We compared extended-spectrum β-lactamase–producing Escherichia coli isolates from meat and fish, gut-colonized women, and infected patients in Cambodia. Nearly half of isolates from women were phylogenetically related to food-origin isolates; a subset had identical multilocus sequence types, extended-spectrum β-lactamase types, and antimicrobial resistance patterns. Eating sun-dried poultry may be an exposure route.

In Europe, evidence for the spread of extended-spectrum β-lactamase (ESBL)–producing Escherichia coli from animals to humans via food is unclear (1). Few studies have been conducted in low- and middle-income countries, where colonization rates can exceed 60% (2). High ESBL colonization rates in low- and middle-income countries such as Cambodia are usually attributed to unrestricted consumer access to and hospital overuse of third-generation cephalosporins (3,4). However, antimicrobial drugs in classes critical for human health (e.g., β-lactams, macrolides, aminoglycosides, polymyxins) are increasingly being used in food animals (5). In Cambodia, weak public health protections and consumption of undercooked animal products could exacerbate the spread of ESBL-producing E. coli or ESBL genes from animals to humans.

We had 2 goals with this study. First, we assessed the prevalence of ESBL-producing or carbapenemase-producing E. coli from fish, pork, and chicken from markets in Phnom Penh, Cambodia. Second, we examined the contribution of food-origin isolates to locally disseminated ESBL E. coli by comparing isolates from food with isolates from healthy, colonized persons and infected patients.

The Study

During September–November 2016, we purchased 60 fish, 60 pork, and 30 chicken samples from 150 vendors at 2 markets in Steung Meanchey district, Phnom Penh (Appendix Table 2, https://wwwnc.cdc.gov/EID/article/25/1/18-0534-App1.pdf) and tested them at the Institut Pasteur du Cambodge for third-generation cephalosporin- and carbapenem-resistant E. coli (Appendix sections 1.1–1.3). We detected ESBL-producing E. coli (all CTX-M-type) among 93 (62%) of 150 food samples, including 32 (53%) of 60 fish, 45 (75%) of 60 pork, and 16 (53%) of 30 chicken samples. We identified carbapenem-resistant E. coli (OXA-type) from 1 pork and 1 fish sample.

We also selected ESBL-producing E. coli from 88 recently pregnant healthy women living in Steung Meanchey and participating in the Bacterial Infections and antibiotic Resistant Diseases among Young children in low-income countries (BIRDY) program, a surveillance program of bacterial infections among young children in low- and middle-income countries (6). During September 2015–December 2016, ESBL-producing E. coli isolates were cultured from rectal swabs or fecal samples collected at or just after delivery (Appendix Table 3). We further included ESBL-producing E. coli from 15 Phnom Penh–based patients who sought care at the Sihanouk Hospital Center of Hope during November 2015–
Figure 1. Distribution of 105 multilocus sequence types (MLSTs) among predominant extended-spectrum β-lactamase (ESBL) and carbapenemase gene types encoded by 196 ESBL-producing *Escherichia coli* from humans and food, Cambodia, 2015–2016. A) CTX-M-55; B) CTX-M15; C) CTX-M-27; D) CTX-M-14; E) carbapenemases. Vertical axes depict MLSTs. Horizontal axes depict the frequency of each observed MLST. CTX-M-3, CTX-M-24, and CTX-M-65 are not shown because these ESBL gene types were rare (<2%). One human colonization isolate (ST394, clan I/B2&D) encoded CTX-M-3, 1 food-origin isolate (ST10, clan II/A) encoded CTX-M-24, and 2 food-origin isolates (ST2207, clan II/A and ST7586, clan III/B1) encoded CTX-M-65.

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December 2016. ESBL-producing *E. coli* were cultured from blood (12 patients), urine (2 patients), and peritoneal fluid (1 patient) (Appendix Table 4).

We performed whole-genome sequencing for 1 ESBL-producing *E. coli* isolate from each food sample and all human-origin ESBL-producing *E. coli* isolates (Appendix sections 1.4–1.6) and compiled genetic and phenotypic characteristics of these 196 isolates (Appendix Tables 6, 7). We also determined the distribution of multilocus sequence types (MLSTs) encoding predominant ESBL- or carbapenemase-gene types (Figure 1).

Phylogenetic analysis of ESBL-producing *E. coli* genomes revealed 3 distinct clans (Figure 2, panel A). Clan I/B2&D (n = 53) comprised mostly human-origin isolates, including isolates from colonized persons and most infected patients. Clans II/A (n = 69) and III/B1 (n = 47) included isolates from colonized persons and from food but not from infected patients. Each clan comprised an exclusive subset of sequence types (STs); clan I/B2&D included ST131 and clonal complex (CC) 38, clan II/A included CC10, and clan III/B1 included CC58 and CC156. Approximately half (21/39) of isolates in clans II/A and III/B1 from colonized patients belonged to STs detected in both humans and meat (Appendix Table 8).

We determined the distributions of ESBL-encoding genes and resistance patterns among isolates from colonized persons by clan (Figure 2, panels B and C). The *bla*<sub>CTX-M-55</sub> gene was more common among colonization isolates belonging to clan II/A than to clan I/B2&D (p<0.05). Amphenicol resistance was more common among colonization isolates belonging to clan II/A than clan I/B2&D (p<0.05) and was most often encoded by *floR* (Appendix Table 7).

Women colonized with amphenicol-resistant (vs. amphenicol-susceptible) ESBL-producing *E. coli* were more likely to report having ever eaten dried poultry (adjusted odds ratio 9.0, 95% CI 1.8–45.2) (Table). Women colonized with CTX-M-55–producing *E. coli* (vs. other ESBL types) were more likely to have handled live poultry (adjusted odds ratio 4.6, 95% CI 1.1–19.3), but this exposure was uncommon (11/88).

Our genomic and epidemiologic findings suggest that ESBL-producing *E. coli* that contaminates meat and fish in Phnom Penh may be disseminating to the community. ESBL-producing *E. coli* were highly prevalent among the meat and fish we sampled. More than 80% of food-origin isolates were amphenicol resistant, and two thirds produced CTX-M-55. When food-origin isolates were compared

Figure 2. Genomic comparisons of extended-spectrum β-lactamase (ESBL)–producing *Escherichia coli* from humans, fish, pork, and chicken from Cambodia and differences in human colonization isolates by phylogenetic clan. All isolates were phenotypically resistant to third-generation cephalosporins (data not shown). A) Whole-genome sequence-based phylogenetic tree of 195 ESBL-producing *E. coli* genomes comprising 87 human colonization isolates, 15 human clinical isolates, and 93 isolates from fish, pork, and chicken meat and resulting phylogenetic clans I/B2&D (n = 53), II/A (n = 69), and III/B1 (n = 47). B) ESBL-encoding genes of human colonization *E. coli* isolates, by phylogenetic clan. C) Phenotypic resistance of human colonization ESBL-producing *E. coli* isolates to antimicrobial drugs of 8 classes, by phylogenetic clan. Clinical isolates are not included in panels B or C. Of 87 human colonization genomes, 13 did not group into a phylogenetic clan and thus are excluded from panels B and C. Prevalence of outcome differed significantly (p<0.05, indicated by *) between 2 indicated clans by post hoc Tukey test. Only statistically significant differences are depicted. 1, quinolone; 2, co-trimoxazole; 3, tetracycline; 4, aminoglycoside; 5, macrolide; 6, amphenicol; 7, carbapenem; 8, colistin.
with human-origin isolates, ≈40% of ESBL-producing E. coli from healthy persons grouped into the same phylogenetic clans that comprised most food-origin isolates. Approximately half of these colonization isolates had MLSTs detected among food, and a substantial portion were more likely to produce CTX-M-55 and amphenicol resistant than colonization isolates that grouped separately. The fact that chloramphenicol has not been used in human medicine for almost 20 years in Cambodia, yet chloramphenicol analogs (e.g., florfenicol, thiamphenicol) are administered to food animals (5,7), suggests a food origin for these colonizing isolates.

Healthy women colonized with amphenicol-resistant ESBL-producing E. coli were more likely to eat poultry meat prepared by sun drying, a process that may not eliminate bacteria (8). Although we did not test dried meat samples for ESBL-producing E. coli contamination, our finding is consistent with those of other studies (8,9). Women reported having prepared dried poultry at home. Especially in low-resource households, sun-dried meat may become cross-contaminated by raw meat, dust, animals, and flies (8).

Our findings are concerning because of growing interest in using chloramphenicol as a drug of last resort for panresistant strains of bacteria (10). In the early 2000s, the Cambodia government stopped purchasing chloramphenicol because of concerns about side effects. Since restriction of this drug, infections in the hospital setting have reverted to a chloramphenicol-susceptible phenotype (11). Nevertheless, our findings suggest that amphenicol resistance genes are circulating in the

### Table. Environmental exposures and colonization with amphenicol-resistant and CTX-M-55–encoding ESBL-producing Escherichia coli among healthy women, Phnom Penh, Cambodia, 2015–2016*

| Variable                     | CHL resistance | ESBL type |
|------------------------------|----------------|-----------|
|                              | Resistant, no. (%), n = 29 | Susceptible, no. (%), n = 59 | OR (95% CI) | aOR (95% CI) | CTX-M-55, no. (%), n = 26 | Other, no. (%), n = 62 | OR (95% CI) | aOR (95% CI) |
| Persons living in home       |                |           |            |             |                    |                       |            |             |
| >8                           | 5 (17)         | 10 (17)   | 1.1        | (0.3–3.7)   | 3 (12)            | 12 (19) | 0.6         | (0.1–2.5) |
| 6–8                          | 9 (31)         | 19 (32)   | 1.1        | (0.3–3.7)   | 10 (38)           | 18 (29) | 1.4         | (0.5–3.7) |
| ≤5                           | 15 (52)        | 30 (51)   | Referent   |               | 13 (50)           | 32 (52) | Referent    |               |
| Place of delivery            |                |           |            |             |                    |                       |            |             |
| Private clinic               | 5 (17)         | 17 (29)   | 0.4        | (0.1–1.4)   | 4 (15)            | 18 (29) | 0.4         | (0.1–1.4) |
| Hospital                     | 11 (38)        | 20 (34)   | 0.8        | (0.3–2.2)   | 9 (35)            | 22 (35) | 0.7         | (0.2–1.9) |
| Health center                | 13 (45)        | 22 (37)   | Referent   |               | 13 (50)           | 22 (35) | Referent    |               |
| Received antimicrobial drugs at delivery† | 2 (7) | 11 (19)   | 0.3        | (0.1–1.3)   | 1 (4)             | 12 (19) | 0.2         | (0.0–1.4) |
| Untreated drinking water     | 5 (17)         | 7 (12)    | 1.5        | (0.4–5.3)   | 4 (15)            | 8 (13)  | 1.2         | (0.3–4.5) |
| Toilet shared‡               | 11 (38)        | 16 (27)   | 1.6        | (0.6–4.2)   | 5 (19)            | 22 (35) | 0.4         | (0.1–1.3) |
| Nonflush toilet              | 26 (90)        | 47 (80)   | 2.2        | (0.6–8.5)   | 24 (92)           | 49 (79) | 3.2         | (0.7–15.3) |
| Pet contact                  | 6 (21)         | 13 (22)   | 0.9        | (0.3–2.7)   | 6 (23)            | 13 (21) | 1.1         | (0.4–3.4) |
| Live poultry contact         | 4 (14)         | 7 (12)    | 1.2        | (0.3–4.4)   | 6 (23)            | 5 (8)   | 3.4         | (0.9–12.4) |
| Consumption habits           |                |           |            |             |                    |                       |            |             |
| Dried pork ≥1×/wk            | 15 (52)        | 32 (54)   | 0.9        | (0.4–2.2)   | 11 (42)           | 36 (58) | 0.5         | (0.2–1.3) |
| Dried beef                   | 17 (59)        | 38 (64)   | 0.8        | (0.3–2.1)   | 20 (77)           | 35 (56) | 2.6         | (0.9–7.3) |
| Dried poultry                | 27 (93)        | 39 (66)   | 7.9        | (1.7–36.4)  | 22 (85)           | 44 (71) | 2.3         | (0.7–7.5) |
| Pork ≥3×/wk                  | 22 (76)        | 53 (90)   | 0.4        | (0.1–1.2)   | 23 (88)           | 52 (84) | 1.5         | (0.4–5.9) |
| Insects                      | 21 (72)        | 33 (56)   | 2.2        | (0.8–5.7)   | 16 (62)           | 38 (61) | 1           | (0.4–2.6) |
| Raw vegetables ≥1×/wk        | 5 (17)         | 8 (14)    | 1.3        | (0.4–4.5)   | 3 (12)            | 10 (16) | 0.7         | (0.2–2.7) |

