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Greater Ciprofloxacin Tolerance as a Possible Selectable Phenotype Underlying the Pandemic Spread of the *H*30 Subclone of *Escherichia coli* Sequence Type 131

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Minimum bactericidal concentrations (MBCs) for ciprofloxacin were significantly higher among 41 members of the *H*30 subclone within *Escherichia coli* sequence type 131 than among 48 other fluoroquinolone-resistant *E. coli* isolates. This MBC difference, which was not explained by ciprofloxacin MICs, *gyrA*, *parC*, and *parE* mutations, the presence of *aac*(6′)-*Ib-cr*, or organic solvent tolerance (a surrogate for efflux pump activity), conceivably could have promoted the pandemic emergence of the *H*30 sequence type 131 subclone.

Escherichia coli is clonally diverse and increasingly antimicrobial resistant (1). The *H*30 subclone within *E. coli* sequence type 131 (ST131) has emerged inexplicably in many locales as the dominant *E. coli* lineage among human clinical isolates, including those resistant to fluoroquinolones or extended-spectrum cephalosporins (2–9).

We recently documented higher fluoroquinolone MICs for the ST131 *H*30 subclone (here, *H*30) isolates than for other fluoroquinolone-resistant *E. coli* isolates (10). Here, we assessed whether *H*30 isolates also have higher minimum bactericidal concentrations (MBCs) for ciprofloxacin and explored the associations between the ciprofloxacin MBCs, MICs, and fluoroquinolone resistance mechanisms.

Isolates. The 89 fluoroquinolone-resistant (i.e., ciprofloxacin nonsusceptible) *E. coli* study isolates were from our study of fluoroquinolone MICs in relation to clonal background and resistance-associated traits (10). They included 41 *H*30 and 48 non-*H*30 *E. coli* isolates and were selected from larger source collections for diversity of *gyrA* and *parC* alleles, multilocus sequence types, and pulsed-field gel electrophoresis profiles (3, 7, 11, 12). The non-*H*30 group included 31 STs from 20 different ST complexes (as determined by eBURST software) and 4 non-*H*30 ST131 strains.

MICs and MBCs. The ciprofloxacin MICs were determined by broth microdilution using standard methods (13), with doubling dilutions of ciprofloxacin (range, 0.5 to 2,048 mg/liter) in cation-adjusted Mueller-Hinton broth, plus turbidity-adjusted suspensions of each test isolate (0.5 McFarland standard; approximately 1×10^8 CFU/ml). The MIC for a given titration series was the lowest ciprofloxacin concentration yielding no visible growth after overnight incubation.

For MBC determination, aliquots from each overnight MIC dilution series underwent quantitative plating to antibiotic-free agar. The MBC was the lowest ciprofloxacin concentration that yielded a ≥99.9% decrease in viable counts (14).

For each isolate, the MIC and MBC were determined initially in duplicate. If the duplicates disagreed by >2-fold for any result, up to 5 total determinations were done. The geometric mean value was used.

Resistance-associated traits. The presence of *aac*(6′)-*Ib-cr* (encoding a fluoroquinolone- and aminoglycoside-modifying enzyme) (15), mutations in the quinolone resistance-determining region (QRDR) of *gyrA*, *parC*, and *parE* (16, 17) and the organic solvent tolerance (OST) (a proxy for efflux pump activity) (18) were determined previously (10).

Statistical analysis. Comparisons involving continuous variables were tested using the Mann-Whitney *U* test. The correlation was analyzed by simple regression.

Distribution of ciprofloxacin MBCs versus resistance-associated traits in *H*30 and non-*H*30 isolates. The 89 study isolates exhibited a broad range of ciprofloxacin MBCs (Fig. 1). Compared with the non-*H*30 isolates, the *H*30 isolates had significantly higher MBCs (median, 4-fold; $P < 0.001$). For both MICs and MBCs, the four non-*H*30 ST131 strains resembled more closely the non-ST131 strains than the *H*30 strains (not shown).

