a subset of patients. After the emergence of vancomycin resistant Enterococci, it was feared that this resistance may spread to Staphylococci which proved to be the case when low level vancomycin resistance was reported in Staphylococci.[4] High level resistance against vancomycin was reported soon after,[5,6] making the treatment of infections by this organism a therapeutic dilemma.

Linezolid is the first approved member of a new generation of antibiotics, synthetic oxazolidinone. These are broad spectrum antibiotics active against a wide variety of Gram-positive organisms, including methicillin resistant Staphylococci, penicillin resistant Pneumococci and the vancomycin resistant Enterococci.[7-9] Linezolid has a bacteriostatic action predominantly by binding to the 50S ribosomal subunit and ultimately inhibiting bacterial protein synthesis by interfering with the formation of the initiation complex in bacterial translation systems.[10] As this agent possesses a novel structure and unique mechanism of action, it does not display cross-resistance with other classes of antimicrobial agents.1 The Clinical and Laboratory Standards Institute11 (CLSI) has established indications for linezolid use and breakpoint interpretive criteria of ≤2 mg/L as susceptible for Streptococci or Enterococci and ≤4 mg/l for Staphylococci.

Linezolid therapy was shown to be successful for MRSA infection in patient with a severe allergic reaction to vancomycin.[12] Plasma concentrations of intravenous and oral linezolid are equivalent, with an average concentration exceeding the MIC for susceptible pathogens throughout the 12 h dosing interval.[10] The excellent oral bioavailability of linezolid and a much lower cost as compared to vancomycin and teicoplanin makes it an extremely attractive antibiotic for the treatment of suspected or confirmed Staphylococcal infections.

The objective of the study was to assess the MIC of Linezolid against MRSA to highlight the efficacy of this therapeutic agent against this deadly organism in our setting.

METHODS

The study was carried out in the Department of Microbiology at Army Medical College, National University of Sciences and Technology, Rawalpindi, Pakistan. A total of 74 clinical strains of methicillin-resistant *S. aureus* were collected from January 2010 to July 2010 from patients attending at the Military Hospital, Rawalpindi. These isolates were identified by conventional methods 11 including colony morphology and hemolysis on 5 per cent sheep blood agar, Gram's staining, catalase production, DNAse and coagulase test. Methicillin resistance of isolated Staphylococci was detected by agar disk-diffusion method (Kirby-Bauer) using a 30 µg cefoxitin disc according to the guidelines established by CLSI.

Staphylococcal isolates were sub cultured on blood agar and after overnight incubation at 37 0C; three to five morphologically similar colonies were then emulsified in sterile isotonic saline. The suspension was adjusted to 0.5 McFarland standards (106 CFU/ml). In order to determine the activity of linezolid, Mueller-Hinton agar plates were inoculated by swabbing of the surface with the suspension of organisms. E-test (AB-Biodisk, Sweden) strips containing linezolid (range 0.016-256 mg/L) were applied onto the surface of the agar. After incubation for 24 h at 37°C in ambient air, the MIC was read directly from the intersection of the inhibition zone with the test strip MIC scale. Results of MIC were interpreted according to the breakpoints given by CLSI 2009 where MIC ≤4 mg/L was taken as susceptible. *S. aureus* ATCC 29213 reference strain was used as control. Results were interpreted by using SPSS version 17.0 and the quantitative variables like the MIC50 and MIC90 were calculated.

RESULTS

The activity of linezolid against the 74 MRSA strains tested is shown in the table (Table 1). All the 74 isolates were found to be highly susceptible to linezolid

(MICs <4.0 mg/L). The range of minimum inhibitory concentration for the strains was 0.023-0.75 mg/L with MIC50 and MIC90 of 0.25 and 0.5 mg/L respectively. Majority of the tested strains were isolated from the pus samples [44 (60%)]) followed by nasobronchial lavage [18 (24%)], urine [8 (10%)] and vaginal swabs [4 (6%)] .

