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Authors

Johnson, Timothy J

Miller, Elizabeth A

Flores-Figueroa, Cristian

et al.

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Extensive Overlap in Avian and Extraintestinal Pathogenic *Escherichia coli* Strains Between Backyard Poultry, Humans, and Dogs in Ecuador

Timothy J. Johnson[A,D], **Elizabeth A. Miller**[A], **Cristian Flores-Figueroa**[A], **Jeannette Munoz-Aguayo**[A], **Heather Amato**[B], **Liseth Salinas**[C], **Gabriel Trueba**[C], **Jay P. Graham**[B]

[A]University of Minnesota, Department of Veterinary and Biomedical Sciences, 1971 Commonwealth Avenue, Saint Paul, MN 55108

[B]University of California, Environmental Health Sciences Division, 2121 Berkeley Way West, Berkeley, CA 94720

[C]Universidad San Francisco de Quito, Instituto de Microbiología, Colegio de Ciencias Biológicas y Ambientales, Quito, Ecuador

SUMMARY.

Escherichia coli is a ubiquitous organism that colonizes a variety of animal hosts and has the ability to persist within the environment. As such, it is not surprising that animals frequently share *E. coli* strains and contribute to environmental *E. coli* ecology. It has been well documented that poultry meat can serve as a reservoir of avian pathogenic *E. coli* (APEC) with the potential to cause human disease. However, the impact of backyard poultry rearing on household and community APEC sharing is less clear. In this study, we examined 1348 *E. coli* isolates from children, dogs, and chickens in 222 households in peri-urban communities of Quito, Ecuador, sampled across five timepoints. Extensive overlap between isolates from all three host sources were identified using Clermont phylotyping and multilocus sequence typing. Human and dog isolates also had a high rate of carriage (37% and 49%, respectively) of genes indicative of APEC. Phylogenetic analyses of dominant sequence types (ST10, ST155, ST117, ST2847, ST162, ST38, and ST354) provided examples of highly related clones found between host sources and households, and spanning timepoints. Overall, this study illustrates the apparent extensive sharing of *E. coli* that occurs across peri-urban communities. The high rates of carriage of APEC by humans and dogs in this study contrasts with previous work examining the carriage of APEC in mammalian hosts and suggests that widespread rearing of, and frequent contact with, backyard chickens may influence the dissemination of APEC within households and communities.

RESUMEN.

Superposición amplia de cepas de *Escherichia coli* patógenas aviares y extraintestinales entre aves de traspatio, humanos y perros en Ecuador.

La bacteria *Escherichia coli* es un organismo ubicuo que coloniza diversos hospedadores animales y tiene la capacidad de persistir en el ambiente. Por lo tanto, no es sorprendente que los animales

[D]Corresponding author. tjj@umn.edu.

compartan con frecuencia cepas de *E. coli* y contribuyan a su ecología ambiental. Está bien documentado que la carne de pollo puede servir como reservorio de *E. coli* patógena aviar (APEC) con potencial para causar enfermedades en humanos. Sin embargo, el impacto de la cría de aves de corral en la compartición de APEC en hogares y comunidades es menos claro. En este estudio, se examinaron 1348 aislamientos de *E. coli* de niños, perros y pollos en 222 hogares de comunidades periurbanas de Quito, Ecuador, muestreados en cinco puntos temporales. Se identificó una amplia superposición entre los aislamientos de las tres fuentes de hospedadores mediante la filotipificación de Clermont y la tipificación de secuencias multilocus. Los aislados humanos y caninos también mostraron una alta tasa de la presencia de genes indicativos de APEC (37% y 49%, respectivamente). Los análisis filogenéticos de los tipos de secuencia dominantes (ST10, ST155, ST117, ST2847, ST162, ST38 y ST354) proporcionaron ejemplos de clones altamente relacionados encontrados entre fuentes hospedadoras y hogares, y abarcando puntos temporales. En general, este estudio ilustra la aparente y extensa distribución de *E. coli* que ocurre entre comunidades periurbanas. Las altas tasas de la presencia de APEC en humanos y perros en este estudio contrastan con trabajos previos que examinaron la presencia de APEC en hospedadores mamíferos y sugieren que la cría generalizada y el contacto frecuente con gallinas de traspatio pueden influir en la diseminación de APEC dentro de hogares y comunidades.

