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Spontaneous Conversion to Quinolone and Fluoroquinolone Resistance among Wild-Type *Escherichia coli* Isolates in Relation to Phylogenetic Background and Virulence Genotype

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Human clinical isolates of *Escherichia coli* that are resistant to quinolone or fluoroquinolone agents typically exhibit fewer extraintestinal virulence factors (VFs) than susceptible isolates, along with a different phylogenetic background. To experimentally assess the basis for this as-yet-unexplained phenomenon, 40 *E. coli* strains (20 *E. coli* Reference collection members and 20 Israeli cystitis isolates) were subjected to serial selective passaging to obtain derivatives resistant to nalidixic acid (NA) and ciprofloxacin (C). PCR-based VF profiling and phylotyping were performed on the parents and their respective resistant derivatives. All 40 susceptible parent strains yielded NA- and C-resistant derivatives after a median of 6 (range, 4 to 12) serial selective passages on agar plates containing increasing concentrations of NA and C. The numbers of passages required for resistance did not differ by collection origin, phylogenetic group, basal VF profile, source (urine versus fecal), or host group (human versus animal). With the development of C resistance, only one VF was lost in a single strain. Resistant derivatives exhibited the same phylotype as their susceptible parents. These findings suggest that the sparse VF profiles and the low-virulence phylogenetic background of NA- and C-resistant *E. coli* clinical isolates probably are not attributable to the loss of VFs from intrinsically high-virulence strains during conversion to resistance or to enhanced emergence of drug resistance among intrinsically low-virulence strains. A more likely explanation is the importation of resistant strains from an as-yet-undefined low-virulence external selection reservoir.

Extraintestinal *Escherichia coli* infections in humans are a major source of morbidity, mortality, and increased health care costs (35). Annually in the United States, they are responsible for millions of physician visits and hospital admissions, an estimated 40,000 deaths from sepsis, and billions of dollars in direct medical costs (35). Most of these infections are caused by distinctive *E. coli* strains termed extraintestinal pathogenic *E. coli* (ExPEC) strains, which possess the requisite arsenal of virulence factors (VFs) to overcome host defenses and injure or invade host tissues, thereby causing extraintestinal disease (16, 36). Such strains derive predominantly from *E. coli* phylogenetic group B2 and, to a lesser extent, from group D, as opposed to (commensal-associated) groups A and B1 (8, 13).

The management of extraintestinal *E. coli* infections has become increasingly challenging in recent years because of emerging resistance to most first-line antimicrobial agents, including fluoroquinolones (3, 5–7, 9, 12, 27, 30, 31, 33, 37). The fluoroquinolones, which are highly bioavailable, well tolerated, and extremely active against susceptible *E. coli* strains, until recently encountered minimal resistance from *E. coli* strains worldwide. However, resistance has now emerged to a concerning extent in many locales (5, 6, 25, 37), and other locales (e.g., the United States) are experiencing a slow but steady increase (26, 42).

Interestingly, quinolone- and fluoroquinolone-resistant *E. coli* isolates typically appear to be less virulent than their susceptible counterparts, according to both clinical behavior and VF content (28, 40, 41). Their typical lack of characteristic ExPEC-associated VFs, such as *pap* (P fimbriae) and *hly* (hemolysin), has led to the suggestion that mutation to quinolone or fluoroquinolone resistance may be accompanied by the loss of VF genes (41). Supporting this notion is the observation that growth of ExPEC strains in the presence of sub-MIC levels of these drugs is associated with the loss of VFs from a small subset of the population (38). Against this notion is the reported absence of detectable VF profile alterations in two wild-type *E. coli* strains after experimental selection for quinolone or fluoroquinolone resistance (19, 28). Also against it is the consistent epidemiological observation that quinolone- and fluoroquinolone-resistant human clinical isolates exhibit a markedly different phylogenetic group distribution than do their susceptible counterparts, deriving predominantly from *E. coli* groups A, B1, and D (14, 18–20, 24, 29).