*Blank cells indicate variable not included in multivariate models. aOR, adjusted (for age) OR; CHL, chloramphenicol; ESBL, extended-spectrum β-lactamase; OR, odds ratio.
†Not reported for 4 women (missing data). All 4 were colonized with CHL-susceptible ESBL-producing Escherichia coli. One woman was colonized with CTX-M-55–type E. coli, whereas the other 3 were colonized with other CTX-M–encoded isolates.
‡With persons in other households.
community, potentially because amphenicol use in food animals has selected for resistant bacteria that can spread to humans (12). This possibility is concerning because physicians in Cambodia are often unable to assess the resistance of infectious agents before prescribing antimicrobial drugs (4).

Our study had several limitations. First, for logistical reasons, we sampled meat and fish during only 1 season. Contamination rates may have differed had we sampled across seasons (13). Second, although we included colonization samples from healthy women, all women had recently given birth in healthcare settings. However, more than half were colonized with ESBL-producing E. coli phylogenotypes A and B1, supporting community-associated, rather than healthcare-associated, acquisition. Third, we were unable to include clinical isolates from the same population that contributed colonization isolates. Thus, differences in colonization and clinical isolates could have resulted from population differences. Fourth, we did not sample food animals, which could have helped confirm that CTX-M-55–type and amphenicol-resistant ESBL-producing E. coli circulate among them. Last, we did not investigate additional potential pathways for ESBL-producing E. coli transmission to colonized women, such as contact with persons employed at farms or slaughterhouses or proximity to such operations.

Conclusions
This study, which integrated epidemiologic and genomic methods to characterize community, clinical, and environmental data, supports concerns that the dissemination of antimicrobial drug–resistant bacteria from food animals to humans may be more likely in low- and middle-income countries (14,15). This finding is concerning because meat consumption is projected to drastically increase in these countries, and animal production that relies on routine antimicrobial drug use is being promoted to meet this demand (14). Particularly for low- and middle-income countries such as Cambodia, implementation of multisectoral strategies to combat antimicrobial resistance from a One Health perspective must be supported, and food safety should be prioritized.

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Appendix

1. Supplementary Methods

1.1. Detection of third generation cephalosporin- and carbapenem-resistant *Escherichia coli* from fish and meat

All samples were processed within two hours of arrival at IPC. First, 10 g of sample were homogenized in 90 ml Brain Heart Infusion Broth (BHIB). For pork and fish, we sub-sampled meat from both the surface and interior. For chicken, we sub-sampled neck skins only, as is typical when sampling whole chicken carcasses (1). Following overnight incubation at 37°C, a sterile loop was used to plate ~10 µl of enriched BHIB onto Drigaliski supplemented with 2 mg/L cefotaxime (DRI-CTX), to select for third-generation cephalosporin -resistant *Enterobactericeae*, and Drigaliski supplemented with 0.5 mg/L ertapenem (DRI-ERT), to select for carbapenemase-producing *Enterobacteriacea*. Plates were incubated overnight at 37°C. We subcultured up to two lactose-producing colonies from DRI-CTX and DRI-ERT for further characterization.

To confirm ESBL production among presumptive *E. coli* selected from DRI-CTX, we performed the double-disk synergy test with aztreonam (monobactam), cefotaxime, ceftazidime (third generation cephalosporins), cefepime (fourth generation cephalosporin), and an amoxicillin-clavulanate disc. Isolates for which we observed an enhanced inhibition zone toward amoxicillin-clavulanate were considered ESBL-producers.

To confirm carbapenemase production among presumptive *E. coli* selected from DRI-ERT, we performed the Carba-NP test (2). Isolates that produced a color change within two hours were considered carbapenemase-producers.
1.2. Species identification

Among ESBL-P and carbapenemase-producing isolates, we used API20E to confirm the species of up to one presumptive *E. coli* per sample.

1.3. Antibiotic resistance testing

One third-generation cephalosporin- and/or carbapenem-resistant *E. coli* isolate per food sample was assessed for resistance to nine antibiotics at IPC, using the Kirby-Bauer disk diffusion method. All human-origin ESBL-*Ec* were assessed for resistance to 30 antibiotics at IP-Paris (Appendix, Table 1). Diameter interpretations were based on 2016 European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations where available, or 2016 Clinical and Laboratory Standards Institute (CLSI) recommendations for those antibiotics for which 2016 EUCAST recommendations did not exist (3,4). MICs (MICs) for azithromycin, nalidixic acid, and ciprofloxacin were additionally determined used E-tests (bioMérieux, France). We defined isolates resistant to ≥3 antibiotic classes (including third-generation cephalosporins) as multidrug-resistant.

We screened all isolates for colistin susceptibility using a 4 mg/L colistin sulfate solution. Among strains that exhibited growth following overnight incubation at 37°C, we used Sensititer colistin microdilution assays (TREK Diagnostic Systems Inc., Cincinnati, OH) to determine colistin MICs.

1.4. Genome characterization

Libraries were constructed using the Nextera XT DNA Library Preparation kit (Illumina, Inc., San Diego, CA) and sequenced on a NextSeq-500 instrument using a 2x150 paired-end protocol. All sequenced paired-ends reads were clipped and trimmed with AlienTrimmer (5), corrected with Musket (6), merged (if needed) with FLASH (7), and subjected to a digital normalization procedure with khmer (8). For each sample, remaining processed reads were assembled and scaffolded with SPAdes (9).

*E. coli* genomes were screened for acquired antimicrobial resistance genes with ResFinder (selected threshold equal to 90% identity), assigned a multilocus-sequence type (MLST) based on the Achtman scheme (10,11), and assigned a core-genome MLST (cgMLST) based on a scheme from Enterobase that uses 2,513 loci. *E. coli* clonal complexes were
determined using goeBURST following the stringent group definition (6/7 shared alleles) (12). We used in-silico PCR to assign phylo-types following the Clermont scheme (13).

Sequence data have been deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under project number PRJEB25898 (Appendix Table 5).

1.5. Phylogenetic analysis

A Minimum Evolution phylogenetic tree was inferred from the pairwise evolutionary distances estimated between each pair of assembled ESBL-Ec genomes. We used this approach because our goal was to examine the general population structure of epidemiologically-unrelated isolates belonging to 100 STs (with no more than 13 isolates belonging to any single ST (Appendix Table 8)), rather to investigate molecular evidence for specific cases of transmission.

The pairwise $p$-distance (i.e., proportion of nucleotide differences) between each pair of whole genome sequences was estimated with Mash (14). To infer accurate $p$-distances, k-mer size = 20 was chosen according to the Mash method recommendation (see Ondov et al, formula (2)), and sketch size = 431,000 was selected by searching for the one that leads to the pairwise distance matrix associated with the optimal overall treelikeness (15). As every pairwise $p$-distance was quite small (i.e., all Mash estimates <0.038), no further correction was required (16,17), and the distance matrix was directly used for a distance-based phylogenetic inference with FastMe (18). One human colonization ESBL-Ec was excluded from genomic comparisons due to insufficient quality.

The resulting tree was visualized using iTOL v4.2 (https://itol.embl.de/) (19). We use the term “clan” rather than “clade” when reporting results to clarify our description of an unrooted tree (20).

To ensure that the inclusion of accessory genomes in our whole genome-based phylogenetic analysis did not bias our results, we performed a phylogenetic analysis of the 2,513 cgMLST loci defined by the Enterobase scheme. For each locus, allele sequences were aligned with MAFFT (21), and a Maximum Likelihood phylogenetic tree was inferred using IQTree (optimal evolutionary model GTR+F+R3) from the concatenation of the 2,513 multiple sequence alignments (Appendix Figure). The “clans” delineated by this cgMLST-based phylogenetic tree were nearly identical to those presented in Figure 2, suggesting that our whole genome-based
phylogenetic approach did not significantly alter the overall, population-level relationships that we were interested in characterizing.

1.6. Statistical analyses

We used Fisher exact tests to compare resistance patterns among ESBL-Ec from different meat types and between human sample types (i.e., colonization, infection).

Among human colonization ESBL-Ec, we examined whether ESBL-encoding genes and phenotypic antibiotic resistance patterns differed between phylogenetic clans using one-way ANOVAs and post-hoc Tukey tests. Both a) amphenicol resistance and b) presence of \( \text{bla}_{\text{CTX-M-55}} \) significantly differed between clans (p < 0.05 by Tukey test). Thus, we constructed univariate logistic regression models examining associations between healthy women’s environmental exposures, including dietary habits, and the presence versus absence of these characteristics in their colonizing ESBL-Ec (i.e., amphenicol resistance versus susceptibility, CTX-M-55 versus other ESBL-type). Additionally, we used multinomial logistic regression models to explore associations between healthy women’s exposures and the phylogenetic clan (i.e., I/B2&D, II/A, or III/B1) to which their colonizing ESBL-Ec belonged. Exposures considered for both model types are listed in Appendix Table 3.

Variables with univariate \( p \)-values ≤0.2 were included in multivariate binary and multinomial logistic regression models, respectively. We conducted backward stepwise elimination of non-significant parameters (p < 0.05). Multivariate models were adjusted for age.

Genomic differences were not explored for clinical ESBL-Ec because most clinical isolates grouped in one phylogenetic clan.

Analyses were performed using SAS version 9.4 (Cary, NC).