Keywords

Escherichia coli ; avian; household; backyard; genomics; humans

Escherichia coli is a ubiquitous inhabitant of the mammalian gastrointestinal tract, particularly in humans and domesticated animals (1). *Escherichia coli* is also extremely diverse, with some strains belonging to an array of pathogenic types and others that act primarily as commensal organisms. A great deal of effort has been put forth toward the classification of the wide variety of *E. coli* strains (2). Advances in *E. coli* research have enabled high-resolution approaches toward understanding its overall ecology and evolution. Its ease of isolation, combined with its ubiquity and enhanced molecular tools, makes it an ideal organism for studying microbial dissemination in humans and other animals.

A number of studies have examined the interconnectivity between gut microbes in humans and domestic animals within the same household (3,4,5,6,7). From this work, it is clear that extensive sharing of *E. coli* strains and clones occurs between humans and domestic animals with intimate contact. Sharing extends outside of the household as well; community sharing of *E. coli* and other microbes is common, especially in health care settings (8,9). Furthermore, these microbes can be shared through the environment and our food supply. Using *E. coli*, it has been shown that humans directly impact environmental *E. coli* populations, and vice versa (10). *Escherichia coli* colonizing food animals also presents a significant impact on humans through foodborne acquisition, with many examples of disease-causing *E. coli* transmitted through our food supply (11,12). Overall, *E. coli* ecology is a highly intertwined web involving humans, animals, and the environment.

Of the various pathotypes of *E. coli*, extraintestinal pathogenic *E. coli* (ExPEC) are unique in that they have the capacity to reside in the animal gastrointestinal tract as commensal

organisms, and the potential to cause diseases such as sepsis, urinary tract infection, and wound infection (2). In poultry, a subset of ExPEC known as avian pathogenic *E. coli* (APEC) are important because they cause colibacillosis, an impactful disease affecting birds of various ages (1). There is ample evidence that some overlap exists between APEC populations and those causing urinary tract infections in humans (12). This connectivity has been postulated to occur through foodborne acquisition of APEC from retail meats. The potential for acquisition of APEC through direct contact with live poultry also certainly exists, but this linkage has not been thoroughly studied.

Although many studies exist focusing on the relatedness of *E. coli* isolated from humans and animals, most of these studies have occurred in highly industrialized countries with centralized sources of disinfected water and standardized sanitation practices. Although these studies are extremely useful, they cannot necessarily be extrapolated to countries in which water disinfection and sanitation practices are lacking. One could logically hypothesize that sharing of *E. coli* is more extensive in the latter countries, and this has been previously demonstrated (13). However, most of those studies have been performed in retrospective fashion using samples of convenience. In this study, we sought to utilize a prospective longitudinal study in Ecuador to examine the inter-relatedness of *E. coli* from households containing humans, dogs, and backyard poultry.

MATERIALS AND METHODS

Collection of isolates.

This study was approved by the Office for Protection of Human Subjects at the University of California, Berkeley (IRB No. 2019–02-11803), the Bioethics Committee at the Universidad San Francisco de Quito (No. 2017–178M), and the Ecuadorian Health Ministry (No. MSPCURI000243–3). Formal written consent was obtained from each primary caregiver of children enrolled in the study prior to participation. Isolates in this study were obtained as a part of a repeated measures observational study conducted in peri-urban communities of Quito, Ecuador, between July 2018 and September 2021 (14). Sampling was performed in 360 households across seven rural parishes, and households were sampled up to five different times, as previously described (14). Households were randomly enrolled in the study with the following inclusion criteria: 1) there was a primary household caregiver at least 18 yr of age present; 2) there was a child living at the household between 6 mo and 5 yr of age; 3) written informed consent was provided by the primary childcare provider. Power calculations were performed for this study and are extensively described in a previous publication, powered to detect expected differences in prevalence of multidrug- and cephalosporin-resistant (MDR) *E. coli* between neighborhoods of different types (14). Households reporting any animal ownership and with at least one animal fecal sample collected were selected for inclusion in this study's analysis. From children, a single stool sample per person was collected at the time of visit. From dogs and chickens, fecal droppings were collected following defecation in their backyard environment, with an effort made to collect feces and avoid soil. To obtain isolated colonies, samples were plated onto MacConkey agar (Difco, Sparks, Maryland) containing 2 mg/ml ceftriaxone. Presumptively positive third-generation cephalosporin-resistant (3GCR) *E. coli* (one colony per sample)

were then confirmed on Chromocult coliform agar (Merck, Darmstadt, Germany). Antibiotic selection was used in this study because of the extensive diversity of *E. coli* present in hosts, to increase the likelihood of recovering strains with clonal relationships across space and time. As previously described, one colony was selected per sample. In total, 3610 samples were collected and processed.