A possible alternative explanation for these observations is that conversion to resistance occurs more readily in non-B2

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TABLE 1. MICs for NA and C and number of selective passages required to obtain resistant derivatives among 40 clinical and reference *E. coli* isolates

Type of data	Characteristic (strain or derivation)	Median result (range)
MIC (mg/liter)	NA MIC (parent)	1.0[a] (0.38–3.0)
	C MIC (parent)	0.008[a,b] (0.002–0.16)
	C MIC (NA-resistant[c] derivative)	0.22[b] (0.032–1.7)
	C MIC (C-resistant derivative)	6.0 (4.0–>32)
No. of selective passages required to obtain characteristic[d]	NA resistance[c] (from parent)	2 (2–6)
	C resistance (from NA resistance[c])	4 (2–6)
	C resistance (from parent)	6 (4–12)

[a] For parent strain's NA MIC versus C MIC; $P < 0.001$ (Wilcoxon rank-sum test).
[b] For C MIC of parent versus that of NA-resistant derivative; $P < 0.001$ (Wilcoxon rank-sum test).
[c] NA resistance defined here as an NA MIC of ≥250 mg/liter.
[d] Data shown are only for passages involving multiple NA- or C-supplemented agar plates containing a range of drug concentrations. Interval passages for amplification purposes are not counted.

strains or in those with fewer baseline VFs (19). We tested these competing hypotheses by determining the ease with which derivatives resistant to nalidixic acid (NA), a representative quinolone, and to ciprofloxacin (C), a representative fluoroquinolone, could be obtained from diverse wild-type *E. coli* strains by in vitro selection in relation to the strains' phylogenetic background and VF profiles. In the process, we also assessed the impact of the transition to resistance on VF profiles to determine whether the development of resistance is associated with the loss of VFs.

MATERIALS AND METHODS

Strains. To provide a diversity of sources while emphasizing recent clinical isolates, 20 wild-type *E. coli* isolates were selected for analysis from each of two different strain collections, the 72-member *E. coli* Reference (ECOR) collection and a collection of 100 urine isolates from women with acute uncomplicated cystitis from Afula, Israel (19). The ECOR collection, which includes human urine and fecal isolates and animal fecal isolates from diverse locales, was assembled in the early 1980s, whereas the Israeli cystitis isolates are from 2001 (19, 32). These collections have been characterized in terms of their phylogenetic groups and VF profiles (11, 14, 18). From each collection, four to six representatives were selected deliberately from each of the four *E. coli* phylogenetic groups (A, B1, B2, and D) according to the following criteria. For both collections, several high-VF-number and low-VF-number representatives (as available) were chosen from each phylogenetic group, with attention given to including a diversity of VF profiles. For the ECOR collection, within which phylogenetic relationships have been defined by multilocus enzyme electrophoresis (11), attention also was given to selecting phylogenetically diverse strains within each phylogenetic group.

Selection and confirmation of resistant derivatives. The selection of spontaneous NA- and C-resistant derivatives of the 40 parent *E. coli* strains was done in a stepwise fashion by serial passaging of dense suspensions of each test strain on agar plates containing increasing concentrations of the target drugs. Initially, the parent strains underwent MIC determinations for NA and C by gradient elution (Etest), as directed by the manufacturer (AB-Biodisk). They were then grown overnight to confluence on nonsupplemented modified Mueller-Hinton (MMH) agar plates (1). The total colony material from each of these plates was harvested and suspended in approximately 1 ml of 0.8% NaCl. From these dense suspensions, 50 μl was spread onto each of three MMH agar plates containing NA at 4 mg/liter, 32 mg/liter, and 256 mg/liter. Colonies from the highest-concentration NA-supplemented plate yielding growth were regrown overnight on the corresponding supplemented MMH agar plate. The above process was then repeated until growth was obtained on plates containing 256 mg/liter NA, which is a more stringent criterion for NA resistance than the CLSI (formerly NCCLS)-specified threshold of ≥32 mg/liter. For each parent strain, an arbitrarily selected isolate from a plate containing NA at 256 mg/liter underwent MIC determinations for NA and C by Etest. To confirm genomic identity with the parent strain, random amplified polymorphic DNA (RAPD) analysis was done using arbitrary decamer 1290, followed by the use of decamer 1247 in case of ambiguous results (2).

Confirmed high-level NA-resistant derivatives (NA MIC of ≥256 mg/liter plus parental RAPD profile) next underwent a similar series of passages onto various C-supplemented agar plates (0.25, 0.5, 1.0, 2.0, and 4.0 mg/liter) until the 4-mg/liter (resistance-defining) level was reached. For each parent strain, an arbitrarily selected isolate from the plate containing 4 mg/liter C underwent (i) MIC determination for C by Etest and (ii) RAPD analysis (as described above) to confirm genomic identity with the parent.