2. Supplementary Results

2.1. Characteristics of ESBL-Ec from food and humans

**ESBL genes.** Among 93 ESBL-Ec from food, CTX-M-55 was the most common ESBL gene type detected, comprising 23/32 ESBL-Ec from fish (72%), 27/45 from pork (60%), and 12/16 (75%) from chicken (Appendix Table 6).
Among 88 human colonization isolates, CTX-M-15 (41/88) and CTX-M-55 (27/88) were the most common ESBL gene types, while among 15 clinical isolates, CTX-M-15 (6/15) was most common and CTX-M-55 (2/15) was least common (Appendix Table 7).

**Antibiotic resistance.** More than two-thirds of ESBL-Ec from food (62/93) expressed resistance to at least five antibiotic classes in addition to third-generation cephalosporins, most commonly tetracycline (89%), co-trimoxazole (86%), fluoroquinolone (80%), aminoglycoside (86%), and amphenicol (83%). We identified 11 phenotypically colistin-resistant ESBL-Ec from three fish and eight pork, but none from chicken. Colistin resistance among 3/3 isolates from fish and 4/8 from pork was mediated solely by mcr-1, resistance among 1/8 pork was mediated solely by mcr-3, and resistance among 2/8 pork was mediated by both mcr-1 and mcr-3 (Appendix Table 6).

Human colonization isolates were more likely to be resistant to amphenicol (p = 0.06) and susceptible to carbapenems (p = 0.04) and azithromycin (p = 0.02) than clinical isolates. Carbapenem resistance was mainly encoded by NDM-type genes. Colistin resistance was rare (<3%) among colonization isolates and was not detected among clinical isolates (Table 4).

**MLST.** We detected 105 distinct STs. Ten of these 105 STs (10%) were detected among both humans and food (i.e., STs 10, 48, 101, 155, 156, 189, 617, 871, 1081, 1196), while 44/105 and 51/105 were detected exclusively among humans or food, respectively (Appendix Table 8). ST10 and single locus variants (collectively, clonal complex (CC) 10) were the most common STs among both food-origin and human colonization ESBL-Ec, comprising 11/93 isolates (12%) and 12/88 isolates, respectively (14%). STs 131 and 410 were more common among clinical isolates, comprising 7/15 (47%) and 2/15 (13%) isolates, respectively.

CC10 encoded all predominant ESBL gene types, although CTX-M-55 was most common (Figure 1; Appendix Table 8). STs that were only common among human-origin isolates (i.e. ST131, CC38, ST410, ST405) rarely or never encoded blaCTX-M-55. Instead, ST131 (n = 11) mostly encoded blaCTX-M-15 (4/11) and blaCTX-M-27 (5/11), CC38 (n = 11) mostly encoded blaCTX-M-15 (7/11), ST410 (n = 6) mostly encoded blaCTX-M-15 (4/6), and ST405 (n = 5) exclusively encoded bladCTX-M-15.
Although only 10/105 (10%) STs were shared between humans and food, 22/88 (25%) of human colonization isolates belonged to these STs. Among colonization isolates that grouped in Clans II/A and III/B1, 21/39 (54%) belonged to nine shared STs.

2.2. Environmental exposures associated with humans’ ESBL-\textit{Ec} colonization patterns

We did not identify consistent associations between any of the environmental or healthcare exposures we examined and women’s colonization with ESBL-\textit{Ec} that belonged to clans II/A or III/B1, versus I/B2&D (referent) (Appendix Table 9).

3. Supplementary Discussion

Our findings differ from previous studies conducted in Europe. Although ESBL-\textit{Ec} are prevalent among poultry (≥80%) in several European countries (22), they are usually genetically distinct from ESBL-\textit{Ec} circulating among healthy humans (23). Conversely, we report a substantial portion of isolates from healthy, gut-colonized persons that were phylogenetically related to food-origin strains. Similar to European studies, we found that only 10% of MLSTs were shared between human- and food-origin ESBL-\textit{Ec}. However, 25% of human colonization isolates belonged to these overlapping STs, and this proportion was even higher (54%) among colonization isolates that were phylogenetically related to food-origin ESBL-\textit{Ec}. In comparison to Europe, we conjecture that weaker public health protections, inadequate regulation of antibiotic use in food animals, and/or consumption of undercooked animal products could be exacerbating the spread of bacterial clones and ESBL-encoding mobile genetic elements from farmed animals to the community in Phnom Penh.

\textit{CTX-M-55} is an increasingly reported ESBL gene type among humans, farmed animals, food, and the environment in Asia (24–26), and was the most common ESBL gene type recovered from fish and meat in this study. We were unable to identify dietary exposures that were associated with women’s colonization with \textit{CTX-M-55}-producing \textit{E. coli}, although women colonized with these isolates were more likely to report direct contact with live poultry. Other work suggests this ESBL-type may be widespread in the environment (25), and thus tracing community exposure pathways may have been difficult.

Among the samples we tested, pork was most commonly contaminated with ESBL-\textit{Ec} (75%). This finding was unexpected because other studies have found poultry and poultry meat
to be most frequently contaminated (22,27). In Europe, ESBL selection is thought to be a consequence of third-generation cephalosporin administration to eggs and young chicks (28), but in Cambodia, farming practices that might select for ESBLs are not monitored (29). Our finding that pork was more contaminated could be a reflection of higher prevalence of ESBL-Ec fecal carriage among pigs compared to chickens, as has been observed in Thailand (30), or the fact that pork is more heavily processed than chicken or fish before sale. Future studies should include samples from the food supply chain to investigate sources of contamination.

Unlike colonization isolates, none of the clinical isolates we examined grouped in the phylogenetic clans that comprised most food-origin isolates (Clans II/A and III/B1). One hypothesis for this finding is that ESBL-Ec with characteristics of food animal origin are less capable of causing infections. However, we lacked sufficient diversity in our clinical isolates to investigate this possibility. Specifically, as gut-colonizing E. coli are more likely to cause urinary tract infections (UTIs) than systemic infections, this hypothesis would have been best explored with the inclusion of a much larger number of UTIs. However, UTIs are difficult to sample in Cambodia and other LMICs where antibiotics can be purchased without a prescription, as sick persons rarely seek medical care for uncomplicated cases. Thus, we were only able to include ESBL-Ec from two UTIs in our present analysis. Future studies in LMICs should prioritize inclusion of UTI samples to fully investigate this hypothesis.

Although all ESBL- Ec from colonized humans that grouped in Clan I were phylo-types B2 or D (commonly associated with infection), we did not identify healthcare exposures associated with women’s colonization with these isolates. Global studies have described a high proportion of ESBL- Ec belonging to phylo-types B2&D, including ST131, among gut-colonized, healthy individuals who lack recent healthcare exposures (31). Indeed, the fact that most clinical isolates that grouped in Clan I/B2&D were community-associated (12/13), rather than hospital-associated, suggests that these phylo-types and STs may be circulating in the community. However, we did observe that women who received antibiotics during delivery were less likely to carry ESBL- Ec with genetic and phenotypic characteristics of food-origin isolates (i.e. CTX-M-55 and amphenicol resistance), although neither of these results were statistically significant. It is possible that antibiotic exposure during delivery altered these women’s intestinal flora, facilitating colonization with ESBL-Ec that encoded different CTX-M-types and resistance patterns than those which predominated among food-origin isolates.
ESBL-Ec we detected on meat and fish could have originated from human contamination, including from farmers (if animals were exposed to human waste), or by slaughterhouse workers and market vendors, through handling. However, >80% of ESBL-Ec from meat and fish were resistant to amphenicols, an antibiotic class that has not been used by humans in Cambodia for almost 20 years. If the meat and fish we sampled were primarily contaminated with human-origin ESBL-Ec, we would have expected a much smaller proportion of these isolates to be amphenicol-resistant (perhaps similar to what we found among colonized women, for example, i.e. 33%). This discrepancy suggests that food animals, who are regularly given amphenicols (32), were the main source of the ESBL-Ec strains we recovered from animal-derived products.

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### 4. Supplementary Tables

#### Appendix Table 1. Antibiotics used for susceptibility testing of human- and food-origin *Escherichia coli* isolates.

| Antibiotic class | Antibiotic tested | Laboratory | Disk Concentration (μg) | Zone Diameter Thresholds (mm) | MIC (MIC) Thresholds (μg/mL) |
|------------------|-------------------|------------|-------------------------|-------------------------------|-----------------------------|
| Amphenicol       | Chloramphenicol   | IPC        | X                       | 30                            | 17-17                       |                             |
| B-lactam         | Amoxacillin       | IP-Paris   | X                       | 25                            | 14-14                       |                             |
|                  |                    |            |                         |                               |                             |                             |
| B-lactam         | Piperacillin       |            | X                       | 30/6                          | 17-20                       |                             |
| B-lactamase      | Amoxicilin+clavulanic acid |            | X                       | 20/10                        | 19-19                       |                             |
| inhibitor        | Ticarcillin+ clavulanic acid |            | X                       | 75/10                         | 23-23                       |                             |
| Carbapenem       | Ertapenem         |            | X                       | 10                            | 22-25                       |                             |
|                  | Meropenem         |            | X                       | 10                            | 16-22                       |                             |
| Cephalosporin    | Cefepime          |            | X                       | 30                            | 21-24                       |                             |
|                  | Cefazidime        |            | X                       | 10                            | 19-22                       |                             |
|                  | Cefamandole       |            | X                       | 30                            | 15-18                       |                             |
|                  | Cefepazarone      |            | X                       | 30                            | 16-21                       |                             |
| Aminoglycoside   | Gentamicin        |            | X                       | 10                            | 14-17                       |                             |
|                  | Streptomycin      |            | X                       | 10                            | 12-15                       |                             |
|                  | Kanamycin         |            | X                       | 30                            | 14-18                       |                             |
|                  | Netilimicine      |            | X                       | 10                            | 12-15                       |                             |
|                  | Amikacin          |            | X                       | 30                            | 15-18                       |                             |
| Fluoroquinolone  | Ciprofloxacin     |            | X                       | 5                             | 19-22                       | 0.5-1                       |
|                  | Nalidic acid      |            | X                       | 30                            | 14-19                       | 16-32                       |
|                  | Perfloxacin       |            | X                       | 5                             | 24-24                       |                             |
| Macrolide        | Azithromycin      |            | X                       | 15                            | 13-13                       | 16-32                       |
| Monobactam       | Aztreonam         |            | X                       | 30                            | 21-24                       |                             |
| Co-trimoxazole   | Sulframidine      |            | X                       | 300                           | 13-17                       |                             |
|                  | Trimethoprim      |            | X                       | 5                             | 15-18                       |                             |
|                  | Sulfamethoxazole/trimethoprim | X | 23.75/1.25 | 13-16                       |                             |
| Tetracycline     | Tetracycline      |            | X                       | 15                            | 12-15                       |                             |
|                  | Tigecycline       |            | X                       | 15                            | 15-18                       |                             |

Note: IPC = Institut Pasteur du Cambodge. IP-Paris = Institut Pasteur in Paris. MIC = MIC. All 103 human ESBL-Ec (88 colonization and 15 infection) were tested at IP-Paris by Kirby Bauer disk diffusion. All 93 fish and meat ESBL-Ec were tested at IPC by Kirby Bauer disk diffusion. Of these, 12/32 fish, 29/45 pork, and 8/16 chicken ESBL-Ec were additionally tested at IP-Paris by Kirby Bauer disk diffusion. MIC testing was only conducted at IP-Paris. Diameter and MIC interpretations were based on 2016 European Committee on Antimicrobial Susceptibility Testing recommendations unless otherwise noted.