Bacterial sequencing and genomic analyses.

DNA extraction and whole genome sequencing of the isolates in this study were previously described (14). Briefly, overnight cultures were used for DNA extractions using the Qiagen DNEasy Blood & Tissue Kit (Qiagen Sciences, German-town, MD). Isolate DNAs were sequenced using either Illumina MiSeq or NovaSeq with Nextera XT libraries (Illumina Inc., San Diego, CA). Sequencing was performed with a target genome coverage of 30×.

For each genome, raw FASTQ files were quality filtered and trimmed using Trimmomatic (v0.33) (15). Trimming was done with a sliding window of 4 and average Phred quality score of 20, and 36 as the minimum read length. Trimmomatic included removal of Illumina adapters. Each genome was assembled using Shovill (v1.0.4), specifying the SPAdes assembler (16), with default parameters (https://github.com/tseemann/shovill). Assembly qualities were assessed with QUAST (v5.0.0) (17).

Genome assemblies were subjected to *in silico* multilocus sequence typing (MLST) using the Achtman seven-gene *E. coli* MLST scheme hosted on the PubMLST website (https://pubmlst.org) (18) using MLST (v2.16.1) (https://github.com/tseemann/mlst). Minimum spanning trees were generated in GrapeTree (v1.5.0) using the MSTree V2 algorithm (19), based on traditional MLST data. Genomes were mined for a variety of genetic traits using ABRicate (v.0.8.13) (https://github.com/tseemann/abricate). A minimum identity of 95% and minimum coverage of 80% was used to consider a genome positive for a given trait. Traits searched using Abricate included selected *E. coli* virulence factors using a database established by the U.S. Food and Drug Administration (https://virulence.preprod.fda.gov/), acquired resistance genes using ResFinder (20), plasmid replicons using PlasmidFinder (21), and APEC-associated virulence factors using a custom database (22). EZClermont was used to predict *E. coli* phylogenetic group for each isolates using default parameters (23).

Phylogenetic analyses.

For dominant sequence types (STs) with substantial overlap in host source, single-nucleotide polymorphisms (SNPs) were used to generate phylogenetic trees. SNPs were identified in each sample using Snippy (v4.4.0), with a minimum sequencing depth of 8× (https://github.com/tseemann/snippy) and *E. coli* strain APEC O78 used as reference (24). Separate core SNP alignments were created for isolates classified as ST10, ST155, ST117, ST2847, ST162, ST38, and ST354. Maximum likelihood trees for datasets were reconstructed with IQ-TREE (v1.6.10), using 1000 ultrafast bootstrap iterations (25). ModelFinder was used to identify the most appropriate substitution models (26). The Interactive Tree of Life was used for tree construction and annotation (27). Pairwise SNP distances between isolates were calculated with snp-dists (https://github.com/tseemann/snp-dists). Clonal relationships were defined as follows. If isolates were less than 25 SNPs different, they were considered clonal

irrespective of APEC virulence gene content. If isolates were less than 50 SNPs different and contained matching APEC virulence gene content, they were also considered clonal. This relatively loose definition was applied because we were examining a diverse collection of *E. coli* isolates across time, space, and hosts.

Data availability.

Raw reads from isolates sequenced in this study are available at the NCBI Short Read Archive under BioProject accession no. PRJNA861272 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA861272). The APEC virulence and fitness gene database is available at https://github.com/JohnsonSingerLab/APEC_VF_database.

Statistical analyses.

All statistical analyses were performed in R (v 3.3.0) with the rstatix package (v 0.7.0). Wilcoxon rank sum tests (*wilcox_test()*) with effect sizes (*wilcox_effsize()*) were used to compare median SNP distances between different populations based on host source, collection timepoints, and households. Fisher's exact tests (*fisher_test()*) were used to compare the prevalence of genetic traits between host source populations and phylotypes. Benjamini-Hochberg corrections were applied to *P* values to account for multiple hypothesis testing. An alpha level of 0.05 was used to assess statistical significance.

