

Mutations within the *rplD* Gene of Linezolid-Nonsusceptible *Streptococcus pneumoniae* Strains Isolated in the United States

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Three invasive *Streptococcus pneumoniae* strains nonsusceptible to linezolid were isolated in the United States between 2001 and 2012 from the CDC's Active Bacterial Core surveillance. Linezolid binds ribosomal proteins where structural changes within its target site may confer resistance. Our study identified mutations and deletions near the linezolid binding pocket of two of these strains within the *rplD* gene, which encodes ribosomal protein L4. Mutations in the 23S rRNA alleles or the *rplV* gene were not detected.

Linezolid was the first oxazolidinone to be licensed in the United States (in 2000) and marketed worldwide (1–3). Linezolid is approved by the U.S. Food and Drug Administration (FDA) for the treatment of complicated skin infections, meningitis, nosocomial pneumonia, endocarditis, sepsis, osteomyelitis, concurrent bacteremia, and bacteremia associated with community-acquired pneumonia (1, 2).

Linezolid blocks the assembly of a functional initiation complex for protein synthesis, thereby preventing mRNA translation. Other antibiotics that prevent mRNA translation include chloramphenicol, tetracycline, macrolides, and lincosamides. They allow the formation of an initiation complex but inhibit subsequent peptide elongation (3, 4).

The LEADER (Linezolid Experience and Accurate Determination of Resistance) program, which monitors linezolid-resistant clinical isolates, reports that, in the United States, linezolid-sensitive *Streptococcus pneumoniae* isolates have an MIC₉₀ of 1 µg/ml (5–9). Therefore, *S. pneumoniae* clinical strains with linezolid MICs of >1 µg/ml should be monitored and investigated for potential mechanisms of resistance. This is consistent with the Clinical and Laboratory Standards Institute (CLSI) breakpoint of 2 µg/ml (10).

The mechanisms of resistance to linezolid that have been described to date include target modification and use of a mobile *cfr* element (2, 8, 11). The linezolid target (the 50S subunit) is composed of 5S and 23S rRNAs and 36 riboproteins (L1 through L36). Linezolid-resistant strains present mutations in one or more alleles of the 23S rRNA gene, decreasing the affinity of ribosomes for the drug (12). A clear correlation between the number of 23S rRNA alleles mutated and increased linezolid resistance has been demonstrated (13, 14). The most frequently reported mutation in linezolid-resistant clinical isolates of staphylococci and enterococci occur by G-to-U substitution in the peptidyl transferase center of 23S rRNA at position 2576 (2, 8). Additional mutations within the same 23S rRNA gene have also been described (e.g., A2059G, C2190T, and G2447T) (15–17).

The *cfr* mobile element includes the *cfr* gene, which encodes a methyltransferase that methylates the 23S rRNA at position A2503. This affects binding of linezolid to the 50S subunit (11, 18, 19). While carried by *Staphylococcus aureus* strains (20, 21) and recently described in *Streptococcus suis* (22), this mobile element has not been described in *S. pneumoniae*.

Only a few *S. pneumoniae* strains with reduced susceptibilities to linezolid have been isolated from disease cases (16, 23). For these strains, it was suggested that mutations in 23S rRNA genes and those encoding ribosomal proteins L4 and L22 confer linezolid resistance (16). However, direct evidence demonstrating deletions within the *rplD* gene of *S. pneumoniae* strain TN33388, encoding ribosomal protein L4, which is linked to reduced susceptibility to linezolid, was published by Wolter et al. (23). Strain TN33388 was identified through the Active Bacterial Core surveillance (ABCs), part of the Centers for Disease Control and Prevention's (CDC's) Emerging Infections Program.

In this study, the CDC *Streptococcus* laboratory identified two other additional *S. pneumoniae* strains (7828-04 and 2008227074) with reduced susceptibilities to linezolid. Overall, 3 of 45,099 pneumococci tested (<1%) were isolated from invasive disease in the United States between 2001 and 2012 through the ABCs, and they showed reduced susceptibilities to linezolid (Table 1). Mutations within demonstrated linezolid targets were investigated in these two isolates.

Strain TN33388 from the CDC (for whom its mechanism of resistance to linezolid had been investigated), two serotype 19A linezolid-susceptible strains, and the reference *S. pneumoniae* strain R6 were utilized as controls (23). The MICs for linezolid, vancomycin, penicillin, amoxicillin, erythromycin, chloramphenicol, clindamycin, and tetracycline were determined using the broth microdilution methodology according to the CLSI (24). The linezolid-susceptible strains shown in Table 1 had linezolid MICs of 0.25 or 1 µg/ml, whereas linezolid-nonsusceptible strains had MICs of 4 µg/ml. The strains were susceptible to penicillin, vancomycin, amoxicillin, and tetracycline. Except for one strain (3084-03), they were also susceptible to clindamycin. Linezolid-nonsusceptible strains were resistant to chloramphenicol and erythromycin (Table 1).

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pneumoniae strains. Similarly, a global study that utilized strains ($n = 636$) isolated in 22 different countries showed susceptibility to linezolid in all *S. pneumoniae* strains (31). Despite many years of exposure to the drug, the very low rate of linezolid resistance in pneumococci suggests that the fitness cost of resistance (32) may be suppressing the successful dissemination of these strains in the pneumococcus.

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