M: Diameters less than the lower bound were considered resistant. Diameters greater than or equal to the upper bound were considered susceptible. All other diameters were considered intermediate.

G: Growth at concentrations less than or equal to the lower bound were considered susceptible. Growth at concentrations greater than or equal to the upper bound were considered resistant.

* Only used at IPC to determine ESBL expression using the double-disk synergy test.

* Diameter and MIC interpretations based on 2016 Clinical and Laboratory Standards Institute recommendations.

#### Appendix Table 2. Characteristics of 150 fish, pork, and chicken samples purchased from two markets in Phnom Penh, Cambodia, 2016.

| Source information | Fish | Pork | Chicken |
|--------------------|------|------|---------|
|                    | N = 60 | N = 60 | N = 30 |
| Market source      |      |      |         |
| Deum Kor           | 36(60) | 36(60) | 20(67) |
| Steung Meanchey    | 24(40) | 24(40) | 10(33) |
| Setting where animal raised         |      |      |         |
| Large scale farm | 29(48) | 57(95) | 23(77) |
| Backyard or village | 6(10) | 3(5) | 7(23) |
| Wild               | 24(40) | 0    | 0       |
| Other types of meat/seafood sold at same stall where sample purchased     |      |      |         |
| Yes                | 14(23) | 2(3) | 1(3)    |
| No                 | 45(75) | 58(97) | 27(90) |

* Reported by meat vendors to the questionnaire administrator at time of purchase, but not independently verified.

* Totals may not sum to 100% due to missing information.

* Fish vendors sold other types of meat/seafood (e.g., crabs, shrimp, frogs), but never pork or chicken. Two pork vendors also sold chicken and one chicken vendor also sold pork.
### Appendix Table 3. Characteristics and exposures among 88 healthy women colonized with ESBL-producing *Escherichia coli* in Phnom Penh, Cambodia, 2015–2016.

| Characteristic                                      | N = 88 |
|-----------------------------------------------------|--------|
| Age in years (mean, SD)                             | 28(5)  |
| Number of household members (mean, SD)              | 6(3)   |
| Number of young children <5 y old (mean, SD)        | 2(1)   |
| hospitalized during pregnancy                       |        |
| Yes                                                  | 2 (2)  |
| No                                                   | 86 (98)|
| Antibiotics during pregnancy                        |        |
| Yes                                                  | 1 (1)  |
| No                                                   | 87 (99)|
| Location of recent childbirth                       |        |
| Health center                                       | 35(40) |
| Hospital                                             | 31(35) |
| Private clinic                                       | 22(25) |
| Given antibiotics at birth*                          |        |
| Yes                                                  | 13(15) |
| No                                                   | 71(81) |
| Unknown                                              | 4(5)   |
| Birth by Cesarian section                           |        |
| Yes                                                  | 16(18) |
| No                                                   | 72(82) |
| Drinking water treatment method*                     |        |
| No treatment                                         | 12(14) |
| Boiling                                              | 41(47) |
| Disinfectant                                         | 14(16) |
| Filtration                                           | 8(9)   |
| Other or unknown                                     | 13(15) |
| Toilet shared with neighboring households            |        |
| Yes                                                  | 27(31) |
| No                                                   | 61(69) |
| Pour flush toilet                                   |        |
| Yes                                                  | 73(83) |
| No                                                   | 15(17) |
| Contact with pets                                    |        |
| Yes                                                  | 19(22) |
| No                                                   | 69(78) |
| Contact with live poultry animals*                   |        |
| Yes                                                  | 11(13) |
| No                                                   | 77(88) |
| Source of household meat and produce                 |        |
| Steung Meanchey market                               | 29(33) |
| Deum Kor market                                      | 3(3)   |
| Neighborhood or street vendors*                      | 56(64) |
| Pork consumption                                     |        |
| ≥3/week                                              | 75(85) |
| <3/week                                              | 13(15) |
| Fish consumption                                     |        |
| ≥3/week                                              | 55(63) |
| <3/week                                              | 33(38) |
| Poultry consumption                                  |        |
| ≥1/week                                              | 49(55) |
| <1/week                                              | 40(45) |
| Beef consumption                                     |        |
| ≥1/week                                              | 18(20) |
| <1/week                                              | 70(80) |
| Dried pork consumption                               |        |
| ≥1/week                                              | 47(53) |
| <1/week                                              | 41(47) |
| Dried fish consumption                               |        |
| ≥1/week                                              | 25(28) |
| <1/week                                              | 63(72) |
| Dried poultry consumption                            |        |
| Ever                                                  | 66(75) |
| Never                                                 | 22(25) |
| Dried beef consumption                               |        |
| Ever                                                  | 55(63) |
| Never                                                 | 33(38) |
### Appendix Table 4. Characteristics of 15 patients with ESBL-producing *Escherichia coli* infections presenting at the Sihanouk Center Hospital Center for Hope in Phnom Penh, Cambodia, between November 2015 and December 2016.

| Characteristic                          | n(%) |
|----------------------------------------|------|
| Age in years (median, SD)              | 64(13) |
| Female                                 | 11(75) |
| Infection type                         |      |
| Blood                                  | 12(80) |
| Urine                                  | 2(13)  |
| Peritoneal fluid                       | 1(7)  |
| Previous hospitalization <2 mo prior  | 2(13)  |

| Infection detected >48 h after intake | 2(13) |

### Appendix Table 5. Accession numbers for sequences of 196 ESBL-producing *Escherichia coli*, deposited in the European Nucleotide Archive under project number PRJEB25898.