RESULTS

Household *E. coli* from humans and animals are diverse.

A total of 1348 genomes from 3GCR *E. coli* were analyzed in this study, including 744 isolates from children, 382 isolates from dogs, and 222 isolates from household chickens. Isolates were first analyzed for their Clermont phylogenetic groups, which is a commonly used typing approach that broadly classifies isolates into eight major groups based on ancestral lineages (Fig. 1) (28). Overall, phylogenetic group A was the most prevalent isolate across all isolates (32.3%), followed by phylogenetic groups B1 (24.0%), D (15.0%), E (7.7%), and F (5.3%). All other phylogenetic groups were found at an overall prevalence of less than 5%. When comparing phylogenetic group by host source, some notable differences in prevalence were observed. For example, isolates from children had a numerically lower prevalence of phylogenetic groups A and B1 than isolates from chickens and dogs and had a numerically higher prevalence of phylogenetic groups B2 and D. Isolates from chickens had numerically higher prevalence of phylogenetic groups E and G than isolates from dogs and children. Overall, though, prevalence was mostly uniform across phylogenetic groups and host sources.

Extensive overlap exists between chicken, human, and dog isolates from Ecuador.

Isolates were next classified according to the Achtman seven-gene MLST (18) scheme to assign STs for each isolate (Fig. 2). A total of 203 different STs were identified across 1178 isolates (87.3%) in the overall collection, and 171 isolates (12.7%) were nontypeable using the MLST scheme. The most prevalent STs across all isolates included ST10 (n = 93), ST155 (n = 76), ST117 (n = 62), ST38 (n = 60), ST2847 (n = 56), and ST354 (n = 45). Among chicken isolates, dominant STs included ST10 (9.5%), ST2847 (7.7%), ST117

(7.2%), and ST155 (6.3%). Among dog isolates, dominant STs included ST155 (7.6%), ST2847 (4.7%), ST10 (4.2%), ST117 (4.2%), and ST162 (4.2%). Among children, dominant STs included ST10 (7.5%), ST38 (7.3%), ST155 (4.4%), and ST117 (4.0%). Of the top 21 most frequently occurring STs, 18/21 (86%) of the STs contained isolates from all three host sources (dogs, chickens, and humans).

Isolates classified as APEC were common among all sources examined.

A previously defined five-gene scheme was used for classifying an isolate as APEC, a pathotype representing virulent *E. coli* capable of causing colibacillosis in poultry (29). Modifications of this scheme here involved using genomic data rather than PCR for this classification and the categorization of APEC for isolates containing four or more genes within the scheme. This criterion was selected because of the imperfectness of draft genome sequences and the potential variability in APEC genes found on its plasmid. Using a criterion of four or more genes being positive for APEC, 51.8% of chicken-source isolates were classified as APEC. In dogs and children, 49.0% and 37.1% of isolates were classified as APEC, respectively.

Phylogenetic analyses indicate extensive community sharing of *E. coli* and APEC.

Most of the dominant STs contained fairly balanced mixtures of isolates (by count) from chicken, dog, and child sources. We further investigated potential relationships between isolates by conducting SNP analyses on dominant STs. Phylogenetic trees were constructed for seven STs with host source overlap, including ST10, ST155, ST117, ST2847, ST162, ST38, and ST354 (Figs. 3–9). Trees were overlaid with host source and possession of APEC virulence genes because of the relatively high prevalence of these genes found across host source populations. Isolate clusters were also highlighted on each tree when they were 25 SNPs or less apart from one another, or 50 SNPs or less apart but containing identical virulence factor gene profiles. This categorization led to numerous groups of isolates sharing high genetic similarities.

Within ST10 (Fig. 3), eight clusters were identified involving 31 isolates. Five of these clusters contained isolates from different host sources. Every cluster contained isolates from at least two different households. One cluster was identified which contained a matching chicken and human isolate collected at the same timepoint from different households with 18 SNP differences (F9 and N19). Another matching isolate was identified in this cluster from a child in the same household at a later timepoint and was 20 SNPs different from the child's isolate sampled at the previous timepoint. Overall, though, most isolate clusters contained isolates from different households. Most of the ST10 isolates would not be classified as APEC, if defined as possessing genes of the APEC plasmid as previously described (29).

