Effects of selection pressure and genetic association on the relationship between antibiotic resistance and virulence in Escherichia coli

Lixin Zhang, University of Michigan
Karen Levy, Emory University
Gabriel Trueba, Universidad San Francisco de Quito
William Cevallos, Universidad Central del Ecuador
James Trostle, Trinity College Hartford
Betsy Foxman, University of Michigan
Carl F. Marrs, University of Michigan
Joseph N.S. Eisenberg, University of Michigan

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Antibiotic selection pressure and genetic associations may lead to the cooccurrence of resistance and virulence in individual pathogens. However, there is a lack of rigorous epidemiological evidence that demonstrates the cooccurrence of resistance and virulence at the population level. Using samples from a population-based case-control study in 25 villages in rural Ecuador, we characterized resistance to 12 antibiotics among pathogenic \((n = 86)\) and commensal \((n = 761)\) *Escherichia coli* isolates, classified by the presence or absence of known diarrheagenic virulence factor genes. The prevalences of resistance to single and multiple antibiotics were significantly higher for pathogenic isolates than for commensal isolates. Using a generalized estimating equation, antibiotic resistance was independently associated with virulence factor carriage, case status, and antibiotic use (for these respective factors: odds ratio \(OR = 3.0\), with a 95% confidence interval \(CI\) of 1.7 to 5.1; \(OR = 2.0\), with a 95% CI of 1.3 to 3.0; and \(OR = 1.5\), with a 95% CI of 0.9 to 2.5). Virulence factor carriage was more strongly related to antibiotic resistance than antibiotic use for all antibiotics examined, with the exception of fluoroquinolones, gentamicin, and cefotaxime. This study provides epidemiological evidence that antibiotic resistance and virulence factor carriage are linked in *E. coli* populations in a community setting. Further, these data suggest that while the cooccurrence of resistance and virulence in *E. coli* is partially due to antibiotic selection pressure, it is also genetically determined. These findings should be considered in developing strategies for treating infections and controlling for antibiotic resistance.

Antibiotic resistance is a major public health problem, identified by the World Health Organization (WHO) as one of the most pressing issues in global health (1). In the United States, more than 2 million people per year acquire bacterial infections that are resistant to antibiotics, resulting in at least 23,000 deaths and expenditures of 20 to 35 billion dollars annually (2). Elsewhere, losses to the gross domestic product (GDP) from antibiotic resistance are estimated to be 0.4% to 1.6% (3). Antibiotic resistance can occur in any bacteria but is of direct concern among pathogens, because of the resulting increased duration of care, cost of treatment, morbidity, and mortality (4, 5). Although selection due to antibiotic use is considered a major factor associated with increased antibiotic resistance among pathogens, studies on the molecular mechanisms of resistance and virulence suggest a genetic association. For example, genes for both resistance and virulence can be colocalized in the genome, indicating coselection (6). While these mostly laboratory-based studies suggest a plausible ecological and biological link, there is a lack of rigorous epidemiological evidence demonstrating this link between antibiotic resistance and virulence in human populations.

Pathogenic *Escherichia coli* and shigellae are responsible for an estimated 840,000 cases of disease per year in the United States (7) and for 583,500 deaths globally (8). Most *E. coli* strains found in the human gut are commensals that do not cause disease. A minority of *E. coli* strains, however, possess virulence factors that are markers of specific pathotypes that can cause either intestinal or extraintestinal infections (9). Diarrheagenic *E. coli* strains are among the best-characterized pathogenic *E. coli* strains and can be identified by the presence of specific virulence gene markers (9, 10). Because these *E. coli* strains are capable of causing disease, they are considered more virulent than commensal *E. coli* strains, even though colonization by diarrheagenic *E. coli* does not always result in disease (10). Some studies have documented relationships (both positive and negative) between antibiotic resistance and virulence in *E. coli* strains causing extraintestinal infections, but much less is known about this relationship in diarrheagenic *E. coli* (11–13).

