

Investigation of linezolid resistance in *Staphylococcus epidermidis*: first reported linezolid resistant coagulase negative staphylococcus in Turkey*

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Linezolid is a member of oxazolidinons and it inhibits protein synthesis at the initiation step. In this step, formation of 70S complex occurs by association of 50S ribosomal subunit, and 30S initiation complex, which is made of 30S ribosomal subunit, mRNA, and methionine carrying tRNA. Linezolid prevents this formation by binding the 50S subunit (1-3).

Although it is a new agent, resistance has been reported from several centers worldwide, but it is still rare. Target modification is the main factor in linezolid resistance. Mutations in V domain of 23S rRNA, and ribosomal proteins such as L3 and L4 emerge as resistance (4,5). Nonmutational oxazolidinon resistance is due to the chloramphenicol-florfenicol resistance (*cf*r) gene. Posttranscriptional methylation of the 23S rRNA at position A2503 by a methyltransferase encoded by the *cf*r gene causes resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics (6,7).

A 61-year-old woman with sarcoidosis was hospitalized after cardiopulmonary resuscitation (CPR) in the intensive care unit of a medical center. Methicillin-resistant coagulase negative staphylococcus (MR-CoNS) was isolated from blood cultures on days 2 and 9 of hospitalization. After the first isolation of MR-CoNS, ceftriaxone therapy (2 gr I.V. once a day) was replaced by linezolid (600 mg I.V. twice a day) and linezolid was used for 14 days until the patient was transferred to our center. Two sets of blood culture samples (1 set from the peripheral vein and 1 set from the central venous catheter) were sent to our laboratory. Blood cultures are incubated in a Bactec (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA)

instrument. Both blood cultures were signaled positive by the instrument. VITEK2 Compact reported the growing organism as *Staphylococcus epidermidis* and it was confirmed by 16S rRNA sequencing.

An antibiotic susceptibility test (AST) was performed by Kirby-Bauer disk diffusion method and minimum inhibitory concentration (MIC) values were obtained by E-test. The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations (7). AST results were also confirmed by VITEK 2 Compact (bioMérieux, Marcy L'Etoile, France).

In our study, sequencing primers 5'-AGAGTTTGATCCTGGCTCAG-3' (0008F) and 5'-TACCGCGGCTGCTGGCAC-3' (0532R) were used for the 16S rRNA gene, and 5-GCGGTCGCCTCCTAAAAG-3 (upper primer) and 5-ATCCCGGTCCTCTCGTACTA-3 (lower primer) were used for the domain V of 23S rRNA region amplifications as described before (8). Both of the amplification results were proved by loading products to 1% agarose gel. The bands were determined for 16S and 23S gene regions. Post-PCR purification was applied to products excised from agarose gel by using a High Pure PCR Product Purification kit (Roche Diagnostics GmbH, Mannheim, Germany). The purified samples were used for sequencing reaction by DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Piscataway, NJ, USA). Samples were loaded to ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). BLAST analysis was applied to the sequencing results.

In the disk diffusion test, we did not observe any inhibition zone around the linezolid disk, and linezolid MIC determined by E-test was 256 µg/mL (Figure 1).

* This research was presented as poster at 1st National Congress of Clinical Microbiology, Joint Meeting held by Turkish Society for Microbiology and Society of Specialists for Clinical Microbiology, and also won the best poster prize given by the American Society for Microbiology.

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Figure 1. Linezolid E-test and disk diffusion results.

VITEK2 Compact reported a MIC value of ≥ 8 $\mu\text{g/mL}$ for linezolid. We also detected reduced susceptibility for teicoplanin by disk diffusion method, and VITEK2 Compact reported an intermediate susceptibility for both vancomycin and teicoplanin, whereas the results of the E-test showed that the isolate was susceptible to glycopeptides with MIC values of 3 $\mu\text{g/mL}$ and 6 $\mu\text{g/mL}$ for vancomycin and teicoplanin, respectively. The isolate was also susceptible to quinupristin/dalfopristin and daptomycin, and resistant to several antibiotics such as chloramphenicol, erythromycin, or rifampicin. AST results were obtained by all 3 methods for these antibiotics. 16S rRNA and 23S rRNA gene regions were amplified and both of the amplification results were proved by loading products to 1% agarose gel. The bands we determined for 16S and 23S were between 500 and 550 and 400 and 450 bp, respectively (Figure 2).