Other STs examined displayed similar patterns of clonal relationships. Within ST155 (Fig. 4), 10 clusters were identified, one of which had matching isolates from the same household and timepoint. This cluster included isolates (BB12 and BB21) from a child and dog within the same household with zero SNP differences and identical APEC gene profiles. Another cluster contained isolates from the same collection timepoint and different households and

included isolates from multiple children and a chicken with identical gene profiles and fewer than 10 SNP differences (RS2139, L7, and RS1409), and isolates from a dog and two different children from different households with identical gene profiles and fewer than 10 SNP differences (M18, V1, and L18).

Within ST117 (Fig. 5), five clusters were identified, all of which contained isolates from different households. Six of these clusters contained isolates which would be considered APEC using plasmid possession as the criteria. Two clusters possessed highly similar isolates with matching gene profiles from chickens and children (B14 and B18; QQ23 and KK6). One cluster contained multiple dog and child isolates, all from different households and timepoints (KK17, GG8, KK2, and GG18).

ST2847 (Fig. 6) contained nine clusters, one of which had matching children isolates from the same household (J7 and RS1364). All nine of these clusters contained isolates that classified as APEC. Several clusters contained mixtures of child and chicken or dog isolates with matching gene profiles. ST162 (Fig. 7) contained four clusters and one set of matching isolates (W7 and W2) from a dog and child in the same household and same timepoint, differing by 18 SNPs with identical APEC gene profiles. This cluster also contained additional isolates from children, dogs, and a chicken, all belonging to different households and timepoints. Another cluster contained isolates from a dog and chicken with matching gene profiles (H9 and RS1337). Again, all four of these clusters contained isolates classified as APEC.

ST38 (Fig. 8) contained six clusters with 49 isolates and in general possessed many closely related isolates falling into three major clades. Examples were found of matching isolates from the same household at different timepoints (G21 and X11, G20 and R2), matching isolates from different host sources (dog and child) from the same household at the same timepoint (E5 and E3, RS1110 and RS1115), and isolates from children in adjacent households with matching genetic profiles (G21 and G20, X11 and R2). There was also a cluster identified containing a chicken-source isolate and child-source isolates with matching gene profiles (W1 and J3). Most isolates within clusters again came from unique households in the study. None of the ST38 clusters contained isolates classified as APEC.

Finally, ST354 (Fig. 9) contained six clusters with 22 isolates, and only one example was found of matching isolates from the same household (dog and child, RS1343 and G19), differing by 11 SNPs and possessing identical APEC gene profiles. One cluster of isolates contained two dog and two child isolates (QQ13, QQ12, RR16, and NN15), all collected from different households at the same timepoint. Three of the ST354 clusters contained isolates classified as APEC.

Across the top-occurring STs (Fig. 2), we analyzed overall median SNP differences within and between host source populations ($n = 438$ isolates). When categorizing isolate pairs as same host source versus different host source, we found that there was a significant difference in median SNPs (20,592 versus 19,289, respectively) between the two categories (Wilcoxon rank sum test: $P < 0.01$), but the effect size was very small ($r = 0.017$). Similarly, comparing isolate pairs from the same collection timepoint versus different collection

timepoints also identified significant differences in median SNPs (20,239 versus 19,567, respectively), but the effect size was again small. Finally, comparing isolates from the same household versus different households identified a difference in median SNPs (16,947 versus 19,617) which was significant (Wilcoxon rank sum test: $P < 0.01$), but again with a small effect size ($r = 0.009$).

Extended APEC genotyping revealed high gene prevalence across multiple host sources.

We then assessed the prevalence of 46 genes associated with APEC virulence, 59 antimicrobial resistance (AMR) genes/alleles, 39 plasmid replicons, and 26 *E. coli* virulence factors of interest across the isolate collection (Table 1). APEC genes previously linked to a transmissible virulence plasmid (30) were generally highly prevalent across all host source populations, including *hlyF* (41%–55%), *ompTp* (40%–55%), *iroBCDEN* (35%–53%), *etsABC* (38%–44%), *cvaABC-cvi* (22%–37%), and *cbi-cib-cmi-cma* (18%–36%). A total of 23/26 APEC virulence factors differed significantly between the host source groups (Fisher's exact tests: adjusted $P < 0.05$). The majority of these differences were driven by the human source population, which mostly contained APEC genes at significantly lower prevalence than dog or chicken sources.

AMR and plasmid replication genes, but not intestinal pathogenic *E. coli* virulence factors, were prevalent and different across host sources.

