
**[Abstract]**

**OBJECTIVES**: Fluoroquinolone-resistant *Streptococcus pneumoniae* are increasing worldwide rapidly. In vitro activities of sitafloxacin were evaluated against clinical isolates of *S. pneumoniae* resistant to levofloxacin (MIC of levofloxacin ≥ 4 mg/L), which were characterized genetically. **METHODS**: The quinolone resistance determining regions (QRDRs) of gyrA, gyrB, parC and parE of these strains were analysed by PCR-based sequencing. MICs of sitafloxacin and other quinolones were determined by a microdilution broth method. **RESULTS**: All 18 strains had at least one amino acid substitution in the QRDRs of GyrA and ParC, which included Ser→Tyr/Phe and Glu→Lys in GyrA and Ser→Phe/Ile/Tyr, Asp→Tyr, Asn→Asp, Ser→Phe, Lys→Asn and Ala→Ser in ParC. Most isolates had Asp→Asn/Ile→Val/Ala→Thr substitutions in ParE, while no amino acid substitution in GyrB was noted in all isolates. Ten isolates for which levofloxacin MICs were 16 or 32 mg/L had multiple mutations in both GyrA and ParC. The MIC80 value of sitafloxacin for levofloxacin-resistant isolates was 0.25 mg/L. The range of MICs of sitafloxacin for isolates resistant to levofloxacin (MIC 4–32 mg/L) was 0.016–0.5 mg/L. **CONCLUSIONS**: These findings warrant further studies to evaluate the usefulness of sitafloxacin in the treatment of levofloxacin-resistant *S. pneumoniae* infection.

Key words: levofloxacin-resistant *S. pneumoniae*, drug resistance, sitafloxacin, garenoxacin, target alteration, efflux pump, gyrase, topoisomerase IV