Selection pressure and genetic mechanisms both contribute to the positive correlation between virulence and antibiotic resistance. Antibiotic use is associated with increased antibiotic resistance (16, 17), and antibiotics are frequently used to treat diarrhea and other conditions in humans (14) and animals (15), but gen-
gerally only when symptoms occur. Therefore, strains with great pathogenicity—those that are more likely to cause symptoms following colonization—have a higher probability of being exposed to antibiotic therapy than nonpathogenic strains and have a higher probability of developing resistance. The use of antibiotics to treat infections could also affect the development of resistance in commensal strains of the hosts. Reducing the use of antibiotics, and therefore reducing selection pressure, is considered an important strategy for controlling resistance levels (18). This strategy could reduce the resistant bacterial population and thus reduce the cooccurrence of resistance and virulence. Genes that determine virulence and antibiotic resistance often occur on mobile genetic elements and occasionally are coregulated (for reviews, see the work of Martinez and Baquero [6] and Beceiro et al. [19]). Plasmids are known to be major vectors for the spread of both antibiotic resistance and virulence genes. For example, the same plasmid within *E. coli* has been observed to carry both resistance genes (e.g., encoding resistance to tetracycline, streptomycin, and sulfonamides) and virulence genes (e.g., encoding heat-labile and heat-stable enterotoxin) (20, 21). Similarly, virulence genes such as those encoding enterotoxin and Shiga toxin are found on transposons (22, 23), genetic elements often associated with antibiotic resistance genes. Additionally, antibiotic pressure plays a role in the dissemination of mobile genetic elements, some of which carry virulence genes (24).

While the genetics underlying resistance and virulence strongly suggest a linkage between these two traits, to the best of our knowledge, no population-based study has examined the population-level association between antibiotic exposure and variability in antibiotic resistance levels by pathogenicity. In hospital settings, high rates of antibiotic resistance are commonly observed among pathogenic bacteria (2), but population-based epidemiological studies can provide insight into how much this occurs in the community setting. In the present study, we take advantage of a unique and extensive data set of pathogenic and commensal *E. coli* isolates from villages in rural Ecuador to examine the cooccurrence of antibiotic resistance and virulence and to assess whether virulence and antibiotic resistance among *E. coli* strains have emerged as coupled phenomena in the general population. Using symptomatic status and antibiotic exposure data from humans, along with information on genotype and the presence of resistance and virulence genes, we assess whether antibiotic resistance is associated with the virulence of *E. coli*.

### MATERIALS AND METHODS

**Study design and population.** As part of an ongoing study of the effect of environmental changes on diarrheal diseases in a rural region of northern coastal Ecuador (25), we conducted a series of case-control studies in 25 communities in the northern Ecuadorian province of Esmeraldas between 2009 and 2010. All households within each village were recruited, except in the larger town of Borbón, where a random sample of 400 households (out of approximately 5,000) was recruited. Each community was visited for 15 days, and stool samples were collected from all identified case patients and 10% of randomly selected control individuals. Cases were defined using the WHO definition of three or more loose stools in a 24-h period; controls were defined as individuals without diarrhea in the previous 6 days. Individual antibiotic usage was ascertained via a structured interview within the previous week, before the onset of diarrhea. Informed consent was obtained from all households, and all study protocols were approved by the University of Michigan and Trinity College Institutional Review Boards and the Universidad San Francisco de Quito Bioethics Committee.

**E. coli isolation, antimicrobial susceptibility testing, and genotyping.** Stool samples were cultured on MacConkey medium within 48 h of collection. After 24 h of incubation, five lactose-positive colonies were randomly selected and tested for β-glucuronidase activity to identify *E. coli*, using Chromocult agar (Merck, Darmstadt, Germany). Suspected *E. coli* isolates were confirmed using API 20E tests (bioMérieux, Marcy l’Étoile, France). The first randomly identified lactose-positive *E. coli* strain (or lactose-negative *E. coli* strain if no lactose-positive *E. coli* strain was identified) from each sample was tested for susceptibility to 12 antibiotics (Table 1) by using the disk diffusion method.