Dominant AMR genes identified in the overall collection included *tet*(A) (51%–67%), *floR*_2 (28%–50%), *aph*(6)-Id (41%–43%), *fosA3* (33%–42%), *sul2* (32%–42%), *ant*(3″)-Ia (32%–42%), *aph*(3″)_Ib_5 (36%–38%), $bla_{\text{CTX-M-55}}$ (27%–37%), $bla_{\text{TEM-141}}$ (24%–32%), and *qnrB19* (22%–29%). A total of 29/59 antimicrobial resistance genes differed significantly between the host source groups (Fisher's exact tests: adjusted $P < 0.05$). Several genes were of significantly higher prevalence in human isolates compared to both dog and chicken isolates, including $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-27}}$, and $bla_{\text{CTX-M-3}}$. A number of genes were significantly higher in the chicken and dog sources compared to human isolates, including *floR*, $bla_{\text{CTX-M-65}}$, *sul3*, $bla_{\text{CTX-M-130}}$, *aph*(4)-Ia, *fosA3*, *aac*(3)-IVa, *cmlA1*_2, *aadA2*, and $bla_{\text{CTX-M-55}}$. Only two antimicrobial resistance genes were found at significantly higher prevalence in chicken isolates compared to both dog and human isolates: *aadA2* and *cmlA1*_1.

Dominant plasmid replicon alleles identified in the isolate collection included IncFIB(AP001918) (62%–68%), Col(MG828) (39%–48%), IncFII(pHN7A8) (25%–36%), IncN (16%–23%), Col440I (23%–27%), pO111 (14%–22%), and IncI1_alpha (19%–25%). There were 13/39 plasmid alleles that differed significantly in prevalence by host source population (Fisher's exact tests: adjusted $P < 0.05$). Three of these alleles were of significantly higher prevalence in the chicken and dog populations as compared to the human isolates, including IncFII(pHN7A8), pO111, and IncN. The IncFII plasmid allele differed in all three host populations, increasing in prevalence from chicken-to-dog-to-human populations. The IncB/O/K/Z_2 allele was significantly higher in the human population compared to both chicken and dog isolates.

Twenty-six additional *E. coli* virulence factors were examined to determine the possible presence of intestinal pathogenic *E. coli* pathotypes in the isolates studied, and some additional genes associated with ExPEC virulence. The overall prevalence of any *E. coli* virulence factor sought never exceeded 10% in any population. Only *papGII* and *espB* had prevalence >1% in any host source population. Only 3/26 *E. coli* virulence factors significantly differed in prevalence by host source (Fisher's exact tests: adjusted $P < 0.05$): *afaC*, *afaC*, and *espB*, all of which were significantly higher in human-source isolates.

DISCUSSION

The primary goal of this study was to examine the relationships between 3GCR *E. coli* from different host sources, collected in prospective longitudinal fashion from 360 different households in seven rural parishes in Quito, Ecuador. In a previous study examining isolates from Ecuador that contained matching phenotypic antimicrobial susceptibility patterns, it was concluded that there was no evidence of the clonal spread of AMR *E. coli* in the Quito community due to the diversity of clones and plasmid types observed (31). Previous work on samples from this study have focused on antimicrobial use and susceptibility (32,33). A study utilizing 264 3GCR *E. coli* from this sample set found 16 clonal relationships among the isolates with connectivity mainly between households, with limited evidence of within-household clonal relationships (32). A second study examined risk factors associated with MDR *E. coli* within the households of this study and found that colonization of children with said bacteria is influenced by exposure to backyard and commercial livestock and poultry (33).

The present study took a broader approach utilizing 1348 3GCR *E. coli* collected throughout this prospective sampling effort, with isolates collected under selection for ceftriaxone resistance. The rationale for using antibiotic selection was that this approach reduces the overall diversity of *E. coli* collected, and thus increases the likelihood of finding clonal relationships between isolates. A limitation of this approach is that it may not be fully reflective of the scope of clonal relationships that exist in the overall *E. coli* populations inhabiting the gastrointestinal tracts of the hosts studied. This may have influenced many traits examined, including phylogenetic types, virulence genes, resistance genes, and plasmid replicons examined. Since plasmids are known to commonly encode antimicrobial resistance, it is possible that initial selection with ceftriaxone may have influenced the recovery of APEC plasmid-containing *E. coli* in this study. Therefore, it should be cautioned that prevalences reported in this study may not reflect overall gene or trait prevalence across all *E. coli* populations within these hosts, particularly regarding antimicrobial resistance genes. However, a positive aspect of this approach is that it enabled us to focus on an important subset of ceftriaxone-resistant *E. coli* and successfully identify clonal relationships between isolates.