| Sample                  | Accession       | Experiment         | Source       | Type       | ST       | ESBL Gene Type(s) |
|-------------------------|-----------------|--------------------|--------------|------------|---------|-------------------|
| ERR2538560              | ERR2367559      | ERX2557097         | Human        | Fecal Swab| 410     | CTX-M-55          |
| ERR2538533              | ERR2367354      | ERX2556670         | Human        | Fecal Swab| 7584    | CTX-M-27          |
| ERR2538534              | ERR2367355      | ERX2556671         | Human        | Fecal Swab| 3075    | CTX-M-14          |
| ERR2538535              | ERR2367357      | ERX2556672         | Human        | Fecal Swab| 405     | CTX-M-15          |
| ERR2538536              | ERR2367358      | ERX2556673         | Human        | Fecal Swab| 1722    | CTX-M-15,CTX-M-27 |
| ERR2538537              | ERR2367359      | ERX2556674         | Human        | Fecal Swab| 38      | CTX-M-15,CTX-M-27 |
| ERR2538538              | ERR2367360      | ERX2556675         | Human        | Fecal Swab| 871     | CTX-M-14          |
| ERR2538539              | ERR2367361      | ERX2556676         | Human        | Fecal Swab| 3052    | CTX-M-27,SHV-12   |
| ERR2538540              | ERR2367362      | ERX2556677         | Human        | Fecal Swab| 6438    | CTX-M-14          |
| ERR2538541              | ERR2367363      | ERX2556678         | Human        | Fecal Swab| 155     | CTX-M-15          |
| ERR2538542              | ERR2367364      | ERX2556679         | Human        | Fecal Swab| 5147    | CTX-M-15          |
| ERR2538543              | ERR2367365      | ERX2556680         | Human        | Fecal Swab| 410     | CTX-M-55          |
| ERR2538544              | ERR2367366      | ERX2556681         | Human        | Fecal Swab| 10      | CTX-M-55          |
| ERR2538545              | ERR2367367      | ERX2556682         | Human        | Fecal Swab| 394     | CTX-M-3           |
| ERR2538546              | ERR2367368      | ERX2556683         | Human        | Fecal Swab| 1722    | CTX-M-14,CTX-M-55 |
| ERR2538547              | ERR2367369      | ERX2556684         | Human        | Fecal Swab| 156     | CTX-M-15          |
| ERR2538548              | ERR2367370      | ERX2556685         | Human        | Fecal Swab| 10      | CTX-M-27          |
| ERR2538549              | ERR2367371      | ERX2556686         | Human        | Fecal Swab| 394     | CTX-M-15          |
| ERR2538550              | ERR2367372      | ERX2556687         | Human        | Fecal Swab| 410     | CTX-M-55          |
| ERR2538551              | ERR2367373      | ERX2556688         | Human        | Fecal Swab| 10      | CTX-M-55          |
| ERR2538552              | ERR2367374      | ERX2556689         | Human        | Fecal Swab| 156     | CTX-M-15          |
| ERR2538553              | ERR2367375      | ERX2556690         | Human        | Fecal Swab| 156     | CTX-M-55          |
| ERR2538554              | ERR2367376      | ERX2556691         | Human        | Fecal Swab| 4456    | CTX-M-55          |
| ERR2538555              | ERR2367377      | ERX2556692         | Human        | Fecal Swab| 1081    | CTX-M-14          |
| ERR2538556              | ERR2367378      | ERX2556693         | Human        | Fecal Swab| 131     | CTX-M-15          |
| ERR2538557              | ERR2367379      | ERX2556694         | Human        | Fecal Swab| 410     | CTX-M-55          |
| ERR2538558              | ERR2367381      | ERX2556695         | Human        | Fecal Swab| 48      | CTX-M-55          |
| ERR2538559              | ERR2367382      | ERX2556696         | Human        | Fecal Swab| 345     | CTX-M-55          |
| ERR2538560              | ERR2367383      | ERX2556697         | Human        | Fecal Swab| 421     | CTX-M-27          |
| ERR2538561              | ERR2367384      | ERX2556698         | Human        | Fecal Swab| 1588    | CTX-M-15          |
| ERR2538562              | ERR2367385      | ERX2556699         | Human        | Fecal Swab| 1163    | CTX-M-27          |
| ERR2538563              | ERR2367386      | ERX2556700         | Human        | Fecal Swab| 38      | CTX-M-15          |
| ERR2538564              | ERR2367387      | ERX2556701         | Human        | Fecal Swab| 38      | CTX-M-14          |
| ERR2538565              | ERR2367389      | ERX2556702         | Human        | Fecal Swab| 636     | SHV-12            |
| ERR2538566              | ERR2367390      | ERX2556703         | Human        | Fecal Swab| 4040    | CTX-M-15          |
| ERR2538567              | ERR2367391      | ERX2556704         | Human        | Fecal Swab| 10      | CTX-M-55          |
| ERR2538568              | ERR2367392      | ERX2556705         | Human        | Fecal Swab| 345     | CTX-M-55          |
| ERR2538569              | ERR2367393      | ERX2556706         | Human        | Fecal Swab| 1196    | CTX-M-55          |
| ERR2538570              | ERR2367394      | ERX2556707         | Human        | Fecal Swab| 695     | CTX-M-55          |
| ERR2538571              | ERR2367395      | ERX2556708         | Human        | Fecal Swab| 3052    | CTX-M-15          |
| ERR2538572              | ERR2367396      | ERX2556709         | Human        | Fecal Swab| 155     | CTX-M-15          |
| ERR2538573              | ERR2367397      | ERX2556710         | Human        | Fecal Swab| 405     | CTX-M-15          |
| ERR2538574              | ERR2367398      | ERX2556711         | Human        | Fecal Swab| 405     | CTX-M-15          |
| Sample   | Accession   | Experiment | Source         | Type       | ST      | ESBL Gene Type(s) |
|----------|-------------|------------|----------------|------------|---------|-------------------|
| ERR2538175 | ERX2556712  | Human      | Fecal Swab     | 3052       | CTX-M-15 |
| ERR2537176 | ERX2556749  | Human      | Fecal Swab     | 10         | CTX-M-15 |
| ERR2538177 | ERX2556714  | Human      | Fecal Swab     | 3580       | CTX-M-15 |
| ERR2538178 | ERX2556715  | Human      | Fecal Swab     | 1656       | CTX-M-55 |
| ERR2538179 | ERX2556716  | Human      | Fecal Swab     | 162        | CTX-M-15 |
| ERR2538180 | ERX2556717  | Human      | Fecal Swab     | 131        | CTX-M-27 |
| ERR2538181 | ERX2556718  | Human      | Fecal Swab     | 10         | CTX-M-55 |
| ERR2538182 | ERX2556719  | Human      | Fecal Swab     | 421        | CTX-M-27 |
| ERR2538183 | ERX2556720  | Human      | Fecal Swab     | 759        | CTX-M-55 |
| ERR2538184 | ERX2556721  | Human      | Fecal Swab     | 13         | CTX-M-15 |
| ERR2538185 | ERX2556722  | Human      | Fecal Swab     | 48         | CTX-M-55 |
| ERR2538186 | ERX2556723  | Human      | Fecal Swab     | 7590       | CTX-M-55 |
| ERR2538187 | ERX2556724  | Human      | Fecal Swab     | 8375       | CTX-M-15 |
| ERR2538188 | ERX2556725  | Human      | Fecal Swab     | 10         | CTX-M-55 |
| ERR2538189 | ERX2556726  | Human      | Fecal Swab     | 1244       | CTX-M-14 |
| ERR2538190 | ERX2556727  | Human      | Fecal Swab     | 38         | CTX-M-55 |
| ERR2538191 | ERX2556728  | Human      | Fecal Swab     | 131        | CTX-M-15 |
| ERR2538192 | ERX2556729  | Human      | Fecal Swab     | 410        | CTX-M-15 |
| ERR2538193 | ERX2556730  | Human      | Fecal Swab     | 617        | CTX-M-15 |
| ERR2538194 | ERX2556731  | Human      | Fecal Swab     | 405        | CTX-M-15 |
| ERR2538195 | ERX2556732  | Human      | Fecal Swab     | 5044       | CTX-M-15 |
| ERR2538196 | ERX2556733  | Human      | Fecal Swab     | 1722       | CTX-M-55 |
| ERR2538197 | ERX2556734  | Human      | Fecal Swab     | 131        | CTX-M-15 |
| ERR2538198 | ERX2556735  | Human      | Fecal Swab     | 969        | CTX-M-27 |
| ERR2538199 | ERX2556736  | Human      | Fecal Swab     | 10         | CTX-M-15 |
| ERR2538200 | ERX2556737  | Human      | Fecal Swab     | 215        | CTX-M-15 |
| ERR2538201 | ERX2556738  | Human      | Fecal Swab     | 421        | CTX-M-27 |
| ERR2538202 | ERX2556739  | Human      | Fecal Swab     | 43         | CTX-M-15 |
| ERR2538203 | ERX2556740  | Human      | Fecal Swab     | 6303       | CTX-M-15 |
| ERR2538204 | ERX2556741  | Human      | Fecal Swab     | 361        | CTX-M-55 |
| ERR2538205 | ERX2556742  | Human      | Fecal Swab     | 442        | CTX-M-14 |
| ERR2538206 | ERX2556743  | Human      | Fecal Swab     | 2083       | CTX-M-55 |
| ERR2538207 | ERX2556744  | Human      | Fecal Swab     | 226        | CTX-M-15 |
| ERR2538208 | ERX2556745  | Human      | Fecal Swab     | 2040       | CTX-M-15 |
| ERR2538209 | ERX2556746  | Human      | Fecal Swab     | 38         | CTX-M-14 |
| ERR2538210 | ERX2556747  | Human      | Fecal Swab     | 7160       | CTX-M-55 |
| ERR2538211 | ERX2556748  | Human      | Fecal Swab     | 2003       | CTX-M-55 |
| ERR2538212 | ERX2556749  | Human      | Fecal Swab     | 155        | CTX-M-15 |
| ERR2538213 | ERX2556750  | Human      | Fecal Swab     | 13         | CTX-M-15 |
| ERR2538214 | ERX2556751  | Human      | Fecal Swab     | 10         | CTX-M-55 |
| ERR2538215 | ERX2556752  | Human      | Fecal Swab     | 457        | CTX-M-27 |
| ERR2538216 | ERX2556753  | Human      | Fecal Swab     | 48         | CTX-M-55 |
| ERR2538217 | ERX2556754  | Human      | Fecal Swab     | 5713       | CTX-M-14 |
| ERR2538218 | ERX2556755  | Human      | Fecal Swab     | 1844       | CTX-M-15 |
| ERR2538219 | ERX2556756  | Human      | Fecal Swab     | 602        | CTX-M-55 |
| ERR2538220 | ERX2556757  | Human      | Fecal Swab     | 10         | CTX-M-55 |
| ERR2538221 | ERX2556758  | Human      | Fecal Swab     | 2705       | CTX-M-55 |
| ERR2538222 | ERX2556759  | Human      | Fecal Swab     | 602        | CTX-M-55 |
| ERR2538223 | ERX2556760  | Human      | Fecal Swab     | 3873       | CTX-M-15 |
| ERR2538224 | ERX2556761  | Human      | Fecal Swab     | 3014       | CTX-M-55 |
| ERR2538225 | ERX2556762  | Human      | Fecal Swab     | 739        | CTX-M-55 |
| ERR2538226 | ERX2556763  | Human      | Fecal Swab     | 2207       | CTX-M-55 |
| ERR2538227 | ERX2556764  | Human      | Fecal Swab     | 155        | CTX-M-55 |
| ERR2538228 | ERX2556765  | Human      | Fecal Swab     | 156        | CTX-M-15 |
| ERR2538229 | ERX2556766  | Human      | Fecal Swab     | 1290       | CTX-M-55 |
| ERR2538230 | ERX2556767  | Human      | Fecal Swab     | 7585       | CTX-M-55 |
| ERR2538231 | ERX2556768  | Human      | Fecal Swab     | 424        | CTX-M-55 |
| ERR2538232 | ERX2556769  | Food       | Fish           | 6706       | CTX-M-55 |
| ERR2538233 | ERX2556770  | Food       | Fish           | 746        | CTX-M-55 |
| ERR2538234 | ERX2556771  | Food       | Fish           | 13         | CTX-M-55 |
| ERR2538235 | ERX2556772  | Food       | Fish           | 48         | CTX-M-15 |
| ERR2538236 | ERX2556773  | Food       | Fish           | 48         | CTX-M-55 |
| ERR2538237 | ERX2556774  | Food       | Fish           | 156        | CTX-M-55 |
| ERR2538238 | ERX2556775  | Food       | Fish           | 8399       | CTX-M-55 |
| ERR2538239 | ERX2556776  | Food       | Fish           | 7586       | CTX-M-55 |
| ERR2538240 | ERX2556777  | Food       | Fish           | 206        | CTX-M-55 |
| ERR2538241 | ERX2556778  | Food       | Fish           | 10         | CTX-M-24 |
| Sample   | Accession   | Experiment   | Source | Type   | ST   | ESBL Gene Type(s) |
|----------|-------------|--------------|--------|--------|------|-------------------|
| ERR2538242 | ERX2556779  | Food         | Fish   | 7370   | CTX-M-55 |
| ERR2538243 | ERX2556784  | Food         | Fish   | 1485   | CTX-M-55 |
| ERR2538244 | ERX2556781  | Food         | Fish   | 1266   | CTX-M-55 |
| ERR2538245 | ERX2556782  | Food         | Fish   | 224    | CTX-M-55 |
| ERR2538246 | ERX2556783  | Food         | Fish   | 3873   | CTX-M-15 |
| ERR2538247 | ERX2556784  | Food         | Fish   | 195    | CTX-M-14 |
| ERR2538248 | ERX2556785  | Food         | Fish   | 2207   | CTX-M-65 |
| ERR2538249 | ERX2556786  | Food         | Fish   | 2690   | CTX-M-55 |
| ERR2538250 | ERX2556787  | Food         | Fish   | 1196   | CTX-M-55 |
| ERR2538251 | ERX2556788  | Food         | Fish   | 2690   | CTX-M-55 |
| ERR2538252 | ERX2556789  | Food         | Fish   | 5834   | CTX-M-55 |
| ERR2538253 | ERX2556790  | Food         | Fish   | 10     | CTX-M-55 |
| ERR2538254 | ERX2556791  | Food         | Fish   | 744    | CTX-M-55 |
| ERR2538255 | ERX2556792  | Food         | Pork   | 101    | CTX-M-27 |
| ERR2538256 | ERX2556793  | Food         | Pork   | 7589   | CTX-M-55 |
| ERR2538257 | ERX2556794  | Food         | Pork   | 641    | CTX-M-14 |
| ERR2538258 | ERX2556795  | Food         | Pork   | 617    | CTX-M-55 |
| ERR2538259 | ERX2556796  | Food         | Pork   | 6799   | CTX-M-55 |
| ERR2538260 | ERX2556797  | Food         | Pork   | 540    | CTX-M-55 |
| ERR2538261 | ERX2556798  | Food         | Pork   | 58     | CTX-M-55 |
| ERR2538262 | ERX2556799  | Food         | Pork   | 165    | CTX-M-14 |
| ERR2538263 | ERX2556800  | Food         | Pork   | 457    | CTX-M-27 |
| ERR2538264 | ERX2556801  | Food         | Pork   | 155    | CTX-M-55 |
| ERR2538265 | ERX2556802  | Food         | Pork   | 7588   | CTX-M-14 |
| ERR2538266 | ERX2556803  | Food         | Pork   | 746    | CTX-M-55 |
| ERR2538267 | ERX2556804  | Food         | Pork   | 1408   | CTX-M-15 |
| ERR2538268 | ERX2556805  | Food         | Pork   | 542    | CTX-M-14 |
| ERR2538269 | ERX2556806  | Food         | Pork   | 58     | CTX-M-55 |
| ERR2538270 | ERX2556807  | Food         | Pork   | 48     | CTX-M-14 |
| ERR2538271 | ERX2556808  | Food         | Pork   | 278    | CTX-M-55 |
| ERR2538272 | ERX2556809  | Food         | Pork   | 4986   | CTX-M-55 |
| ERR2538273 | ERX2556810  | Food         | Pork   | 515    | CTX-M-14 |
| ERR2538274 | ERX2556811  | Food         | Pork   | 8401   | CTX-M-55 |
| ERR2538275 | ERX2556812  | Food         | Pork   | 542    | CTX-M-14 |
| ERR2538276 | ERX2556813  | Food         | Pork   | 515    | CTX-M-14 |
| ERR2538277 | ERX2556814  | Food         | Pork   | 1081   | CTX-M-14 |
| ERR2538278 | ERX2556815  | Food         | Pork   | 540    | CTX-M-55 |
| ERR2538279 | ERX2556816  | Food         | Pork   | 101    | CTX-M-14 |
| ERR2538280 | ERX2556817  | Food         | Pork   | 58     | CTX-M-55 |
| ERR2538281 | ERX2556818  | Food         | Pork   | 1237   | CTX-M-55 |
| ERR2538282 | ERX2556819  | Food         | Pork   | 195    | CTX-M-14 |
| ERR2538283 | ERX2556820  | Food         | Pork   | 641    | CTX-M-14 |
| ERR2538284 | ERX2556821  | Food         | Pork   | 540    | CTX-M-55 |
| ERR2538285 | ERX2556822  | Food         | Pork   | 7589   | CTX-M-55 |
| ERR2538286 | ERX2556823  | Food         | Pork   | 113    | CTX-M-55 |
| ERR2538287 | ERX2556824  | Food         | Pork   | 398    | CTX-M-55 |
| ERR2538288 | ERX2556825  | Food         | Pork   | 1258   | CTX-M-15 |
| ERR2538289 | ERX2556826  | Food         | Pork   | 354    | CTX-M-55 |
| ERR2538290 | ERX2556827  | Food         | Pork   | 398    | CTX-M-14 |
| ERR2538291 | ERX2556828  | Food         | Pork   | 744    | CTX-M-55 |
| ERR2538292 | ERX2556829  | Food         | Pork   | 4417   | CTX-M-55 |
| ERR2538293 | ERX2556830  | Food         | Pork   | 10     | CTX-M-55 |
| ERR2538294 | ERX2556831  | Human         | Urine  | 410    | CTX-M-15 |
| ERR2538295 | ERX2556832  | Human         | Urine  | 410    | CTX-M-15 |
| ERR2538296 | ERX2556833  | Human         | Blood  | 393    | CTX-M-55 |
| ERR2538297 | ERX2556834  | Human         | Blood  | 131    | CTX-M-27 |
| ERR2538298 | ERX2556835  | Human         | Blood  | 2011   | CTX-M-15 |
| ERR2538299 | ERX2556836  | Human         | Blood  | 131    | CTX-M-27 |
| ERR2538300 | ERX2556837  | Human         | Blood  | 4456   | CTX-M-55 |
| ERR2538301 | ERX2556838  | Human         | Blood  | 131    | CTX-M-27 |
| ERR2538302 | ERX2556839  | Human         | Blood  | 131    | CTX-M-14 |
| ERR2538303 | ERX2556840  | Human         | Blood  | 131    | CTX-M-15 |
| ERR2538304 | ERX2556841  | Food         | Poultry | 6962  | CTX-M-55 |
| ERR2538305 | ERX2556842  | Food         | Poultry | 5855  | CTX-M-27 |
Appendix Table 6. Antibiotic resistance profiles of 93 ESBL-producing *Escherichia coli* from meat and fish purchased from markets and the distribution of acquired resistance genes encoding these phenotypes, Phnom Penh, Cambodia, 2015–2016.