To identify the mechanistic correlates, the MBCs were compared statistically with the fluoroquinolone resistance mechanisms. All 41 *H*30 strains carried *gyrA* and *parC* QRDR mutations, and 10 of these additionally carried *aac*(6′)-*Ib-cr*. None carried a *parE*458 mutation. In contrast, of the 48 non-*H*30 strains, 27 carried *gyrA* and *parC* QRDR mutations only, 21 carried a *parE*458 mutation (in addition to *gyrA* and *parC* QRDR mutations, except in 1 strain), and 4 carried *aac*(6′)-*Ib-cr* (3 with *gyrA* and *parC* mutations only and 1 with *gyrA*, *parC*, and *parE*458 mutations).

aac(6′)-*Ib-cr* was associated with significantly higher MBCs among the *H*30 isolates ($P = 0.04$), and a similar trend was exhib-

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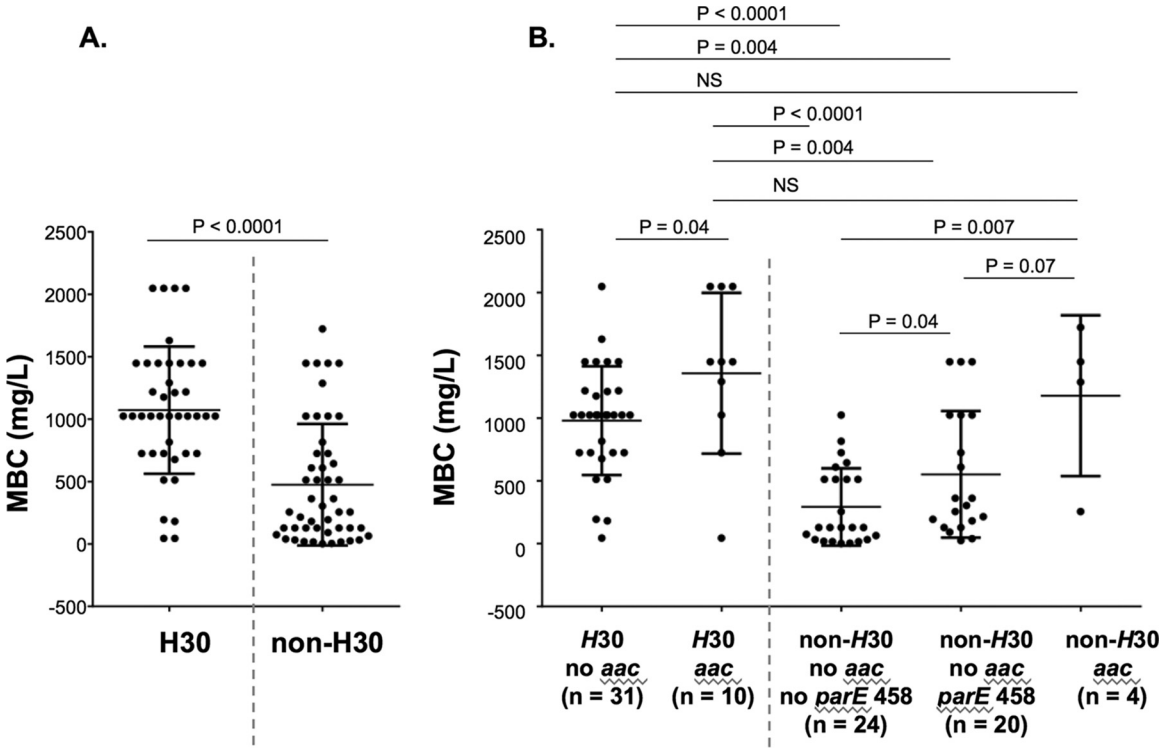

