for efflux pump overexpressed (EPO) phenotype. FQ-R was defined as levofloxacin minimum inhibitory concentration (MIC) of 8 µg/mL or more by broth microdilution. EPO was defined as 8-fold lower MIC in the presence of an EPI. Human lung epithelial cell line A549 was infected with PA, and cytotoxicity was measured using the Cytotox96 assay kit at 5, 1, 1.5, 2, 2.5, and 3 hours. PAO1 and PA103 were control strains. Isolates were grouped by the rate (fast vs. slow) at 1 hour and extent (weak vs. strong) at 3 hours of cytotoxicity.

**RESULTS:** Almost all (98%) isolates had EPO phenotype. Only 10% had no target site mutation (MIC 16–32 µg/mL); all showed slow-weak cytotoxicity. Isolates with a single mutation (11 [28%] of 40) were 73% gyrA (MIC 4–64 µg/mL) and 27% parC mutants (MIC 64–128 µg/mL). Half (52%) had two mutations (mostly gyrA+parC) with MIC 8–64 µg/mL. All four isolates with three target site mutations (gyrA+parC+parE) had MIC 64–128 µg/mL. As the number of mutations increases, the rate and extent of cytotoxicity increases, as indicated by the proportion with a fast rate (0, 18, 40, and 75%) and strong extent of killing (0, 36, 50, and 75%) for strains with zero, one, two, and three target site mutations, respectively.

**CONCLUSIONS:** Target site mutations, as they accumulate, appear to confer an additive cytotoxic potential to respiratory isolates of PA that are FQ resistant. Our data suggest that the expression of virulence and resistance genes is coregulated.
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