### Table 1

**Summary of antibiotics tested and genes screened for each *E. coli* isolate**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Comment</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics tested (n = 12)</td>
<td>Amoxicillin-clavulanate, ampicillin, cefotaxime, cephalothin, chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin, streptomycin, sulfamethoxazole-trimethoprim, sulfisoxazole, tetracycline</td>
<td>Tested using the disk diffusion method and interpreted as resistant, intermediate, or susceptible according to standard criteria</td>
<td>26, 27</td>
</tr>
<tr>
<td>Genes screened (n = 32)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathogen-specific genes</td>
<td>stx1, stx2, eae, bfp, h, virF, ipaH, aafII, afa</td>
<td>Used to identify six diarrheagenic pathotypes (ETEC, EIEC, EPEC, STEC/EHEC, EAEC, and DAEC) according to published criteria</td>
<td>10, 28</td>
</tr>
<tr>
<td>Genes for phylogenetic grouping</td>
<td>chuA, yjaA, tspf4.C2</td>
<td>Used to categorize isolates into one of the four main <em>E. coli</em> phylogenetic groups: A, B1, B2, and D</td>
<td>29</td>
</tr>
<tr>
<td>Resistance genes</td>
<td>tetA, tetB, qnrB, intI1</td>
<td>Tetracycline resistance, Tetracycline resistance, Quinolone resistance, Integron 1, frequently with genes for sulfamethoxazole-trimethoprim resistance</td>
<td>30, 31, 32</td>
</tr>
<tr>
<td>Others</td>
<td>iucD, c0286, c0311, c1600, c3389, capII, cnp1, fyuA, hly, iha, iverin, ompF, prs, s3187, sfa, usp</td>
<td>Additional genes used for genotyping</td>
<td>33, 34</td>
</tr>
</tbody>
</table>

* A binary string of 28 bits, representing the presence or absence of 28 genes (excluding the 4 resistance genes), was used to represent the isolate’s genotype.

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method and was genotyped using a binary typing method based on the presence or absence of 28 previously described genes (Table 1) (33, 34). Nine of these were virulence genes, which we used to identify the following six diarrheagenic E. coli pathotypes according to published criteria: enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), Shiga-toxigenic/enterohemorrhagic (STEC/EHEC), enteraggreggative (EAEC), and diffuse adherent (DAEC) E. coli (10, 28). Three genes were used to categorize isolates into one of the four main E. coli phylogenetic groups: A, B1, B2, and D (29). In addition to the 28 genes used for genotyping, we screened for the presence of three antibiotic resistance genes (tetA, tetB, and qnrB) as well as the intI1 gene, a marker for class 1 integron and its associated resistance genes (Table 1). The presence or absence of all 32 genes was determined using a high-throughput dot blot hybridization assay on the Library-on-a-Slide (LOS) array platform developed in our laboratory, using PCR-amplified DNA fragments internal to each of the genes as hybridization probes (35).

**Statistical analysis.** Clinical and Laboratory Standards Institute (CLSI) standards (27) were used to determine susceptibility profiles, and isolates with intermediate clinical susceptibility were classified together with sensitive isolates. Because the probabilities of sampling of cases and controls were not equal, a weighted prevalence was calculated for antibiotic resistance in overall samples, using the unbiased Horvitz-Thompson estimator (36). Chi-square tests were used for group comparisons or trend analyses. Associations between various antibiotic resistance outcomes (e.g., resistance to a single antibiotic, any antibiotic, or multiple antibiotics) and three main predictors of interest (isolate virulence factor carriage, case status, and reported antibiotic use) were evaluated using generalized linear models (PROC GENMOD [SAS, version 9.1]; SAS Institute, Cary, NC). Model outcomes were at the isolate level and were adjusted for clustering of samples within villages and households by using the generalized estimating equation (GEE) method implemented in PROC GENMOD. The other predictors of interest (i.e., virulence factor carriage, case status, and antibiotic use) were controlled for as covariates in each model, as they could influence the levels of resistance seen in the E. coli strains collected.

### RESULTS
A total of 847 E. coli isolates were collected from 137 case patients and 710 control individuals. The prevalence of resistance varied by antibiotic (Table 2), antibiotic class (see Table S1 in the supplemental material), and case control status (see Table S2).

