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Selection of Fluoroquinolone-Resistant Methicillin-Resistant *Staphylococcus aureus* with Ciprofloxacin and Trovafloxacin

Resistance to ciprofloxacin in methicillin-resistant *Staphylococcus aureus* emerges more rapidly than to ofloxacin, levofloxacin, or sparfloxacin (1, 2). We compared emergence of resistance to trovafloxacin with that to ciprofloxacin.

We examined 13 methicillin-resistant *S. aureus* isolates for which the ciprofloxacin and trovafloxacin MICs were ≤1 and ≤2 μg/ml, respectively, and for which unique genotypes were found by pulsed-field gel electrophoresis.

Spontaneous single-step mutation rates were determined by growing bacteria in antibiotic-free broth and plating 0.1-ml aliquots on Mueller-Hinton agar containing four or eight times the MIC of ciprofloxacin or trovafloxacin. The resistance frequency was calculated by dividing the number of colonies at 48 h by the inoculum. The emergence of resistance during serial transfer was studied by growing bacteria in antibiotic-free medium, adjusting the cultures to 10^8 CFU/ml, and spreading 0.2-ml aliquots onto Mueller-Hinton agar containing twofold dilutions (0.0625 to 32 μg/ml) of antibiotic. All colonies appearing at 48 h at the highest antibiotic concentration were collected by swab, regrown in antibiotic-free medium, and reinoculated onto another set of antibiotic-containing plates. All experiments were performed in duplicate. Single-step resistance rates and changes in the MIC with serial transfer were compared by the Wilcoxon signed rank test after log transformation of the data. The number of strains developing single-step resistance was compared by using Fisher's exact test.

Single-step resistance occurred in all strains exposed to 4× the MIC of ciprofloxacin but in only 4 (31%) strains exposed to trovafloxacin ($P < 0.05$). There was no difference in resistance rates at 8× the MIC of the antibiotics. The mean resistance rates at 4× the MIC of ciprofloxacin and trovafloxacin were 6.8×10^{-7} (range, $<2.1 \times 10^{-8}$ to 3.1×10^{-6}) and 8.5×10^{-9} (range, $<3.3 \times 10^{-10}$ to 1.1×10^{-7}), respectively ($P < 0.05$) (Table 1).

In serial transfer experiments, for all strains the mean MIC of ciprofloxacin increased 6.7 ± 0.9-fold, and the MIC of trovafloxacin increased 3.3 ± 0.9-fold ($P < 0.05$). The MICs for all strains exposed to ciprofloxacin increased by ≥fourfold after a single transfer; the strains became resistant (MIC, ≥4 μg/ml) (3) within 4 transfers, and the MIC was ≥32 μg/ml after 10 transfers. In contrast, the MIC was 4 μg/ml for only one strain exposed to trovafloxacin. The mean MIC for the remaining strains was 2 μg/ml after 10 transfers.

Single-step resistance and the emergence of resistance with serial transfer appear to be more common with ciprofloxacin than with trovafloxacin.

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TABLE 1. Single-step resistance rates in 13 methicillin-resistant *S. aureus* strains exposed to ciprofloxacin (cipro) or trovafloxacin (trova)

Genotype	Cipro MIC (μg/ml)	Trova MIC (μg/ml)	Inoculum (CFU/ml)	Resistance rate			
				Cipro, 4× MIC	Trova, 4× MIC	Cipro, 8× MIC	Trova, 8× MIC
A	1	0.125	5.6×10^8	1.6×10^{-7}	1.1×10^{-7}	6.3×10^{-8}	7.3×10^{-8}
B	0.25	0.0625	6.0×10^9	7.5×10^{-8}	$<6.0 \times 10^{-9}$	$<6.0 \times 10^{-9}$	$<6.0 \times 10^{-9}$
C	0.25	0.0625	3.8×10^9	2.1×10^{-8}	$<3.8 \times 10^{-9}$	$<3.8 \times 10^{-9}$	$<3.8 \times 10^{-9}$
D	0.5	0.0313	4.6×10^8	3.1×10^{-6}	$<4.6 \times 10^{-8}$	2.2×10^{-9}	$<4.6 \times 10^{-8}$
E	0.125	0.0625	7.8×10^8	6.6×10^{-7}	$<7.8 \times 10^{-8}$	$<7.8 \times 10^{-8}$	$<7.8 \times 10^{-8}$
F	0.25	0.0625	1.2×10^{10}	2.3×10^{-8}	3.3×10^{-10}	$<1.2 \times 10^{-10}$	$<1.2 \times 10^{-10}$
G	0.5	0.0313	8.4×10^8	1.9×10^{-6}	$<8.4 \times 10^{-8}$	1.2×10^{-9}	$<8.4 \times 10^{-8}$
H	0.5	0.0625	2.5×10^9	6.4×10^{-7}	$<2.5 \times 10^{-9}$	1.2×10^{-9}	$<2.5 \times 10^{-9}$
I	0.25	0.0625	4.4×10^9	5.3×10^{-8}	1.6×10^{-9}	$<4.4 \times 10^{-9}$	$<4.4 \times 10^{-9}$
J	0.25	0.0625	8.0×10^9	5.8×10^{-8}	1.2×10^{-9}	$<8.0 \times 10^{-9}$	$<8.0 \times 10^{-9}$
K	0.125	0.125	1.9×10^9	1.0×10^{-6}	$<1.9 \times 10^{-9}$	$<1.9 \times 10^{-9}$	$<1.9 \times 10^{-9}$
L	0.5	0.0625	9.0×10^8	1.0×10^{-6}	$<9.0 \times 10^{-8}$	1.3×10^{-8}	$<9.0 \times 10^{-8}$
M	0.25	0.0625	7.8×10^9	1.6×10^{-7}	$<7.8 \times 10^{-9}$	$<7.8 \times 10^{-9}$	$<7.8 \times 10^{-9}$

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