Virulence genotyping and phylotyping. The 40 parent strains and their respective confirmed NA- and C-resistant derivatives were tested in parallel for 40 VFs by using a multiplex PCR-based assay (16, 23). Independently prepared boiled lysates were used as template DNA. Appropriate positive and negative controls were included within each run. Each sample was scored for the presence or absence of each VF by visual inspection of ethidium bromide-stained agarose gels for PCR products of the appropriate sizes. The VF score was the number of VFs detected in an isolate, adjusted for the multiple detection of the *pap* (P fimbriae), *sfa*/*foc* (S and F1C fimbriae), and *kps* (capsule) operons. Isolates were classified as ExPEC if positive for two or more of five key VFs, including *papA* and/or *papC* (P fimbriae structural subunit and assembly), *sfa*/*foc*, *afa*/*dra* (Dr-binding adhesins), *iutA* (aerobactin receptor), and *kpsM* II (group 2 capsule) (22). Phylogenetic group was assessed by using a triplex PCR-based assay (4).

Statistical analysis. First, baseline MICs (to NA and C) and the C MIC of the derivatives with NA MICs of ≥256 mg/liter were analyzed in relation to molecular traits (individual VFs, aggregate VF score, phylogenetic group) and collection origin (Israeli cystitis or ECOR). Next, the number of selective passages required to obtain resistant derivatives was analyzed in relation to molecular traits, collection, and baseline MICs. Comparisons of proportions were tested using Fisher's exact test (two-tailed). Two-group comparisons involving continuous variables were tested using the Mann-Whitney U test for unpaired comparisons and the Wilcoxon rank-sum test for paired comparisons. The correspondence between continuous variables was assessed by using the Spearman correlation test. The criterion for statistical significance was a P value of <0.05.

RESULTS

Baseline MICs for NA and C. Among the 40 parent *E. coli* strains, median MICs for NA and C, respectively, were 1.0 mg/ml and 0.008 mg/ml ($P < 0.001$; Wilcoxon rank-sum test) (Table 1). The parental (NA MIC-to-C MIC) ratios ranged from 83.3 to 500 (median, 125). Parental NA and C MICs did not differ significantly according to collection origin (ECOR [$n = 20$] or Israeli cystitis [$n = 20$]), phylogenetic group (A [$n = 11$], B1 [$n = 9$], B2 [$n = 10$], or D [$n = 10$]), or aggregate VF score (overall median, 6.0 [range, 0 to 16]). Despite the use of 68 separate comparisons (34 molecular characteristics for each of two MICs), only five individual VFs, plus ExPEC status, exhibited a statistically significant association with a lower parental NA or C MIC, and these differences were quite modest (Table 2).

Spontaneous development of NA and C resistance. From the (NA-susceptible) parent strains, derivatives exhibiting NA

TABLE 2. MICs for NA and C among 40 clinical and reference *E. coli* isolates, and their NA-resistant derivatives, according to VF content

MIC endpoint (strain)	VF(s) (no. positive, of 40)[b]	Median MIC (range) (mg/liter) when:		*P* value(s)[c]
		VF(s) present	VF(s) absent	
NA MIC (parent)	K1 (4)	0.63 (0.38–1.0)	1.0 (0.75–3.0)	0.009
	ExPEC (26)	1.0 (0.38–3.0)	1.25 (1.0–3.0)	0.02
C MIC (parent)	*pap* genes (13–18)[d]	0.007 (0.002–0.012)	0.008 (0.004–0.016)	0.005–0.02[d]
	iha (17)	0.008 (0.002–0.012)	0.008 (0.004–0.016)	0.014
	sat (15)	0.006 (0.002–0.012)	0.008 (0.004–0.016)	0.01
	iutA (17)	0.006 (0.002–0.012)	0.008 (0.004–0.016)	0.005
C MIC (NA-resistant derivative[e])	*afa*/*dra* (4)	0.45 (0.3–1.0)	0.21 (0.03–1.7)	0.009
	K1 (4)	0.13 (0.03–0.25)	0.24 (0.12–1.7)	0.047

[a] *afa*/*dra* codes for Dr-binding adhesins; ExPEC is defined by the presence of two or more of the following: *papA* and/or *papC* (P fimbriae structural subunit and assembly), *sfa*/*foc* (S fimbriae/F1C fimbriae), *afa*/*dra, kpsM* II (group 2 capsule), and *iutA* (aerobactin system). *iha* codes for putative siderophore adhesin; K1 is a *kpsM*II variant; *sat* codes for secreted autotransporter toxin.