Diarrheagenic virulence factors for E. coli were identified in 86 of 847 isolates; these 86 isolates were classified into five diarrheagenic E. coli pathotypes, as follows: 17 ETEC, 17 EPEC, 1 EIEC, 25 EAEC, and 26 DAEC isolates. No STEC/EHEC isolates were identified. The weighted prevalences of diarrheagenic pathotypes ranged from 0.1% for EIEC to 2.8% for EAEC (see Table S3 in the supplemental material). Overall, diarrheagenic E. coli strains were isolated significantly more often from case patients than from controls (21.9% versus 7.9%; odds ratio [OR] = 3.3; 95% confidence interval [CI] for OR, 2.0 to 5.3 for any pathotype). Likewise, case isolates had generally higher resistance prevalences than control isolates for most antibiotics (see Table S2).

For 8 of the 12 antibiotics examined, resistance prevalence among pathogenic E. coli isolates (defined as having one or more virulence factors present) was significantly higher than the prevalence among commensal E. coli isolates (defined as having no virulence factors present), regardless of whether or not they were isolated from case patients or controls (Table 3). For five of the eight antibiotics (tetracycline, ampicillin, sulfisoxazole, sulfamethoxazole-trimethoprim, and cephalothin), the prevalences of resistance ranged from 1.2 to 2.5 times higher for pathogenic than for commensal E. coli. For three of the eight antibiotics (streptomycin, chloramphenicol, and amoxicillin-clavulanate), the prev-

### TABLE 2 Prevalences of resistance to 12 antibiotics in E. coli strains isolated from villagers in northwest Ecuador in 2009–2010

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Prevalence of resistance (%) (n = 847)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>33.6</td>
<td>27.8–39.5</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>28.9</td>
<td>22.8–34.9</td>
</tr>
<tr>
<td>Sulfisoxazole</td>
<td>27.8</td>
<td>21.7–33.9</td>
</tr>
<tr>
<td>Sulfamethoxazole-trimethoprim</td>
<td>23.4</td>
<td>17.1–29.7</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>16.7</td>
<td>10.1–23.2</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>12.9</td>
<td>6.2–19.6</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>5.0</td>
<td>0.0–12.0</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>4.7</td>
<td>0.0–11.7</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>3.1</td>
<td>0.0–10.2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2.4</td>
<td>0.0–9.5</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2.2</td>
<td>0.0–9.4</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.3</td>
<td>0.0–7.4</td>
</tr>
</tbody>
</table>

### TABLE 3 Prevalences of resistance to 12 tested antibiotics in E. coli strains isolated from villagers in northwestern coastal Ecuador in 2009–2010, stratified by case/control status (with and without diarrhea) and further stratified by whether the isolate carried a virulence factor (VF) for diarrheagenic E. coli

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Case data (n = 137)</th>
<th>Control data (n = 710)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VF+ (n = 30)</td>
<td>VF− (n = 107)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>53.3</td>
<td>43.9</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>90.0</td>
<td>41.1</td>
</tr>
<tr>
<td>Sulfisoxazole</td>
<td>86.7</td>
<td>40.2</td>
</tr>
<tr>
<td>Sulfamethoxazole-trimethoprim</td>
<td>80.0</td>
<td>35.5</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>46.7</td>
<td>21.5</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>46.7</td>
<td>9.3</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30.0</td>
<td>4.7</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>33.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>6.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

$^a$ Ratio not calculated because some cells had zeros.
The prevalences of resistance to the remaining four antibiotics were low; most of these resistances were from controls and were from commensal E. coli isolates.

Compared to commensal isolates, pathogenic isolates were more likely to be resistant to multiple antibiotics. Among isolates resistant to at least one antibiotic, pathogens were resistant to an average of five antibiotics, versus three for commensals. A significantly higher percentage of pathogenic than commensal isolates were resistant to five or more antibiotics ($P < 0.001$), and a significantly higher percentage of commensal than pathogenic isolates were resistant to two or fewer antibiotics ($P < 0.001$) (Fig. 1). The prevalence ratio comparing pathogenic to commensal isolates increased with an increasing number of antibiotics to which isolates were resistant ($P < 0.001$).

A total of 105 unique antibiotic resistance profiles (specific combinations of resistance to the 12 antibiotics tested) were observed, and 66 were observed only 1 or 2 times. The most common profile of multiple resistance was against ampicillin–tetracycline–sulfisoxazole–streptomycin–trimethoprim-sulfamethoxazole (3.3%), occurring 2.5 times more frequently in pathogens than in commensals ($P = 0.027$).