| Antibiotic Class | Resistant Phenotype and Detected Genes<sup>a,b</sup> | Fish<sup>c</sup> | Pork<sup>c</sup> | Chicken<sup>c</sup> | p-value<sup>c</sup> |
|------------------|--------------------------------------------------|----------------|----------------|----------------|-----------------|
| **Third-generation cephalosporin** | Resistant Phenotype | 32(100) | 45(100) | 16(100) | 1.00 |
| **Detected Genes** | Bla<sub>CTX-M-95</sub> | 23(72) | 27(60) | 12(75) | |
| | Bla<sub>CTX-M-14</sub> | 2(6) | 13(29) | 1(6) | |
| | Bla<sub>CTX-M-15</sub> | 4(13) | 2(4) | 1(6) | |
| | Bla<sub>CTX-M-24</sub> | 1(3) | 0 | 0 | |
| | Bla<sub>CTX-M-27</sub> | 0 | 3(7) | 2(13) | |
| | Bla<sub>CTX-M-65</sub> | 2(6) | 0 | 0 | |
| | Bla<sub<c><sub>CMY-2</sub></sub> | 1(3) | 1(2) | 0 | |
| **Aminoglycoside** | Resistant Phenotype | 26(81) | 42(93) | 12(75) | 0.12 |
| **Detected Genes** | Aph(3')-Ia | 16(50) | 11(24) | 6(38) | |
| | Stra | 11(34) | 14(31) | 8(50) | |
| | StraB | 21(66) | 16(36) | 10(63) | |
| | AadA1 | 9(28) | 12(27) | 1(6) | |
| | AadA2 | 10(31) | 27(60) | 4(25) | |
| | AadA22 | 5(16) | 0 | 2(13) | |
| | Aac(3)-IId | 18(54) | 27(60) | 6(38) | |
| | Aac(6')-Ib-cr | 3(9) | 0 | 1(6) | |
| **Amphenicol** | Resistant Phenotype<sup>d</sup> | 26(81) | 40(89) | 11(69) | 0.18 |
| **Detected Genes** | CatA1 | 3(9) | 0 | 1(6) | |
| | CatA2 | 5(16) | 6(13) | 1(6) | |
| | FloR | 17(53) | 27(60) | 9(56) | |
| | CmlA | 6(19) | 25(56) | 2(13) | |
| **Carbapenem** | Resistant Phenotype<sup>e</sup> | 1(3) | 0 | 0 | |
| **Detected Genes** | Bla<sub>OXA-181</sub> | 1(3) | 0 | 0 | |
| **Colistin** | Resistant Phenotype | 3(9) | 8(18) | 0 | 0.16 |
| **Detected Genes** | Mcr1 | 3(9) | 6(13) | 0 | |
| | Mcr3 | 0 | 3(7) | 0 | |
| **Fluoroquinolone** | Resistant Phenotype | 28(88) | 32(71) | 14(88) | 0.15 |
| **Detected Genes** | |

<sup>a</sup>Note: ST = Multilocus sequence type. ESBL = Extended-spectrum β-lactamase.

<sup>b</sup>n = Number of samples.

<sup>c</sup>p < 0.05 was considered significant.

<sup>d</sup>i.e., resistance to ampicillin or amoxicillin.

<sup>e</sup>i.e., resistance to aztreonam.

<sup>f</sup>i.e., resistance to imipenem.

<sup>g</sup>i.e., resistance to gentamicin.
### Appendix Table 7. Antibiotic resistance profiles of ESBL-producing Escherichia coli from 88 healthy, colonized humans and 15 infected patients, and the distribution of acquired resistance genes encoding these phenotypes, Phnom Penh, Cambodia, 2015–2016.

| Antibiotic Class         | Resistant Phenotype and Detected Genes\(^{a,b}\) | Colonization N = 88 | Clinical N = 15 | p-value\(^{c}\) |
|--------------------------|--------------------------------------------------|---------------------|-----------------|-----------------|
| **Third-generation cephalosporin** |                                                  |                     |                 |                 |
|                          | Detected Genes                                   |                     |                 |                 |
|                          | **bla\(_{CTX-M-3}\)**                             | 1(1)                | 0               | 1.00            |
|                          | **bla\(_{CTX-M-55}\)**                            | 27(31)              | 2(13)           |                 |
|                          | **bla\(_{CTX-M-14}\)**                            | 9(10)               | 3(20)           |                 |
|                          | **bla\(_{CTX-M-15}\)**                            | 41(47)              | 6(40)           |                 |
|                          | **bla\(_{CTX-M-27}\)**                            | 13(15)              | 4(27)           |                 |
|                          | **bla\(_{CMY-2}\)**                              | 3(3)                | 3(20)           |                 |
|                          | **bla\(_{CMY-42}\)**                             | 3(3)                | 0               |                 |
|                          | **bla\(_{HAV-12}\)**                             | 2(2)                | 0               |                 |
|                          | **Aminoglycoside**                                |                     |                 |                 |
|                          | Detected Genes                                   |                     |                 |                 |
|                          | **aph(3')-la**                                    | 10(11)              | 1(7)            | 0.69            |
|                          | **strA**                                          | 43(49)              | 10(67)          |                 |
|                          | **strB**                                          | 42(48)              | 10(67)          |                 |
|                          | **aadA1**                                         | 7(8)                | 0               |                 |
|                          | **aadA2**                                         | 20(23)              | 1(7)            |                 |
|                          | **aadA5**                                         | 39(44)              | 12(80)          |                 |

Note: ESBL = Extended-spectrum β-lactamase. All 93 isolates produced ESBLs; 2/93 additionally produced Amp-C β-lactamases (CMY-type).

\(^{a}\)The frequency of resistance genes detected may exceed the total number of isolates exhibiting resistance to a given antibiotic class because many isolates carried multiple genes encoding resistance to the same antibiotic class.

\(^{b}\)Isolates were categorized as “Resistant” if they demonstrated intermediate or complete phenotypic resistance to any antibiotic within the stated class.

\(^{c}\)p-values were generated using Fisher exact tests comparing the distributions of phenotypic antibiotic resistance patterns between samples types.

\(^{d}\)We recovered an additional carbapenemase-producing (OXA-48), non-ESBL producing E. coli from one pork sample (data not shown here).

\(^{e}\)Frequency of detected resistance genes may not sum to total number of isolates exhibiting resistant phenotype. Some resistance phenotypes may be encoded by point mutations, but these were not investigated.

\(^{f}\)Phenotypic resistance to this antibiotic class was assessed for 49/93 ESBL-Ec isolates. For 20/32 ESBL-Ec from fish, 16/45 from pork, and 8/16 from poultry, phenotypic resistance is reported based on the occurrence of one or more genes conferring resistance to this antibiotic class.