[b] Only VFs yielding *P* values of <0.05 with at least one MIC endpoint are shown. VFs detected in ≥1 isolate but not yielding *P* values of <0.05 include the following (no. of isolates shown in parentheses): *astA*, coding for enteroaggregative *E. coli* heat-stable toxin 1 (2); *cdtB*, coding for cytolethal distending toxin (3); *cnf1*, coding for cytotoxic necrotizing factor (5); *cvaC*, coding for microcin (colicin) V; *fimH*, coding for type 1 fimbriae (39); *focG*, coding for F1C fimbriae (5); *fyuA*, coding for yersiniabactin system (25); H7 *fliC*, coding for flagellin variant (1); *hly*, coding for hemolysin (9); *ireA*, coding for siderophore receptor (6); *iroN*, coding for siderophore receptor (9); *iss*, coding for increased serum survival (1); *kpsMT*III, coding for group 3 capsule (1); *malX*, pathogenicity island marker (12); *ompT*, coding for outer membrane protease T (16); *papG* allele III, coding for P adhesin variant (7); *rfc*, coding for O4 lipopolysaccharide synthesis (2); *sfaS*, coding for S fimbriae (2); *traT*, which is serum resistance associated (17); and *usp*, coding for uropathogenic-specific protein (10). Not detected were the following: *afaE8*, coding for afimbrial adhesin VIII; *bmaE*, coding for M fimbriae; *clpG*, coding for CS31A adhesin; F17, coding for F17c fimbriae; *gafD*, coding for G fimbriae; *ibeA*, associated with invasion of brain endothelium; K2, *kpsM*II variant; and *papG* allele 1, coding for P adhesin variant I.

[c] *P* values determined by Mann-Whitney U test (exact, two-tailed).

[d] *papA* ($n = 18$; $P = 0.006$), *papC* ($n = 18$; $P = 0.006$), *papEF* (P fimbriae tip pilins: $n = 19$; $P = 0.02$), *papG* (P adhesin; $n = 17$; $P = 0.014$), and *papG* allele II (P adhesin variant; $n = 13$; $P = .005$) yielded identical comparative C MIC results (as shown) with the parent strain.

[e] NA resistance defined here as an NA MIC of ≥250 mg/liter.

MICs of ≥256 mg/liter were obtained after a median of two (range, two to six) serial passages on agar plates containing increasing concentrations of NA. From these NA-resistant derivatives, derivatives with C MICs of ≥4 mg/liter were obtained after a median of an additional four (range, two to six) selective passages on C-containing agar plates. In total, this gave a median of 6 (range, 4 to 12) selective passages required to obtain C-resistant derivatives (Table 1).

The initial NA-resistant derivatives exhibited significantly increased C MICs (median, 0.22 mg/liter [range, 0.032 to 1.7]) compared with the corresponding NA-susceptible parent ($P < 0.001$; Wilcoxon rank-sum test), representing a median 27.5-fold increase (range, 12.5- to 212.5-fold) (Table 1). Only 2 of the 34 bacterial characteristics analyzed, i.e., *afa*/*dra* (Dr-binding adhesins) and K1 (group 2 capsule variant), significantly predicted the C MICs of the NA-resistant derivatives (Table 2), and these were associated with only a twofold increase or decrease in C MIC (Table 2).

Predictors of spontaneous development of NA and C resistance. The number of selective passages on C-containing agar required to obtain a C-resistant derivative from an NA-resistant derivative was significantly correlated with the C MIC of the NA-resistant derivative. That is, a median of four additional passages (range, two to six) was needed to obtain C resistance in NA-resistant derivatives with below-median C MICs, while a median of three such passages (range, two to five) was required for those with above-median C MICs ($P = 0.02$). Obtaining a C-resistant derivative from an NA-resistant derivative also required slightly fewer selective passages for isolates containing *papA*, *papC*, or *papG* than for other isolates (median of three passages [range, two to six] versus a median of four passages [range, two to six], respectively; $P = 0.045$ or $P = 0.03$, respectively). With these exceptions, none of the other 38 bacterial characteristics analyzed, including parental MICs for NA or C, collection origin, phylogenetic group, individual VFs, ExPEC status, or aggregate VF score, was significantly associated with the number of selective passages required to obtain an NA- or C-resistant derivative from a susceptible parent or a C-resistant derivative from an NA-resistant derivative (not shown).

