In vitro susceptibility to methicillin, vancomycin and linezolid of staphylococci isolated from bloodstream infections in eastern Turkey

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Abstract

Staphylococcus species are one of the major causes of bacterial bloodstream infections. Multi-resistant staphylococci infections are major therapeutic problems. This study was aimed to detect methicillin, linezolid and vancomycin susceptibilities of Staphylococcus isolates. A total of 870 Staphylococcus strains isolated from blood cultures of hospitalized patients with BSI. Antimicrobial susceptibilities of methicillin, linezolid and vancomycin were detected according to the Clinical and Laboratory Standards Institute (CLSI). A total of 771 (88.6%) isolates were coagulase-negative staphylococci (CoNS). 700 (80.5%) isolates were methicillin-resistant (MR) and 170 (19.5%) were methicillin-susceptible (MS). All the MS isolates were also susceptible to linezolid. However 15 (1.7%) of MR strains were resistant to linezolid. The minimum inhibitory concentration range for the linezolid-resistant isolates by Etest was 6-32 μg/mL. The difference between linezolid susceptibilities for MS and MR staphylococci was not quite statistically significant (p = 0.052). There was no statistically significant difference between S. aureus and CoNS isolates for linezolid susceptibility. All of the isolates were susceptible to vancomycin. In conclusion, linezolid is currently an efficient option for the treatment of methicillin-resistant staphylococci infections.

Key words: Staphylococcus, methicillin, linezolid, vancomycin.
Moreillon, 2010). These problematic pathogens have acquired resistance to methicillin and some of which to many non-beta-lactam antibiotics (Boyle-Vavra and Daum, 2007; Niemeyer et al., 1996; Winn et al., 2006). Methicillin resistance in staphylococci is caused by expression of the penicillin-binding protein 2a (PBP2a) encoded by the mecA gene which is located in the staphylococcal cassette chromosome mec (SCCmec) and can be transmitted via horizontal transfer between Staphylococcus species (Boyle-Vavra and Daum, 2007; Niemeyer et al., 1996; Yok-Ai Que and Moreillon, 2010). Currently, over 80% of clinical Staphylococcus isolates throughout the world are resistant to penicillin due to β-lactamase production (Winn et al., 2006). Until recently, glycopeptides such as vancomycin were the first choice drug to treat infections caused by methicillin-resistant staphylococci (Yok-Ai Que and Moreillon, 2010). However, vancomycin-resistant Staphylococcus isolates have been detected in various countries (Centers for Disease Control and Prevention (CDC), 1997, 1999, 2002, 2004; Gemmell et al., 2001; Hiramatsu et al., 1997; Palazzo et al., 2005).

Linezolid was introduced by Ford et al. (1997) as a member of the oxazolidinone class of antibiotics. This antibiotic demonstrates potent antimicrobial activity against most multi-resistant Gram-positive microorganisms, including methicillin-resistant coagulase-negative staphylococci (MRCNS), methicillin-resistant S. aureus (MRSA), multidrug-resistant (MDR) Streptococcus pneumoniae and vancomycin-resistant enterococci (VRE) (Ford et al., 1997). Linezolid inhibits bacterial protein synthesis by binding to the domain V region of 23S rRNA at an early step, and prevents the formation of the N-formylmethionyl-tRNA-mRNA-70S ribosomal tertiary complex. Mutations in the central loop of the domain V region are the most frequent causes of linezolid resistance (Khan et al., 2012; Kloss et al., 1999).

This study presented here aimed to detect methicillin, linezolid and vancomycin susceptibilities for CoNS and S. aureus isolates collected from blood cultures in Dicle University Hospital, Diyarbakir-Turkey.