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### Appendix Table 7.

| Antibiotic Class | Resistant Phenotype and Detected Genes | Colonization N = 88 | Clinical N = 15 | p-value |
|------------------|---------------------------------------|---------------------|-----------------|---------|
| **Macrolide**    |                                       |                     |                 |         |
|                  | Detected Genes                        |                     |                 |         |
|                  | **erm(B)**                            | 3(9)                | 0               | 1(6)    |
|                  | **mph(A)**                            | 17(53)              | 10(22)          | 7(44)   |
|                  | **mef(B)**                            | 4(13)               | 18(40)          | 0       |
|                  | **lnu(F)**                            | 14(44)              | 5(11)           | 4(25)   |
| **Sulphamethoxazole/Trimethoprim** |   |                     |                 |         |
| **Detected Genes** |                                      |                     |                 |         |
| **su1**          | 9(28)                                | 0                   | 4(25)           |         |
| **su2**          | 17(53)                               | 23(51)              | 9(56)           |         |
| **su3**          | 17(53)                               | 32(71)              | 4(25)           |         |
| **dfrA12**       | 7(22)                                | 30(67)              | 2(13)           |         |
| **dfrA14**       | 20(63)                               | 8(18)               | 8(50)           |         |
| **dfrA17**       | 3(9)                                 | 1(2)                | 2(13)           |         |
| **Tetracycline** |                                       |                     |                 |         |
| **Detected Genes** |                                      |                     |                 |         |
| **tet(A)**       | 26(81)                               | 39(87)              | 13(81)          |         |
| **tet(B)**       | 3(9)                                 | 4(9)                | 1(6)            |         |
| **tet(M)**       | 2(6)                                 | 23(51)              | 1(6)            |         |

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Note: ESBL = Extended-spectrum β-lactamase. All 93 isolates produced ESBLs; 2/93 additionally produced Amp-C β-lactamases (CMY-type).

\(^{a}\)The frequency of resistance genes detected may exceed the total number of isolates exhibiting resistance to a given antibiotic class because many isolates carried multiple genes encoding resistance to the same antibiotic class.

\(^{b}\)Isolates were categorized as “Resistant” if they demonstrated intermediate or complete phenotypic resistance to any antibiotic within the stated class.

\(^{c}\)p-values were generated using Fisher exact tests comparing the distributions of phenotypic antibiotic resistance patterns between samples types.

\(^{d}\)We recovered an additional carbapenemase-producing (OXA-48), non-ESBL producing E. coli from one pork sample (data not shown here).

\(^{e}\)Frequency of detected resistance genes may not sum to total number of isolates exhibiting resistant phenotype. Some resistance phenotypes may be encoded by point mutations, but these were not investigated.

\(^{f}\)Phenotypic resistance to this antibiotic class was assessed for 49/93 ESBL-Ec isolates. For 20/32 ESBL-Ec from fish, 16/45 from pork, and 8/16 from poultry, phenotypic resistance is reported based on the occurrence of one or more genes conferring resistance to this antibiotic class.
### Antibiotic Class and Resistance Phenotype

| Antibiotic Class | Resistant Phenotype | Detected Genes | Colonization N = 88 | Clinical N = 15 | p-value<sup>c</sup> |
|------------------|---------------------|----------------|---------------------|-----------------|-------------------|
| **Amphenicol**   | Resistant Phenotype<sup>d</sup> | 29(33)         | 3(3)                | 1(7)            | 0.06              |
| **Detected Genes** |                        |                |                     |                 |                   |
| catA1            | 7(8)                | 0              |                     |                 |                   |
| catA2            | 8(9)                | 1(7)           |                     |                 |                   |
| floR             | 15(17)              | 0              |                     |                 |                   |
| cmlA             | 6(7)                | 0              |                     |                 |                   |
| **Carbapenem**   | Resistant Phenotype | 3(3)           | 32(36)              | 4(27)           |                   |
| **Detected Genes** |                        |                |                     |                 |                   |
| blaKDM-1         | 1(1)                | 0              |                     |                 |                   |
| blaKDM-5         | 2(2)                | 1(7)           |                     |                 |                   |
| blaOXA-181       | 0                   | 1(7)           |                     |                 |                   |
| **Colistin**     | Resistant Phenotype | 2(2)           |                     | 0               |                   |
| **Detected Genes** |                        |                |                     |                 |                   |
| mcr1             | 1(1)                | 0              |                     |                 |                   |
| mcr3             | 1(1)                | 0              |                     |                 |                   |
| **Fluoroquinolone** | Resistant Phenotype<sup>d</sup> | 86(98)         | 15(100)             | 15(100)         | 1.00              |
| **Detected Genes** |                        |                |                     |                 |                   |
| qnrS1            | 46(52)              | 1(7)           |                     |                 |                   |
| aac(6')Ib-cr     | 12(14)              | 6(40)          |                     |                 |                   |
| **Macrolide**    | Resistant Phenotype<sup>d</sup> | 40(45)         |                     | 12(80)          | 0.02              |
| **Detected Genes** |                        |                |                     |                 |                   |
| erm(B)           | 6(7)                | 2(13)          |                     |                 |                   |
| mph(A)           | 42(49)              | 12(80)         |                     |                 |                   |
| mef(B)           | 5(6)                | 0              |                     |                 |                   |
| lnu(F)           | 9(10)               | 1(7)           |                     |                 |                   |
| **Sulfonamide/Trimethoprim** | Resistant Phenotype<sup>d</sup> | 75(85)         |                     | 13(87)          | 1.00              |
| **Detected Genes** |                        |                |                     |                 |                   |
| sul1             | 45(51)              | 12(80)         |                     |                 |                   |
| sul2             | 47(53)              | 10(67)         |                     |                 |                   |
| sul3             | 15(17)              | 1(7)           |                     |                 |                   |
| dfrA1            | 4(5)                | 0              |                     |                 |                   |
| dfrA12           | 18(20)              | 1(7)           |                     |                 |                   |
| dfrA14           | 22(25)              | 1(7)           |                     |                 |                   |
| dfrA17           | 38(43)              | 12(80)         |                     |                 |                   |
| **Tetracycline** | Resistant Phenotype<sup>d</sup> | 71(81)         |                     | 14(93)          | 0.46              |
| **Detected Genes** |                        |                |                     |                 |                   |
| tet(A)           | 49(56)              | 10(67)         |                     |                 |                   |
| tet(B)           | 27(31)              | 4(27)          |                     |                 |                   |
| tet(D)           | 2(2)                | 0              |                     |                 |                   |
| tet(M)           | 5(6)                | 0              |                     |                 |                   |

Note: ESBL = Extended-spectrum β-lactamase. All 103 isolates produced ESBLs; 9/103 additionally produced Amp-C β-lactamases (CMY-type).

*The frequency of resistance genes detected may exceed the total number of isolates exhibiting resistance to a given antibiotic class because some isolates carried multiple genes encoding resistance to the same antibiotic class.

*Isolates were categorized as "Resistant" if they demonstrated intermediate or complete phenotypic resistance to any antibiotic within the stated class.

*p-values were generated using Fisher exact tests comparing the distributions of phenotypic antibiotic resistance patterns between samples types.

*Frequency of detected resistance genes may not sum to total number of isolates exhibiting resistant phenotype. Some resistance phenotypes may be encoded by point mutations, but these were not investigated.
### Appendix Table 8. Multilocus sequence types of ESBL-producing *Escherichia coli* detected among humans and food in Phnom Penh, Cambodia, by phylogenetic clan.

| Clan I/B2&D | MLST CC<sup>ab</sup> | ST | Human colonization n = 35 (%) | Human infection n = 13 (%) | Meat n = 5 (%) | β-lactamase gene type(s) detected | Phylo-type<sup>c</sup> |
|-------------|----------------------|----|-----------------------------|---------------------------|--------------|----------------------------------|---------------------|
| n = 53      | 38                   | –  | 10 (29)                     | 0                         | 1 (20)       | CTX-M-14 (2), CTX-M-15 (4), CTX-M-27 (1) | D                   |
|             | 38                   | 6 (17) | 0 | 0 | 0 | CTX-M-15 (1) | D |
|             | 2003                 | 1 (3)  | 0 | 0 | 0 | CTX-M-55 (1) | D |
|             | 3052                 | 3 (9)  | 0 | 0 | 0 | CTX-M-15 (2), CTX-M-27 (1), SHV-12 (1) | D |
|             | 3268                 | 0      | 0 | 0 | 1 (20) | CTX-M-15 (1) | D |
| Singletons  | 12                   | 0      | 1 (8) | 0 | 0 | CTX-M-14 (1) | B2 |
|             | 131                  | 4 (11) | 7 (54) | 0 | 0 | CTX-M-14 (2), CTX-M-15 (4), CTX-M-27 (5), CMY-2 (1) | B2/D |
|             | 354                  | 0      | 0 | 0 | 1 (20) | CTX-M-55 (1) | D |
|             | 393                  | 0      | 1 (8) | 0 | 0 | CTX-M-55 (1) | D |
|             | 394                  | 2 (6)  | 0 | 0 | 0 | CTX-M-3 (1), CTX-M-15 (1), CMY-2 (1) | D |
|             | 405                  | 4 (11) | 1 (8) | 0 | 0 | CTX-M-15 (5), NDM-5 (1) | D |
|             | 421                  | 3 (9)  | 0 | 0 | 0 | CTX-M-27 (3) | B2 |
|             | 457                  | 0      | 0 | 2 (40) | 0 | CTX-M-27 (2) | D |
|             | 636                  | 1 (3)  | 0 | 0 | 0 | SHV-12 (1) | D |
|             | 969                  | 1 (3)  | 0 | 0 | 0 | CTX-M-27 (1) | B2 |
|             | 1163                 | 1 (3)  | 0 | 0 | 0 | CTX-M-27 (1), CMY-2 (1) | D |
|             | 1193                 | 0      | 1 (8) | 0 | 0 | CTX-M-27 (1), CTX-M-55 (1) | B2 |
|             | 1485                 | 0      | 0 | 1 (20) | 0 | CTX-M-55 (1) | D |
|             | 1588                 | 1 (3)  | 0 | 0 | 0 | CTX-M-15 (1) | D |
|             | 1722                 | 3 (9)  | 0 | 0 | 0 | CTX-M-14 (1), CTX-M-15 (1), CTX-M-27 (1), CTX-M-55 (2) | D |
|             | 2011                 | 0      | 1 (8) | 0 | 0 | CTX-M-15 (1) | D |
|             | 4040                 | 1 (3)  | 0 | 0 | 0 | CTX-M-15 (1) | D |
|             | 4456                 | 1 (3)  | 1 (8) | 0 | 0 | CTX-M-55 (2) | B2 |
|             | 5147                 | 1 (3)  | 0 | 0 | 0 | CTX-M-15 (1) | D |
|             | 6303                 | 1 (3)  | 0 | 0 | 0 | CTX-M-15 (1) | D |
|             | 8375                 | 1 (3)  | 0 | 0 | 0 | CTX-M-15 (1) | D |