Changes in VF profiles among NA- and C-resistant derivatives. Comparison of the 80 NA- and C-resistant derivatives with the corresponding 40 parent strains according to extended VF profiles identified only 1 resistant derivative that lacked a VF present in the parent. Strain A612, an Israeli cystitis isolate from group D, had lost *ompT* (outer membrane protease T) during the transition from NA resistance to C resistance. Thus, of the 361 total nonredundant VFs detected within the collection, none (95% confidence interval [CI], 0 to 1.0%) were lost during the transition from susceptibility to NA resistance, and 1/361 (CI, 0.007 to 1.5%) was lost during the transition from NA to C resistance. (Small numbers precluded an analysis of predictors of loss of VFs during transition to NA or C resistance.) Since a mean of 6.125 selective passages (or 12.25 passages total, including the amplification step that preceded each selection step) separated the parents and their C-resistant derivatives, this represented a net VF loss rate of one VF per 2,211 selective passages or one VF per 4,422 total passages (i.e., 2.3×10^{-4}). These values are similar to the observed rate of the spontaneous deletion of pathogenicity islands from reference ExPEC strains during nonselective serial passage (10). Repeated PCR-based phylotyping showed that each resistant derivative retained the phylogenetic signature of the parent strain (data not shown).

DISCUSSION

This molecular analysis of 40 diverse wild-type *E. coli* strains and their experimentally derived NA- and C-resistant derivatives yielded two main findings. Specifically, the spontaneous

emergence of NA or C resistance during selective passage on antimicrobial-supplemented agar plates both (i) occurred largely independently of underlying strain characteristics, including collection origin, phylogenetic group, and VF profile, and (ii) was accompanied by negligible alterations in VF content or inferred phylogenetic group. These findings provide strong evidence against several hypothesized mechanisms for the negative associations consistently observed among human clinical *E. coli* isolates between quinolone or fluoroquinolone resistance and virulence-associated traits, such as extraintestinal VFs and membership in phylogenetic group B2 (14, 18–20, 24, 28, 29, 41). Consequently, they suggest that alternative mechanisms must underlie these associations.

We found that only 1 of 40 parent strains lost any VF in association with the development of NA or C resistance and that collectively, only 0.2% of all parental VFs was absent from the corresponding resistant derivatives. This provides the best evidence to date that the loss of VF genes as a concomitant of a transition to quinolone or fluoroquinolone resistance is quite rare and thus is an improbable explanation for the striking paucity of VFs consistently encountered among resistant human clinical isolates relative to the quantity encountered among susceptible isolates (28, 41). This complements observational data showing that shifts in phylogenetic distribution toward intrinsically VF-poor phylogenetic groups can explain most or all of the observed low virulences of quinolone- and fluoroquinolone-resistant clinical isolates (14, 18–20, 24, 29). Note that our data do not exclude the possibility that the stress of exposure to quinolones or fluoroquinolones may sometimes precipitate the spontaneous excision of a VF-bearing pathogenicity island, or of a fragment thereof, within a clonal population of VF-rich cells (38). However, this phenomenon appears probably to be sufficiently rare as not to significantly affect VF profiles at the population level.

If the paucity of VFs among resistant human clinical *E. coli* isolates involves shifts in phylogenetic distribution rather than loss of VFs during conversion to resistance, mechanisms that include phylogenetic group must be considered. We tested two such hypothetical mechanisms: (i) that transition to NA or C resistance is inherently more likely among strains from low-virulence phylogenetic groups (A, B1, and D) than from group B2, regardless of VF content, and (ii) that this transition occurs more readily among strains with sparser VF profiles (which are typically non-B2). Both hypotheses were rejected. That is, no convincing difference was apparent in the numbers of selective passages required to obtain NA- or C-resistant derivatives according to phylogenetic group or VF profile, whether VF profile was assessed dichotomously (as individual VFs or ExPEC status) or continuously (VF score). The only suggested differences involved weak associations of *pap* positivity with a more rapid transition from NA resistance to C resistance (which is counter to the hypothesized direction and may have occurred by chance alone because of multiple comparisons). It should be noted that we observed this almost complete absence of statistical differences despite the adoption of a liberal definition of statistical significance ($P < 0.05$, uncorrected for multiple comparisons), which substantially raised the possibility of type I error. Similarly, no such difference was observed in relation to the other baseline bacterial characteristics analyzed, including basal MICs for NA and C and collection origin, i.e., ECOR versus Israeli cystitis. Finally, we excluded the hypothetical possibility that the transition to NA or C resistance produces artifactual shifts in the inferred phylogenetic group measured by the multiplex PCR assay.