FIG 1 Ciprofloxacin minimum bactericidal concentration (MBC) in relation to *H*30 subclone status and resistance mechanisms. (A) *H*30 versus non-*H*30 isolates. (B) Subgroups based on *H*30 status and the presence of *aac*(6′)-*Ib-cr* (*aac*) and/or nonsynonymous mutations at *parE*458. The circles indicate individual isolates. The horizontal bars show group medians (wide bars) and the 25th and 75th percentiles (narrow bars). *P* values (two-tailed) are by the Mann-Whitney *U* test. NS, not significant.

ited among the non-*H*30 isolates (Fig. 1B). Among the *aac*(6′)-*Ib-cr*-positive isolates, the MBCs did not differ significantly between the *H*30 and non-*H*30 isolates. Among the *aac*(6′)-*Ib-cr*-negative non-*H*30 isolates, the presence of a *parE*458 mutation was also associated with a higher MBC ($P = 0.04$). Nonetheless, among the *aac*(6′)-*Ib-cr*-negative isolates, the MBCs still were significantly higher among the *H*30 than the non-*H*30 isolates, whether the latter lacked or contained *parE*458 mutations ($P = 0.004$ and $P < 0.001$, respectively).

Thus, even in the absence of *aac*(6′)-*Ib-cr*, *H*30 strains have significantly higher MBCs than non-*H*30 strains, despite lacking *parE*458 mutations. This indicates a possible MBC-determining role for the unique combination of QRDR mutations and/or other factors in *H*30 strains.

Correlation between MBCs and MICs in *H*30 and non-*H*30 strains. For more closely matched comparisons of MICs and MBCs in *H*30 versus non-*H*30 isolates, analyses were restricted to the 55 isolates (24 *H*30 and 31 non-*H*30) with QRDR replacement mutations involving only *gyrA* and *parC* (not *parE*458) and without *aac*(6′)-*Ib-cr*. Within this population, in each subset (*H*30 and non-*H*30), MBCs and MICs were correlated only moderately (Fig. 2). Interestingly, however, although the two regression curves were nearly parallel, the *H*30 curve was shifted significantly upward to higher MBCs. This shows that at a given MIC, the MBCs tended to be much higher among the *H*30 than the non-*H*30 isolates. An even weaker correlation between the MBCs and MICs was observed when outliers with very low MICs/MBCs were excluded (data not shown).

The OST score was associated positively with the MIC, both overall and among the *H*30 and non-*H*30 isolates separately (Table 1). However, it was associated with the MBC only among the non-*H*30 isolates.

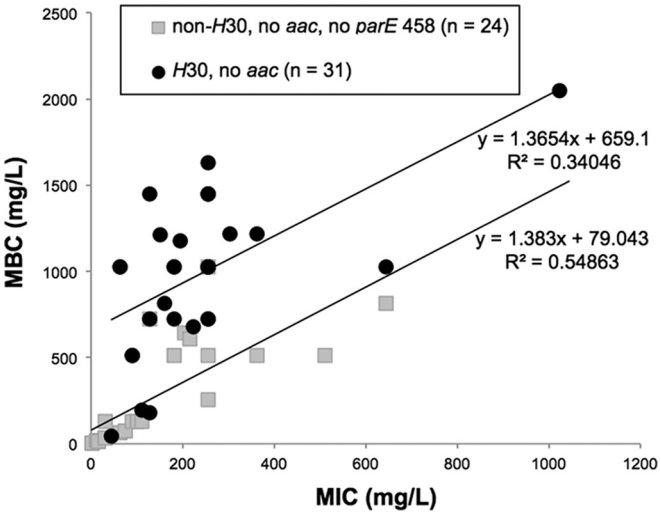

FIG 2 Ciprofloxacin minimum bactericidal concentrations (MBCs) in relation to the MICs among 89 ciprofloxacin-resistant *Escherichia coli* isolates. *H*30 subclone members and non-*H*30 isolates are shown separately. Isolates containing *aac*(6′)-*Ib-cr* (*aac*) and/or nonsynonymous mutations at *parE*458 were excluded.