A total of 870 staphylococcal isolates collected from blood cultures of hospitalized patients in Dicle University Hospital between January 2007 and August 2011 were included retrospectively in this study. Dicle University Hospital is a tertiary care center with capacity of 1400 beds. Among 870 staphylococcal isolates included in this study, 442 were collected from adult patients (ranged 17-65 years), 237 from pediatric patients (≤ 16 years) and 191 from newborn babies admitted into the neonatal intensive care unit (NICU). Only isolates from clinically significant BSI (one isolate per patient) were included in this study. Clinically significant BSI can be diagnosed when two or more of the following criteria are present with positive blood cultures taken from both arms: -body temperature ≤ 36 °C or ≥ 38 °C; -heart rate > 90 beats/min; -respiratory rate > 20 breaths/min or an arterial partial pressure of carbon dioxide (PaCO₂) < 4.3 kPa (32 mmHg); -leukocytes < 4,000 cells/mm³ (4x10⁹ cells/L) or > 12,000 cells/mm³ (12x10⁹ cells/L) or > 10% immature neutrophils (band forms) (American college of chest physicians / society of critical care medicine consensus conference, 1992). Blood culture bottles were incubated in Bactec™ BD 9120 and 9240 (Becton Dickinson, MD, USA) automated blood culture systems at 37°C for 7-10 days. After growth, the culture was inoculated onto 5% sheep blood agar (Oxoid Ltd., Basingstoke, UK) and the plate incubated at 35 ± 2 °C for 18-24 h. Isolate identification was performed by routine methods of Gram staining, catalase activity, slide and tube coagulase tests, DNAse test, and also BD Phoenix™ 100 (Becton Dickinson, MD, USA) the fully automated microbiology system by using the manufacturers’ protocol.

Antimicrobial susceptibility testing for methicillin and linezolid were performed by Kirby-Bauer’s disk diffusion method and BD Phoenix™ 100 the fully automated microbiology system by using the manufacturers’ protocol according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) (2009). Methicillin susceptibility was investigated with 1 μg oxacillin and 30 μg cefoxitin disk (Oxoid Ltd., Basingstoke, UK). Linezolid susceptibility was tested using 30 μg linezolid disk (Oxoid Ltd., Basingstoke, UK) and linezolid resistance was confirmed by Etest strips (bioMerieux SA, Marcy l’Etoile, France) (Abb, 2002; CLSI, 2009). In addition, vancomycin susceptibility was also investigated using Etest strips and the fully automated microbiology system according to the CLSI breakpoints (2009) For disk diffusion method and Etest strips, a 0.5 McFarland standard suspension was inoculated onto Mueller-Hinton agar (Merck KGaA, Darmstadt, Germany) plates as described by CLSI (2009), MHA plates were incubated at 35 ± 2 °C for 24 hours. Inhibition zone diameter was measured at 24 hours in transmitted light for linezolid.

For methicillin susceptibility, in accordance with CLSI guidelines, inhibition zone diameter for oxacillin ≥ 13 mm was considered as susceptible, 11-12 mm as intermediate, ≤ 10 mm as resistant; for cefoxitin ≥ 22 mm was considered as susceptible, ≤ 21 mm as resistant for S. aureus and S. lugdunensis, ≥ 25 mm was considered as susceptible, ≤ 24 mm as resistant for the other CoNS except S. lugdunensis. Inhibition zone diameter for linezolid ≥ 21 mm was considered as susceptible, < 21 mm as resistant. MIC value for linezolid ≤ 4 μg/mL was considered as susceptible, > 4 μg/mL as resistant. According to the MIC interpretive standard of CLSI, MIC value for vancomycin ≥ 2 μg/mL was considered as susceptible, 4-8 μg/mL as intermediate, ≥ 16 μg/mL as resistant.

S. aureus ATCC 25923 was used for quality control in the fully automated microbiology system, Etest strips and disk diffusion method. Data were analyzed by Epi
A recent study in Japan (Hiramatsu et al., 1986) reported approximately one two-fold dilution lower than the mean microdilution MIC. Also Tenover et al. (2007) reported that further studies of the agar-based methods (disk diffusion method and Etest) are needed to better define the optimal endpoints for interpreting results of testing for linezolid resistance. Arias et al. (2008) stated that disk diffusion susceptibility tests or Etest might not detect cfr-mediated linezolid resistance when standard procedures are used and that a longer time of incubation may be needed.

