

IN VITRO SUSCEPTIBILITY OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TO LINEZOLID BY E-TEST METHOD

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SUMMARY

The incidence of methicillin-resistant gram-positive infections continue to increase and linezolid may provide new options to treat patients. The aim of this study was to investigate the in vitro susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from clinical specimens to linezolid. The in vitro susceptibility of a total of 88 MRSA isolates to linezolid was investigated by the E-test. All MRSA isolates were found to be susceptible to linezolid (Minimal inhibitory concentration < 4.0 mg/l). The results of the present study demonstrated that linezolid has a good in vitro activity against MRSA in Turkey. This drug could be a promising therapeutic option against MRSA.

Keywords: in vitro susceptibility, linezolid, methicillin-resistant *Staphylococcus aureus*

ÖZET

E-test Yöntemiyle Metisiline Dirençli *Staphylococcus aureus* suşlarının Linezolide in-vitro Duyarlılığı

Metisiline dirençli Gram pozitif infeksiyonların insidansındaki artış devam etmekte ve linezolid hastalara yeni tedavi seçeneği sağlamaktadır. Çalışmanın amacı; klinik örneklerden soyutlanan metisiline dirençli *Staphylococcus aureus* (MRSA) suşlarının linezolide in-vitro duyarlılığını araştırmaktır. Toplam 88 MRSA suşunun linezolide in-vitro duyarlılığı E-test yöntemiyle araştırılmış, tüm suşlar duyarlı bulunmuştur (MİK<4.0 mg/l). Bu çalışmadaki sonuçlar linezolidin Türkiye'deki MRSA suşlarına in-vitro iyi etkinliği olduğunu göstermektedir. Bu ilaç MRSA infeksiyonlarında iyi bir tedavi seçeneği sağlamaktadır.

Anahtar sözcükler: in-vitro duyarlılık, linezolid, metisiline dirençli *Staphylococcus aureus*

INTRODUCTION

Methicillin-resistant gram-positive cocci have emerged and become increasingly a problematic cause of hospital-acquired infections. Linezolid is the first approved member of a new generation of antibiotics, synthetic oxazolidinone. Oxazolidinones are broad spectrum antibiotics with in vitro activity against gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), and linezolid has a bacteriostatic action predominantly⁽¹⁾. There are numerous studies which showed that the E-test, disk diffusion and broth microdilution methods were comparable in accuracy for the

susceptibility testing of MRSA against linezolid⁽¹⁾. Clinical and Laboratory Standards Institute (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⁽²⁾.

MATERIALS AND METHODS

A total of 88 isolates of MRSA strains were recovered from blood cultures of the patients hospitalized for longer than 48 h in the hospital, from February to September 2007. These isolates were identified by conventional methods. These

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methods include colony morphology and haemolysis on 5 per cent sheep blood agar, Gram's staining, catalase production, and coagulase reaction. The commercial identification system, BBL Crystal MRSA ID (Becton Dickinson), was also carried out according to the manufacturer's instructions. Methicillin resistance of isolated staphylococci was detected by agar disk-diffusion method (Kirby-Bauer) according to guidelines established by CLSI. In order to determine the activities of linezolid, Mueller-Hinton agar plates supplemented with 2 % NaCl were inoculated by swabbing of the surface with a suspension of organisms adjusted to 0.5 McFarland opacity standard. 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 22 to 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. CLSI breakpoints were used to interpret MICs (MIC \leq 4 mg/l as susceptible)⁽³⁾. *S.aureus* ATCC 29213 reference strain was used as control.

RESULTS

All of the 88 MRSA isolates were found susceptible to linezolid (MICs < 4.0 mg/l). All isolates showed a value of MIC between 0.125 mg/l and 1 mg/l (Table).

Table. The MIC values of MRSA isolates.

MIC value (mg/l)	Number of isolates
0.125	1
0.25	3
0.38	16
0.50	40
0.75	26
1	2

DISCUSSION

MRSA is a nosocomial pathogen that causes morbidity and mortality worldwide. The treatment of the MRSA infections has limited options. Vancomycin remains the reference standard for the treatment of systemic infection caused by

the MRSA. However, vancomycin has limited tissue distribution, also the emergence of isolates with reduced susceptibility and in vitro resistance to vancomycin, resulted in need of new antibiotics. Linezolid is an antibacterial agent of oxazolidinone group and has a spectrum limited to gram-positive bacteria^(6,7). Linezolid is a new option for MRSA and our study has shown that linezolid has a good in vitro effect to MRSA. All MRSA strains were susceptible to linezolid. Previous studies support our study reporting that linezolid has a good in vitro activity to MRSA. Mouton and Jansz⁽⁸⁾ reported that the values of MIC₉₀ for linezolid was 1.5 mg/l for *S.aureus* and cross-resistance with other antibiotics was not detected. Cuevas et al⁽⁴⁾ studied 866 staphylococcal isolates (463 *S.aureus*) from 1986 to 2006 in Spain and found that only one strain was linezolid-resistant. This study showed that linezolid resistance was emerging. Another study from Norway declared that 214 *S.aureus* strains were susceptible to linezolid by the E-test, and the values of MIC₅₀ and MIC₉₀ were found as 0.25 and 2.0 mg/l, respectively. The result of this study showed a high prevalence of in vitro susceptibility to linezolid which is necessary for its use in the treatment of infections with resistant gram-positive pathogens including *S.aureus*⁽⁹⁾. Our study determined the value of MIC as 0.125-1 mg/l and the most frequently detected MIC value was 0.50 mg/l. Zhanel et al⁽¹²⁾ found the value of MIC₉₀ as 2 mg/l for MRSA and suggested that linezolid was a good choice for Canadian intensive care unit patients. Dizbay et al⁽⁵⁾ found that all 120 MRSA isolates studied were susceptible to linezolid. Another study from Turkey determined that all 124 MRSA strains were susceptible to linezolid and the value of MIC₉₀ was 0.5-4 mg/l⁽¹⁰⁾.

In conclusion; the results of the present study demonstrate that linezolid has a good in vitro activity against MRSA in Turkey and this drug can be a promising therapeutic option against MRSA infections.

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