TABLE 1 *P* values for associations of MICs and MBCs with organic solvent tolerance among ciprofloxacin-resistant *Escherichia coli* isolates

Parameter	*P* value[b]		
	Total ($n = 89$)	Non-*H*30 ($n = 48$)	*H*30 ($n = 41$)
MIC	0.006	0.007	0.01
MBC[a]	NS	0.04	NS

[a] MBC, minimum bactericidal concentration.
[b] *P* values (by Pearson correlation) are shown where the *P* value is <0.05. NS, not significant ($P \geq 0.05$).

Thus, although there were modest correlations between the MBCs and MICs, the sizeable MBC differences between the *H*30 and non-*H*30 isolates could not be explained by their MIC differences, indicating that the MBC is a separate phenotype from the MIC.

Comment. We found that, compared with fluoroquinolone-resistant non-*H*30 isolates, the fluoroquinolone-resistant *H*30 isolates typically have approximately 4-fold higher MBCs that are not explained by their corresponding MICs. This identifies a novel fitness mechanism that may underlie the striking epidemiological success of the ST131 *H*30 lineage.

Our findings suggest that even when inhibited by ciprofloxacin, *H*30 strains are more likely than other fluoroquinolone-resistant *E. coli* isolates to survive and regrow once the ciprofloxacin concentrations drop below the MIC. This tolerance phenomenon may contribute to the *H*30 strain-associated clinical scenario of same-strain recurrent urinary tract infection, with the causative organism disappearing during ciprofloxacin therapy and then re-emerging after treatment completion (19, 20; J. R. Johnson, unpublished data).

Regarding mechanisms, our data suggest that *aac*(6′)-*Ib-cr* may differentially raise the MBC, which could explain why *aac*(6′)-*Ib-cr* is being selected in strains with preexisting QRDR mutations (10). Additionally, *parE*458 replacement mutations were associated with increased MBCs. However, neither *aac*(6′)-*Ib-cr* carriage nor *parE*458 (absent in *H*30 strains) (10) could explain the *H*30 versus non-*H*30 MBC gap. Likewise, the OST, which appeared to have less impact on the MBC than on the MIC, was associated with MBCs only marginally (non-*H*30 isolates) or not at all (total population and *H*30 isolates). Thus, to the extent that OST reflects the net efflux pump activity, efflux pumps also are unlikely to underlie the higher MBCs of the *H*30 isolates. As such, the mechanism(s) for higher MBCs among *H*30 isolates remains undefined.

Antimicrobial tolerance, which was studied initially mostly in relation to beta-lactam agents and staphylococci, conceivably could result from technical artifacts (21). Our findings are unlikely to represent technical artifacts, since we noted strong associations of the MBCs with the *H*30 subclone, independently from the MICs, when the *H*30 and non-*H*30 isolates were tested in parallel. Studies of quinolone tolerance in *E. coli* (22–25) have identified SOS-regulated genes (25), *hipA* (24), and defects in DNA repair (22) as possible contributors; these warrant future assessment in *H*30 isolates.

The study limitations include the small numbers in certain subgroups, the reliance on the OST as a proxy for efflux pump activity (versus measuring expression of multiple specific pumps), and the absence of attention to porin expression or to MBCs and the MIC/MBC discrepancy for drugs other than ciprofloxacin. The strengths include the well-characterized study population and the analysis of resistance mechanisms in relation to MBCs.

In summary, we newly documented higher ciprofloxacin MBCs for members of the *H*30 subclone of ST131, compared with those for other fluoroquinolone-resistant *E. coli* isolates. These phenotypes could not be explained by differences in the MICs, *gyrA*, *parC*, and *parE* mutations, or OST or the presence of *aac*(6′)-*Ib-cr*. Our findings identify a selectable phenotype that may have contributed to the emergence and pandemic spread of *H*30.

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