These findings suggest that, in *E. coli*, transition to NA and C resistance occurs randomly, without regard to background bacterial traits. If this is so, then the characteristics of a drug-resistant population should reflect the source population within which resistance initially emerged. Therefore, since naturally occurring drug-resistant and drug-susceptible human isolates differ considerably with respect to VF profile and phylogenetic background, resistant isolates must derive from (and, hence, must have been selected from within) a source population distinct from the susceptible isolates. Although this conceivably could be the intestinal florae of humans receiving fluoroquinolone therapy, the skew of the phylogenetic distribution and the paucity of VFs observed among resistant human clinical isolates are extreme even for human fecal *E. coli* (24) and therefore are more consistent with an animal or environmental source. This possibility is consistent with the use of fluoroquinolones in the United States in poultry production and with the corresponding abundance of NA- and C-resistant *E. coli* strains in retail poultry products compared with their occurrence in other foods (6, 15, 21, 22, 39). It also is consistent with the similar phylogenetic and VF characteristics of NA- and C-resistant *E. coli* isolates from retail foods and food animals, relative to those of susceptible *E. coli* isolates from these sources (15, 21). Additional molecular comparisons are needed to test the hypothesis of a food animal source for NA- and C-resistant *E. coli* strains in humans.

The limitations of the study must be considered. The selection for resistance was experimental, using in vitro conditions that may not faithfully mimic naturally occurring selection in the environment or animate host. Isolates had been subcultured multiple times and had undergone prolonged freezer storage prior to testing, possibly altering their behaviors with respect to the development of resistance. The (uncharacterized) mutation(s), or other alterations, accounting for NA and C resistance may have differed from those occurring in naturally derived resistant isolates. Actual mutation frequencies were not measured; instead, the ease of resistance was assessed, using a standardized but not rigorously quantitative method, as the number of selective passages required to obtain resistant derivatives. (Notably, the fact that fewer selective passages were required for transition from NA to C resistance for those NA-resistant derivatives with above-median C MICs indirectly validates our use of the number of selective passages as a relevant metric of the ease of conversion to resistance.) Since VF analysis was limited to a single arbitrarily selected representative of each resistant population, minority variants experiencing more-extensive loss of VFs could have been overlooked. Virulence was inferred from VF content rather than being experimentally assessed, as supported by previous experimental data (17, 34). Finally, the sample size limited the power for detecting small differences. Strengths of the study include the attention to phylogenetic group, VF profile, basal MICs, and source collection as predictors of the development of resistance; the extensive panel of VFs analyzed; and the confirmation of phylogenetic group among resistant derivatives.

In summary, our molecular analysis of 40 diverse wild-type and reference *E. coli* isolates showed that spontaneous transition to NA or C resistance during selective passage on antimicrobial-supplemented agar plates (i) occurred essentially independently of underlying strain characteristics (including collection origin, phylogenetic group, and VF profile) and (ii) was accompanied by negligible alterations in VF content or inferred phylogenetic group. These findings provide novel experimental evidence against several hypothesized mechanisms accounting for the paucity of extraintestinal VFs and for the shifts away from phylogenetic group B2 consistently observed among NA- and C-resistant human clinical *E. coli* isolates, which include the direct loss of VFs during transition to resistance and a greater ease of transition to resistance among low-virulence or non-B2 strains. Consequently, they suggest that alternative mechanisms must underlie the observed associations. A plausible possibility is the importation of resistant strains into humans from an external selection reservoir (e.g., food animals), one in which the susceptible source population exhibits the same low-VF-number, non-group-B2 profile as typifies resistant human clinical isolates.

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