Previous work characterizing human fecal isolates found APEC carriage to be relatively low in healthy hosts. For example, Logue *et al.* found the carriage of APEC plasmid genes in 204 human fecal isolates ranging from 4% to 17% (34). Similar findings have been observed for isolates from urinary tract infections in human hosts. For example, Johnson *et al.* found the carriage of APEC plasmid genes in 200 isolates from human urinary tract infections

ranging from 5% to 27% (35). Collectively, these studies suggest that human carriage of APEC-like strains is likely a spillover event linked to consumption of poultry or contact with live poultry. However, few if any studies have examined the linkage between carriage of APEC and frequent contact with live poultry. To our surprise, we found in this study that 37% of isolates from humans were classified as APEC, and 49% of isolates from dogs were classified as APEC. Although this study was not designed to prove that frequent contact with backyard poultry increases human and domestic animal carriage of APEC, the findings are intriguing. Certainly, other factors in the study design could have contributed to this finding, including selection of isolates, demographic studied, and geographical location. It is also impossible from this study to prove directionality of transmission, or whether transmission occurred directly between animals or indirectly through the environment. Irrespective of this, the relatively high carriage of APEC in humans and dogs contrasts previous findings and warrants further investigation. Additionally, the human subjects in this study were children aged 4 yr or less. It is possible that susceptibility to APEC colonization differs between children and adults, and the frequency of direct contact with household animals in children is almost certainly different compared to adults. This may also warrant further investigation.

Even though antibiotic selection was utilized for retrieving isolates, those isolates collectively were very diverse, belonging to all of the known *E. coli* phylogenetic types. Not surprisingly, more than 50% of the isolates belonged to phylogenetic types A and B1, irrespective of host source. It is well established that phylogenetic types A and B1 are dominant groups in the gastrointestinal tracts of animals (36,37). It was also not surprising that children harbored numerically higher proportions of phylogenetic groups B2 and D isolates than chicken and dog isolates. Group B2 and D isolates are typical for ExPEC in humans, and their primary reservoir is the gastrointestinal tract (36). Chicken-source isolates possessed higher percentages of isolates belonging to phylogenetic groups E and G compared to dog and human isolates. Phylogenetic group G includes ST117, which is a diverse sequence type that includes high-risk clones dominating the clinical landscape of colibacillosis in poultry (38). ST2847 and ST57 both belong to phylogenetic group E, and there were high proportions of chicken-source isolates belonging to these two STs. Other studies have previously identified relatively high percentages of poultry clinical isolates belonging to ST2847 (39) and ST57 (40). Both of these STs were found to contain an APEC-like plasmid uniformly present across isolates from this study (Fig. 5), further supporting the idea that these STs are likely successful clonal groups present in poultry in Ecuador, with evidence of dissemination to other animal sources within the community.

This study was unique in the sense that it compared fecal isolates from different host sources in a prospective and longitudinal fashion. Many previous studies have compared clinical isolates from humans (primarily from urinary tract infection) with isolates from retail poultry products. For example, a recent study examined overlap between such isolates over the course of a 1-yr longitudinal study conducted in Flagstaff, Arizona (41). That study identified a small subset of STs where there was substantial overlap of human versus meat isolates, and it also identified specific accessory genome elements which could be used to identify potential source attribution of strains. In the present study, substantial overlap of host source was found across many STs examined. For example, STs in Liu *et al.* that were

primarily comprised of poultry-source isolates included ST117, ST57, and ST155 (41). In our study, these STs contained isolates from all host sources examined. There are multiple possible contributing factors to this contrast. First, human UTI isolates do not represent the entire fecal *E. coli* cohort in the human host, and one would therefore expect there to be less overlap between human clinical versus retail meat isolates as compared to true fecal isolates from multiple host sources. Second, most previous studies have compared isolates in the context of commercial meat production in highly industrialized countries. The present study examined isolates in a community context that included households across a single community, using poultry raised in backyards, in a middle-income country with an expanding market for food animals and food animal products. Finally, some studies have isolated *E. coli* without use of selection such as an antibiotic and have found fewer linkages between isolates within the cohort. The present study specifically utilized ceftriaxone selection to narrow the *E. coli* population studied, in an effort to identify successful clonal relationships across host sources.