Case status was associated with a more frequent use of antibiotics during the week before the onset of diarrhea. A total of 21.5% of the case patients, versus 6.1% of the controls, reported antibiotic use during the week before the onset of diarrhea, most commonly beta-lactams (12.6% of all cases and 4.1% of all controls) and sulfa drugs (3.0% of all cases and 0.6% of all controls).

Predicting antibiotic resistance. Separate generalized linear models were fitted using the GEE method for different resistance outcomes, including resistance to any antibiotic, multidrug resistance (to three or more antibiotics), and resistance to specific individual antibiotics or combinations of antibiotics (Table 4). Virulence factor carriage, case status, and antibiotic use were each independently associated with any resistance and multidrug resistance (Table 4); virulence factor carriage was the strongest predictor of resistance (highest ORs observed). Virulence factor carriage was also the only consistent predictor of resistance for all but one outcome modeled (fluoroquinolone resistance). Resistances to gentamicin and cefotaxime were not modeled due to very low prevalences. Antibiotic use was the weakest predictor of resistance. When we modeled the use of specific antibiotic classes in...

**FIG 1** Percentages of pathogenic (virulence factor for diarrheagenic E. coli present; white bars) and commensal (no virulence factor for diarrheagenic E. coli present; gray bars) E. coli, by resistance level to no antibiotics (none), 1 antibiotic, 2 antibiotics, 3 antibiotics, 4 antibiotics, or 5 antibiotics. *, $P$ value from the Mantel-Haenszel chi-square test for trend.
stead of any antibiotic, we did not observe an increase in the strength of the associations with resistance.

**Association between resistance genes and virulence factor genes.** The tetA, tetB, and intI1 (a marker of the presence of class 1 integron and its associated resistance genes) genes were strongly associated with the expected phenotypic resistance to tetracycline and sulfamethoxazole-trimethoprim. Odds ratios (95% CI) for the tetA-tetracycline resistance, tetB-tetracycline resistance, intI1-sulfa resistance, and intI1-sulfamethoxazole-trimethoprim resistance combinations were 66.6 (33.4 to 148.6), 31.2 (14.9 to 74.1), 13.2 (8.6 to 20.8), and 9.8 (6.5 to 14.7), respectively. The association of the qnrB gene with quinolone resistance was not as strong as the other associations but was still statistically significant (OR = 5.1; 95% CI, 1.6 to 13.8). The association of the resistance gene with any virulence factor gene was positive and statistically significant for the tetA (OR = 1.7; 95% CI, 1.0 to 2.8) and intI1 (OR = 2.5; 95% CI, 1.5 to 4.1) genes but was not statistically significant for tetB (OR = 1.6; 95% CI, 0.8 to 2.9). The qnrB gene had a negative but not statistically significant association with virulence factor genes (OR = 0.2; 95% CI, 0.0 to 1.1).

**Phylogenetic lineages and genotypic diversity of pathogenic and commensal E. coli isolates.** All phylogroups were represented, but pathogenic isolates (25%) were approximately twice as likely to belong to the D group as commensal isolates (12%) (P = 0.0002). The differences between pathogenic and commensal isolates in the other phylogroups were not as large: for the A group, 39% versus 52% (P = 0.046); for the B1 group, 18% versus 27% (P = 0.082); and for the B2 group, 13% versus 8% (P = 0.088). Significant differences in phylogroup distribution were seen for ampicillin, cephalothin, sulfisoxazole, and sulfamethoxazole-trimethoprim resistance but not for resistance to other antibiotics (Table 5). Antibiotic resistance in phylogroup D was higher than that in other groups for a number of antibiotics, as was resistance to multiple antibiotics.

A total of 199 unique genotypes were identified in commensal E. coli strains, and 54 were identified in pathogenic E. coli strains. However, there was no difference in Simpson’s diversity index (37) between the two groups (pathogen index, 0.985 [95% CI, 0.977 to 0.992]; and commensal index, 0.984 [95% CI, 0.981 to 0.987]).