| Clan II/A | MLST CC<sup>ab</sup> | ST | Human colonization n = 20 (%) | Human infection n = 0 (%) | Meat n = 49 (%) | Phylotype<sup>c</sup> |
|-----------|----------------------|----|-------------------------------|--------------------------|---------------|------------------------|
| n = 69    | 10                   | 12 (60) | 0 | 11 (22) | 5 (10) | CTX-M-15 (2), CTX-M-55 (9), CTX-M-27 (1), OXA-181 (1), CTX-M-24 (1) | – |
|           | 43                   | 1 (5)  | 0 | 0 | 0 | CTX-M-15 (1) | A |
|           | 48                   | 2 (10) | 0 | 3 (6) | 0 | CTX-M-14 (1), CTX-M-55 (4) | A |
|           | 215                  | 1 (5)  | 0 | 0 | 0 | CTX-M-15 (1) | A |
|           | 744                  | 0      | 2 (4) | 0 | 0 | CTX-M-55 (2) | A |
|           | 5713                 | 0      | 0 | 1 (2) | 0 | CTX-M-14 (1) | A |
| Singletons| 189                  | 0      | 0 | 2 (4) | 0 | CTX-M-55 (2) | A |
|           | 195                  | 0      | 0 | 3 (6) | 0 | CTX-M-14 (3) | A |
|           | 206                  | 0      | 0 | 1 (2) | 0 | CTX-M-55 (1) | A |
|           | 361                  | 1 (5)  | 0 | 0 | 0 | CTX-M-55 (1) | A |
|           | 398                  | 0      | 0 | 2 (4) | 0 | CTX-M-14 (1), CTX-M-55 (1) | A |
|           | 540                  | 0      | 0 | 3 (6) | 0 | CTX-M-55 (3) | A |
|           | 542                  | 0      | 0 | 2 (4) | 0 | CTX-M-14 (2) | A |
|           | 617                  | 1 (5)  | 0 | 1 (2) | 0 | CTX-M-15 (1), CTX-M-55 (1) | A |
|           | 695                  | 1 (5)  | 0 | 0 | 0 | CTX-M-55 (1) | A |
|           | 746                  | 0      | 2 (4) | 0 | 0 | CTX-M-55 (2), CMY-2 (2) | A |
|           | 871                  | 1 (5)  | 0 | 1 (2) | 0 | CTX-M-14 (1) | A |
|           | 1139                 | 0      | 0 | 1 (2) | 0 | CTX-M-55 (1) | A |
| Clan | MLST CC<sup>a,b</sup> | ST | Human colonization<sup>n = 25 (%)</sup> | Human infection<sup>n = 0 (%)</sup> | Meat<sup>n = 5 (%)</sup> | β-lactamase gene type(s) detected | Phylotype<sup>c</sup> |
|------|-----------------|----|-------------------------------|---------------------------------|----------------------|---------------------------------|--------------------|
|      |                 |    |                               |                                 |                      |                                 |                    |
| 1244 | 58              | 2  | 1 (5)                         | 0                               | 0                    | CTX-M-14 (1)                   | A                  |
| 1266 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | B2                 |
| 1290 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | A                  |
| 1408 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | A                  |
| 2207 | 58              | 2  | 0                             | 0                               | 2 (4)                | CTX-M-55 (1), CTX-M-65 (1)     | A                  |
| 2345 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | A                  |
| 2690 | 58              | 2  | 0                             | 0                               | 2 (4)                | CTX-M-55 (2)                   | A                  |
| 2705 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | A                  |
| 3014 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | A                  |
| 3075 | 58              | 2  | 1 (5)                         | 0                               | 0                    | CTX-M-14 (1)                   | A                  |
| 5834 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | A                  |
| 5855 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-27 (1)                   | A                  |
| 6390 | 58              | 2  | 1 (5)                         | 0                               | 0                    | CTX-M-55 (1)                   | A                  |
| 6438 | 58              | 2  | 1 (5)                         | 0                               | 0                    | CTX-M-14 (1)                   | A                  |
| 6706 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | A                  |
| 7369 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | A                  |
| 7370 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | A                  |
| 7585 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | A                  |
| 7588 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-14 (1)                   | A                  |
| 7589 | 58              | 2  | 0                             | 0                               | 2 (4)                | CTX-M-55 (2)                   | A                  |
| 8377 | 58              | 2  | 0                             | 0                               | 1 (2)                | CTX-M-55 (1)                   | A                  |
|      |                 |    |                               |                                 |                      |                                 |                    |
|      |                 | 58 | 3 (16)                        | 0                               | 4 (14)               | CTX-M-15 (3), CTX-M-55 (2)     | B1                 |
|      |                 | 3873| 3 (16)                       | 0                               | 2 (7)                | CTX-M-15 (2)                   | B1                 |
|      |                 | 58 | 3 (16)                        | 0                               | 7 (25)               | CTX-M-15 (1), CTX-M-55 (3)     | B1                 |
|      |                 | 155| 3 (16)                        | 0                               | 3 (11)               | CTX-M-15 (2), CTX-M-27(1), CTX-M-55 (3) | B1 |
|      |                 | 13 | 2 (11)                        | 0                               | 0                    | CTX-M-15 (2)                   | B1                 |
|      |                 | 101| 1 (5)                         | 0                               | 2 (7)                | CTX-M-15 (1), CTX-M-15(1), NDM-1 (1), CTX-M-27 (1), | B1 |
|      |                 |    |                               |                                 |                      |                                 | B1/Unknown         |
|      |                 |    |                               |                                 |                      |                                 |                    |
| 162  | 58              | 2  | 1 (5)                         | 0                               | 0                    | CTX-M-15 (1)                   | B1                 |
| 224  | 58              | 2  | 0                             | 0                               | 1 (4)                | CTX-M-55 (1)                   | B1                 |
| 278  | 58              | 2  | 0                             | 0                               | 1 (4)                | CTX-M-55 (1)                   | B1                 |
| 345  | 58              | 2  | 1 (11)                        | 0                               | 0                    | CTX-M-55 (2)                   | B1                 |
| 424  | 58              | 2  | 0                             | 0                               | 1 (4)                | CTX-M-55 (1)                   | B1                 |
| 442  | 58              | 2  | 1 (5)                         | 0                               | 0                    | CTX-M-14 (1)                   | B1                 |
| 602  | 58              | 2  | 0                             | 0                               | 2 (7)                | CTX-M-55 (2)                   | B1                 |
| 641  | 58              | 2  | 0                             | 0                               | 1 (4)                | CTX-M-14 (1), CTX-M-55 (1)     | B1                 |
| 1081 | 58              | 2  | 1 (5)                         | 0                               | 1 (4)                | CTX-M-14 (2)                   | B1                 |
| 1196 | 58              | 2  | 1 (5)                         | 0                               | 1 (4)                | CTX-M-55 (2)                   | B1                 |
| 1258 | 58              | 2  | 0                             | 0                               | 1 (4)                | CTX-M-15 (1)                   | B1                 |
| 2040 | 58              | 2  | 1 (5)                         | 0                               | 0                    | CTX-M-15 (1)                   | B1                 |
| 3580 | 58              | 2  | 1 (5)                         | 0                               | 0                    | CTX-M-15 (1)                   | B1                 |
| 4956 | 58              | 2  | 0                             | 0                               | 2 (7)                | CTX-M-55 (2)                   | B1                 |
| 6799 | 58              | 2  | 0                             | 0                               | 1 (4)                | CTX-M-55 (1)                   | B1                 |
| 7586 | 58              | 2  | 0                             | 0                               | 1 (4)                | CTX-M-55 (1)                   | B1                 |
| 7590 | 58              | 2  | 1 (11)                        | 0                               | 0                    | CTX-M-55 (2)                   | B1                 |
| 8399 | 58              | 2  | 0                             | 0                               | 1 (4)                | CTX-M-55 (1)                   | B1                 |
| 8401 | 58              | 2  | 0                             | 0                               | 1 (4)                | CTX-M-55 (1)                   | B1                 |
|      |                 |    |                               |                                 |                      |                                 |                    |
|      |                 | 165| 1 (7)                         | 0                               | 1 (9)                | CTX-M-14 (1)                   | A                  |
|      |                 | 189| 1 (7)                         | 0                               | 0                    | CTX-M-55 (1)                   | A                  |
|      |                 | 226| 1 (7)                         | 0                               | 0                    | CTX-M-15 (1)                   | A                  |

<sup>a</sup> MLST, multilocus sequence typing; CC, clonal complex; ST, sequence type.

<sup>b</sup> Clan III/B1.

<sup>c</sup> Phylotype from 16S rRNA gene sequence analysis.
### Table A9. Environmental exposures and healthy women’s colonization with ESBL-producing *Escherichia coli* belonging to Clan II/A or Clan III/B1 (versus Clan I/B2&D), Phnom Penh, Cambodia, 2015–2016.

| Exposure                                      | Clan I/B2&D (reference) | Clan II/A | Clan III/B1 |
|-----------------------------------------------|-------------------------|-----------|-------------|
| People living in home                         | N = 35                  | N = 20    | N = 19      |
| >8                                            | 4(11)                   | 5(25)     | 2(11)       |
| 6–8                                           | 12(34)                  | 15(10)    | 9(47)       |
| <5                                            | 19(54)                  | 12(60)    | 8(42)       |
| Place of delivery                             |                         |           |             |
| Private clinic                                | 9(26)                   | 10(52)    | 6(32)       |
| Hospital                                      | 12(34)                  | 3(15)     | 5(25)       |
| Health center                                 | 14(40)                  | 10(50)    | ref         |
| Antibiotics at birth                          | 6(17)                   | 2(10)     | ref         |
| Toilet shared                                 | 10(29)                  | 6(30)     | ref         |
| Non-flush toilet                              | 26(74)                  | 18(90)    | ref         |
| Pet contact                                   | 7(20)                   | 5(25)     | ref         |
| Live poultry contact                          | 2(6)                    | 4(20)     | ref         |
| Consumption habits                            |                         |           |             |
| Dry fish ≥1/week                              | 6(17)                   | 6(30)     | 2(11)       |
| Dry pork ≥1/week                              | 18(51)                  | 13(65)    | 11(58)      |
| Dry beef                                      | 23(66)                  | 9(45)     | 13(68)      |
| Dry poultry                                   | 27(77)                  | 15(75)    | 15(79)      |
| Shellfish                                     | 23(66)                  | 11(55)    | 17(80)      |
| Fish ≥3/week                                  | 25(71)                  | 11(55)    | 10(53)      |
| Pork ≥3/week                                  | 30(86)                  | 17(85)    | 17(89)      |
| Beef ≥1/week                                  | 7(20)                   | 3(15)     | 6(32)       |
| Poultry ≥1/week                               | 16(46)                  | 9(45)     | 11(58)      |
| Insects                                       | 20(57)                  | 12(60)    | 11(58)      |
| Seafood                                       | 28(80)                  | 14(70)    | 16(84)      |
| Raw beef                                      | 11(31)                  | 6(30)     | 2(11)       |
| Raw veg ≥1/week                               | 4(11)                   | 3(15)     | 3(16)       |
5. Supplementary Figure

Appendix Figure. Core genome MLST-based phylogenetic tree of 195 ESBL-producing *E. coli* genomes comprising 87 human colonization isolates, 15 human clinical isolates and 93 isolates from fish, pork, and chicken meat, and resulting phylogenetic Clans I/B2&D (*n* = 53), II/A (*n* = 72), and III/B1 (*n* = 52).

*Note:* ESBL = Extended-spectrum β lactamase. ST = Sequence type.