Thus, linezolid is still an effective agent for staphylococcal isolates obtained from blood culture in our hospital.
Table 1 - Bacterial species and hospital setting for methicillin-linezolid resistant staphylococcal isolates.

| Isolate no | Species     | Inpatient Clinics       |
|------------|-------------|-------------------------|
| DUH1       | S. kloosii  | Plastic Surgery         |
| DUH2       | S. aureus   | Nephrology              |
| DUH3       | S. hyicus   | Pediatric Infectious Diseases |
| DUH4       | S. cohnii   | Oncology                |
| DUH5       | S. cohnii   | Chest Diseases          |
| DUH6       | S. kloosii  | Neonatal Intensive Care Unit |
| DUH7       | S. cohnii   | Cardiology              |
| DUH8       | S. kloosii  | Neonatal Intensive Care Unit |
| DUH9       | S. schleiferi | Neonatal Intensive Care Unit |
| DUH10      | S. kloosii  | Pediatric Infectious Diseases |
| DUH11      | S. capitis  | Neonatal Intensive Care Unit |
| DUH12      | S. kloosii  | Neonatal Intensive Care Unit |
| DUH13      | S. capitis  | Endocrinology           |
| DUH14      | S. cohnii   | Adult Infectious Disease |
| DUH15      | S. hominis  | Hematology              |

Data from LEADER surveillance program in USA, from linezolid program (ZyvoxR Annual Appraisal of Potency and Spectrum; ZAAPS) in European countries and from many studies in Spain and in our country found that linezolid sensitivity rates in staphylococci were approximately 99% (Jones et al., 2007; Ross et al., 2011). It is important to remark that five linezolid-resistant S. kloosii isolates were obtained from hospitalized patients in the plastic surgery (one isolate), pediatrics clinic (one isolate) and neonatal intensive care unit (NICU; three isolates). This result seems to indicate the occurrence of S. kloosii small outbreak in the NICU of the studied hospital. Recently, Peer et al. (2011) reported a case of sepsis with an intracranial bleed in a 60-year-old male from whom linezolid-resistant S. kloosii was repeatedly isolated from blood cultures, demonstrating the potential of this staphylococcal species to cause BSI in immunocompromised host. *S. epidermidis* was not detected among the linezolid-resistant isolates in the present study. However, it is possible that the fully automated identification system used may have failed to detect some of the resistant isolates.

Due to the emergence of vancomycin-intermediate and -resistant isolates, poor tissue penetration, and the fact that vancomycin must be given intravenously, new therapeutic options are necessary for the treatment of staphylococcal infections. Our data showed that, in our hospital, linezolid can be a therapeutic option for treatment of infections cause by both MRCoNS and MRSA. Linezolid is a well-tolerated agent currently available in oral and intravenous forms. The oral form is 100% bio-available and the earlier switching from intravenous to oral therapy is possible (Weigelt et al., 2005). In a cohort study of Lodise et al. (2008), the incidence of nephrotoxicity with higher daily (≥4 g/day) and lower daily doses of vancomycin < 4 g/day were compared with nephrotoxicity of linezolid. According to this study, nephrotoxicity was significantly higher in the high-dose vancomycin group (34.6%) when compared with the low-dose vancomycin group (10.9%) or with the linezolid group (6.7%).

In conclusion, the treatment of methicillin-resistant staphylococcal infections has been a growing problem in both hospital- and community-acquired diseases. For this reason, alternative antimicrobial agents with anti-staphylococcal activity are needed. The results presented here are in line with data of previous national and international investigations suggesting that linezolid is an efficient option for the treatment of a number of infections caused by methicillin-resistant staphylococci (Lin et al., 2008; Weigelt et al., 2005; Zorgani et al., 2012). Finally, due to the increasing antimicrobial resistance among staphylococcal isolates, resistance rates should be reported periodically.

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