The obtained sequence of 23S rRNA was compared with sequences from susceptible *Staphylococcus epidermidis* strain ATCC 12228 (GenBank accession

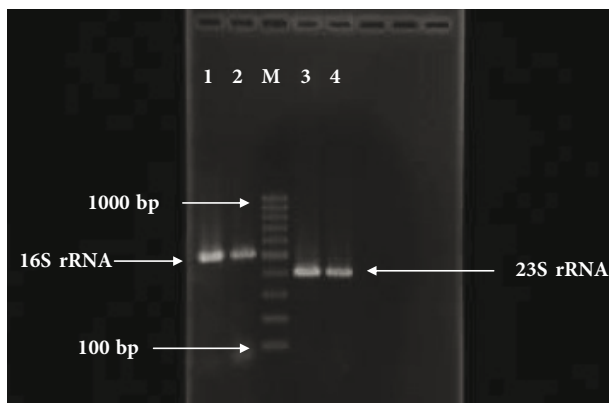


Figure 2. Ethidium bromide staining of PCR products.

number NC_004461.1). The isolate had a G to T mutation at position 2576 (*E. coli* numbering) and A to G mutation at position 2309 (*E. coli* numbering) (Figure 3).

After reporting linezolid resistance, the therapy was replaced by daptomycin (500 mg I.V. once a day) and the central venous catheter present was removed. *Staphylococcus epidermidis* was not isolated from any sample including the central venous catheter of our patient thereafter.

Linezolid is bacteriostatic against staphylococci and enterococci but shows bactericidal activity against pneumococci (9). Despite its bacteriostatic effect it can be used for the treatment of bacteremia and even endocarditis (9,10).

Linezolid-resistant strains of methicillin-resistant *Staphylococcus* spp. are reported. G2576T is the most common mutation related with linezolid resistance in staphylococci and enterococci (11). It is also reported in enterococci in Turkey (12). Furthermore, there are several mutations reported for staphylococci: G2447T in *Staphylococcus aureus* or G2631T and C2534T in *Staphylococcus epidermidis* (5). G2576T mutation together with the *cfr* gene can also be seen *Staphylococcus epidermidis* (13). To the best of our knowledge, no linezolid resistant MR-CoNS strain has been reported in Turkey (14,15).

In our isolate there is an additional mutation at position 2309. It is thought that linezolid binds to any location in the vicinity of the peptidyl transferase center (PTC) (4). The PTC is located in domain V of 23S rRNA, and this center plays a key role in peptide bond formation and peptide release (16). Although PTC is known as the primer binding site of the drug, it is controversial that different sites of ribosome can interact with linezolid. The A-site and P-site of large ribosomal units are among these interaction regions (17). Nucleotide A2309 is located in

A2309G

S_epi (ATCC 12228)
23S-S_epi_23S[1].ab1
E.coli

TTCCT-CAG-AATGGTTGGAAATCATTATAGAGTGTAAGGCATAAGG
TTCCTTCAGAAATGGTTGGAAATCATTATAGAGTGTAAGGCATAAGG
TTGGCT--AATCCTGGTCGGACATCAGGAGGTTAGTGAATGGCATAAGC 2338

G2576T

S_epi (ATCC 12228)
23S-S_epi_23S[1].ab1
E.coli

CCAAGGGTTGGGCTGTCGCCATTAAAGCGGTACGCAAGCTGGGTTTACG
CCAAGGGTTGGGCTGTCGCCATTAAAGCGGTACGCAAGCTGGGTTTACG
CCAAGGGTATGGCTGTCGCCATTAAAGTGGTACGCAAGCTGGGTTTACG 2588

Figure 3. Detected mutations obtained by BLAST analysis.

helix 84 of 23S rRNA. This nucleotide was identified as a point of cross-linking from P-site or A-site bound tRNA (18,19).

Several cross-linking points were detected, and further studies are needed to demonstrate the relationship between mutations, especially in these regions, and linezolid resistance. Prolonged use of linezolid as in our patient plays an important role in selecting resistant

strains. Precautions such as pervading rational antibiotic use and establishing infection control measures are crucial in controlling this emerging problem.

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