**DISCUSSION**

The interplay between antibiotic resistance and virulence can have profound effects on the evolution of bacterial pathogens and our ability to combat them. Our analysis of a large population sample of E. coli isolates from rural Ecuador provides epidemiological evidence that resistance and virulence phenotypes are linked in a community setting. As expected due to selection pressure, we found higher odds of both any- and multidrug resistance in E. coli isolates from individuals reporting use of antibiotics. However, when we controlled for antibiotic use, we found higher rates of resistance in isolates collected from individuals with symptomatic diarrhea, and when we controlled for both antibiotic use and symptomatic status, we found higher rates of resistance for strains of E. coli with diarrheagenic virulence genes present (Table 4). These data suggest that both selection pressure and genetic association are involved in the cooccurrence of resistance and virulence.

**Selection pressure.** Diarrhea patients reported more frequent use of antibiotics in the week prior to the onset of diarrhea than did controls (21.5% versus 6.1%), and antibiotic use was associated with some of the resistance outcomes examined (Table 4). These data provide evidence that selection pressure is occurring in our study population. While only oral rehydration therapy is recommended in most cases of diarrhea, antibiotic therapy is often used to treat acute infectious gastroenteritis (38). Surveys of participating villages found antibiotics available in 16 of 19 study villages but oral rehydration sachets in only 6 of 19. Moreover, ethnographic interviews revealed that a common household remedy used in the area for treatment of diarrhea involves mixing oxytetracycline with lemon juice and water (39).

The observation that people with diarrhea had more frequent use of antibiotics before the onset of diarrhea is also interesting. This may suggest that antibiotic use predisposes individuals to the

**TABLE 5 Resistance to different antibiotics by E. coli phylogroup in E. coli isolates from villagers in northwestern coastal Ecuador in 2009–2010**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>% of isolates with resistance</th>
<th>A (n = 431)</th>
<th>B1 (n = 224)</th>
<th>B2 (n = 76)</th>
<th>D (n = 116)</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>47.3</td>
<td>54.9</td>
<td>60.5</td>
<td>68.1</td>
<td></td>
<td>0.0004</td>
</tr>
<tr>
<td>Amp</td>
<td>27.8</td>
<td>30.8</td>
<td>31.6</td>
<td>51.7</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ceph</td>
<td>9.0</td>
<td>24.6</td>
<td>22.4</td>
<td>37.1</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clo</td>
<td>4.9</td>
<td>5.8</td>
<td>5.3</td>
<td>9.5</td>
<td></td>
<td>0.31</td>
</tr>
<tr>
<td>Str</td>
<td>13.2</td>
<td>11.6</td>
<td>17.1</td>
<td>16.4</td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>Tet</td>
<td>33.4</td>
<td>33.5</td>
<td>39.5</td>
<td>40.0</td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>Sul</td>
<td>28.3</td>
<td>28.6</td>
<td>38.2</td>
<td>41.4</td>
<td></td>
<td>0.021</td>
</tr>
<tr>
<td>Sxt</td>
<td>25.5</td>
<td>21.0</td>
<td>28.9</td>
<td>39.7</td>
<td></td>
<td>0.0026</td>
</tr>
<tr>
<td>Amp or Eno</td>
<td>4.9</td>
<td>5.4</td>
<td>2.6</td>
<td>2.6</td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>Amp + Tet + Sul + Sxt</td>
<td>12.5</td>
<td>13.8</td>
<td>9.2</td>
<td>29.3</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

a Any, resistance to at least 1 of 12 tested antibiotics; Amp, ampicillin; Ceph, cephalothin; Clo, chloramphenicol; Str, streptomycin; Tet, tetracycline; Sul, sulfisoxazole; Sxt, sulfamethoxazole-trimethoprim; Cip, ciprofloxacin; Eno, enrofloxacin.

b The chi-square test of independence was used to test the difference in phylogroup composition between E. coli isolates resistant and nonresistant to the listed antibiotic categories.
development of diarrhea by disrupting the gastrointestinal flora and allowing overgrowth or colonization of a resistant pathogen.

**Genetic association.** The most striking observation in our multivariate analysis was that the presence of diarrheagenic virulence genes was an independent and much stronger predictor of antibiotic resistance than was individual antibiotic use. Furthermore, after controlling for antibiotic use and symptomatic status, the presence of a virulence gene was more strongly associated with antibiotic resistance than was a symptomatic status (Table 4). Consistent with previous reports of cooccurrence of virulence and resistance genes (11, 40, 41), we observed a positive correlation between three resistance genes (tetA, tetB, and intI) and the presence of any virulence gene, although only correlations of the intI and tetA genes were statistically significant.