Table 1. Minimum inhibitory concentrations of linezolid against MRSA (n=74)

Isolates (n)	Antimicrobial agent	% of isolates Susceptible at MIC (mg/L)													Concentrations (mg/L)		
		0.016	0.023	0.032	0.064	0.094	0.125	0.19	0.25	0.38	0.50	0.75	1	2	Range	MIC_{50}	MIC_{90}
MRSA	Linezolid	-	2	2	6	8	4	12	26	24	12	4	-	-	0.023-0.75	0.25	0.5

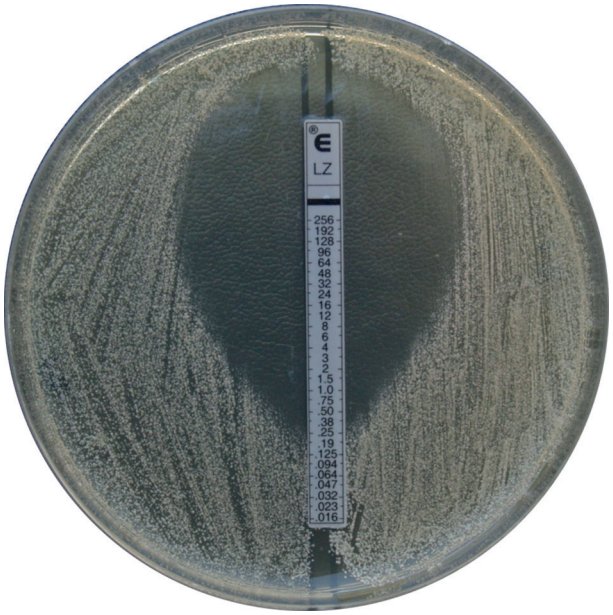

Figure 1. Linezolid E-strip

DISCUSSION

Methicillin-resistant *S. aureus* is a major nosocomial pathogen causing significant morbidity and mortality. Methicillin resistant Staphylococcus has become a global problem limiting the treatment modalities to a large extent. Once the β-lactam fails, the main option against methicillin-resistant Staphylococcus (MRSA) infections is the use of glycopeptides, vancomycin and teicoplanin.[13] However, the emergence of clinical infection due to MRSA with decreased susceptibility to vancomycin is a recent and certainly worrying fact. Treatment of Staphylococcus infections has become more difficult because of multidrug-resistant strains that are resistant to ≥3 antibiotics tested at the same time.[14]

Parenteral vancomycin has so far been the only reliable treatment option in many cases of serious MRSA infections.[15] However, *S. aureus* with intermediate resistance to vancomycin [VISA] (MIC 8 mg/L), was first reported from Japan and then from the United States. This has given the cause for alarm in the health care community.[16,17] In addition to that, vancomycin-resistant Staphylococcal strains (VRSA) have been reported from Brazil, Jordan and India.[18-20] Shajari et al reported that 18.4% of the methicillin-resistant *S. aureus* strains were resistant to vancomycin.[21] This situation warranted the urgent search for new, safe and reliable agents for the treatment of infections caused by multidrug-resistant Gram-positive pathogens. Therefore, the development and subsequent approval of linezolid for clinical use against Gram-positive bacteria is a great achievement.

The excellent in vitro activity of linezolid found in this study agrees with the findings of previous studies,[22] although the MICs are slightly lower than those reported by Afşar et al.[23] A previous study done in Lahore, Pakistan also had similar results.[13] Mouton and Jansz reported that MIC90 of 1.5 mg/L for linezolid for *S. aureus* and cross-resistance with other antibiotics was not detected in their study.[24] Cuevas et al studied 866 Staphylococcal isolates (463 *S. aureus* strains) from 1986 to 2006 in Spain and found that only one strain was linezolid-resistant.[25] Linezolid has an acceptable safety profile for both intravenous as well as oral administration and has proven to be effective in the treatment of infections due to methicillin-resistant Staphylococcal species in critically ill patients. It is a good alternative to vancomycin in the acute renal injury patients.[16] All of these data, together with the results of this study, suggest that linezolid could be useful for the treatment of infections due to methicillin-resistant *S. aureus*.

In conclusion, this study demonstrates that linezolid has an excellent in vitro activity against methicillin-resistant *S. aureus*. The MRSA was most prevalent in the pus samples and the MIC90 was 0.5 mg/dL. This drug is a promising therapeutic option in an era of rapidly growing antibiotic resistance.

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