We also observed that isolates in phylogenetic group D were more strongly associated with multidrug resistance, and pathogenic isolates were twice as likely as commensal isolates to belong to phylogenetic group D. This is consistent with previous observations by other researchers (42). The propensity for a phylotype to be overrepresented in both virulent and resistant *E. coli* strains may suggest an ancient genetic link between virulence and resistance, because phylogroups diverged millions of years ago (43), long before human use of antibiotics. Alternatively, some phylogroups might have more genome plasticity and therefore are better at incorporating resistance genes over time through horizontal gene transfer. At least in our study population, there was a stronger effect observed for a genetic association than for selection via recent antibiotic use.

**Resistance to specific antibiotics.** High prevalences of antibiotic resistance in human diarrheagenic *E. coli* infections have been reported in other countries (16, 44). The highest resistance frequencies observed for pathogenic *E. coli* strains in our study were to the commonly used antibiotics ampicillin (90% in cases and 62.5% in controls) and sulfamethoxazole-trimethoprim (80% in cases and 51.8% in controls) (Table 3). This finding is similar to what has been described for Peru (85% and 79%, respectively) (16) and Mexico (73% and 65%, respectively) (44).

Our analysis extends these studies by showing that pathogenic *E. coli* isolates were associated with higher prevalences of resistance than commensal *E. coli* isolates across most antibiotics examined. However, pathogenic *E. coli* isolates were less resistant to fluoroquinolones. Consistent with the fluoroquinolone result, the qnrB gene was negatively correlated with the presence of virulence genes. A similar inverse relationship between quinolone resistance and virulence has been shown for *Pseudomonas aeruginosa* (11). Although the qnr family of genes confer only low-level fluoroquinolone resistance, they provide a selective advantage in the presence of these antibiotics (45). Soto reported that quinolone resistance was associated with increased virulence in *Pseudomonas aeruginosa* but decreased virulence in *E. coli* (46). Two possibilities have been proposed to explain the negative relationship between resistance to quinolones and virulence, as follows: (i) mutations in DNA gyrase reduce the DNA supercoiling that interferes with the expression of certain virulence factors (46); and (ii) exposure to quinolones induces elimination of virulence genes containing pathogenicity islands by an SOS-dependent pathway, a common bacterial stress response mechanism (46). Negative associations have also been observed between virulence potential and CTX-M beta-lactamase production in *E. coli* (12, 13).

**Study limitations.** Our ability to show the linkage between specific virulence and resistance genes is limited, since we examined only four resistance gene elements and we lacked power to examine individual virulence genes. Additionally, pathogenic *E. coli* strains were identified by detecting the presence of marker genes without confirming the expression of the virulence phenotypes. However, we previously demonstrated that these genotypically identified pathotypes were associated with symptomatic diarrhea, even after taking into account the detection of rotavirus and *Giardia* in samples from the same study region (25), and we showed their virulence phenotypes in an *in vitro* laboratory assay (47). Furthermore, while the diarrheagenic pathotypes we identified as pathogenic *E. coli* are well recognized (10), *E. coli* isolates that we defined as commensal might include unrecognized pathogenic *E. coli* strains. Such potential misclassification would have resulted in an underestimation of the difference between pathogenic and commensal *E. coli* resistance levels.

**Conclusions.** Our population-based study showed a strong correlation between virulence and antibiotic resistance in communities in northern coastal Ecuador. While antibiotic use contributed to the selection of resistance, the link between virulence and resistance was also independent of selection pressure. This suggests that reducing the use of antibiotics will not necessarily reduce resistant pathogens in the population. The maintenance of resistance phenotypes in bacteria could also be influenced by the fitness cost associated with resistance (48) and the likelihood of developing compensatory fitness-improving mutations (49). These can potentially make it difficult to control antibiotic resistance in pathogenic *E. coli* and, more generally, in pathogenic infections. Further studies are needed for a more robust understanding of what drives the link between resistance and virulence so that better antimicrobial stewardship can be developed to balance treatment needs while preserving antibiotic effectiveness.

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