Our hypothesis entering this study was that there would be a high level of clonal relatedness between isolates from multiple host sources within, but not necessarily between, households in this study. To our surprise, most of the clonal relationships identified here spanned across households, many of which were separated by relatively great distance, and these relationships often spanned across multiple timepoints during the study. This highlights the potential community spread of *E. coli* to an extent not previously observed. There are many possible reasons for this observation, but one possible reason is differences in wastewater collection and treatment between this community and those in other previous studies. In communities such as the ones studied here, high-quality piped water is delivered to homes; however, household wastewater is released into the environment with no treatment. Certainly, ExPEC have been found in wastewater and surface waters (42). However, future studies would need to include additional sampling in these waters to validate this suggestion. With increasing urbanization this may contribute to the scenario where *E. coli* clones can be rapidly spread across a community, including those from environmental and domestic animal sources (43). This also increases the opportunity for the dissemination of clones in human hosts that are selected for in an animal host population. Thus, practices such as unregulated antibiotic use in domestic animals are likely to have a greater impact on the human populations in such communities.

We identified numerous examples of clonal relationships between isolates in this study, many of which were between different hosts within different households in the study. Some of these isolates were virtually identical to one another, while others differed by as many as 50 whole genome SNPs. Our definition of “clonal relationships” was perhaps loose compared to what might be used in a foodborne outbreak or other type of study focused on short-term, small-scale genomic differences indicating recent transmission. This was intentional because it was realized over the course of the study that linkages between isolates suggested extensive community spread versus specific connections within households. The limitation of this approach is that some of these identified clusters may contain isolates in which their common origin (or point of commonality) is years apart. However, use of this approach made it clear that community spread of these clones is extensive and ongoing. This again highlights perhaps the most interesting finding of this study, which is

the high prevalence of what appear to be poultry-derived isolates across human and domestic dog populations. This finding certainly warrants further investigation into the contributing factors, and why these findings contrast other work in this area.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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Abbreviations:

AMR	antimicrobial resistance
APEC	avian pathogenic *Escherichia coli*
ExPEC	extraintestinal pathogenic *Escherichia coli*
MDR	multidrug resistant
MLST	multilocus sequence typing
SNP	single-nucleotide polymorphism
ST	sequence type
3GCR	third-generation cephalosporin resistant

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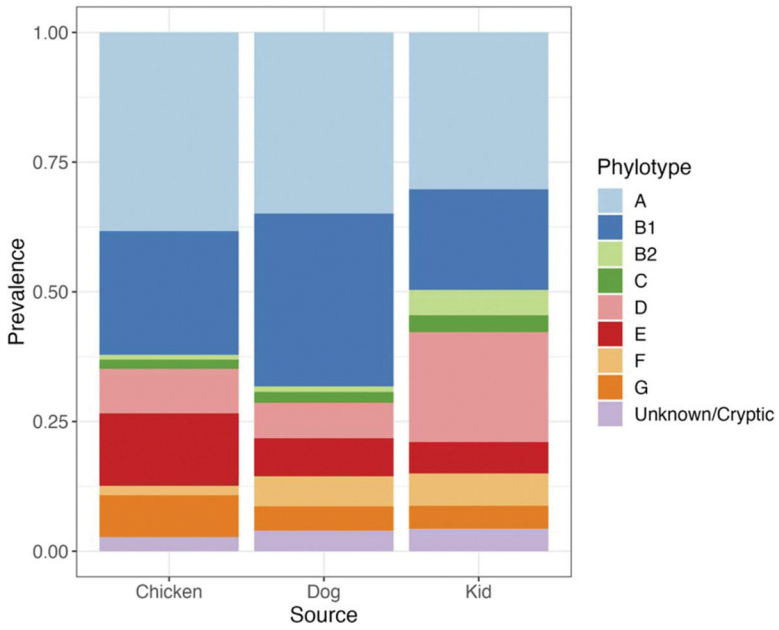

Fig. 1.

In silico assignment of isolates to Clermont phylogenetic group (Phylotype), stratified by host source (chicken = backyard chicken, dog = canine, and kid = human).

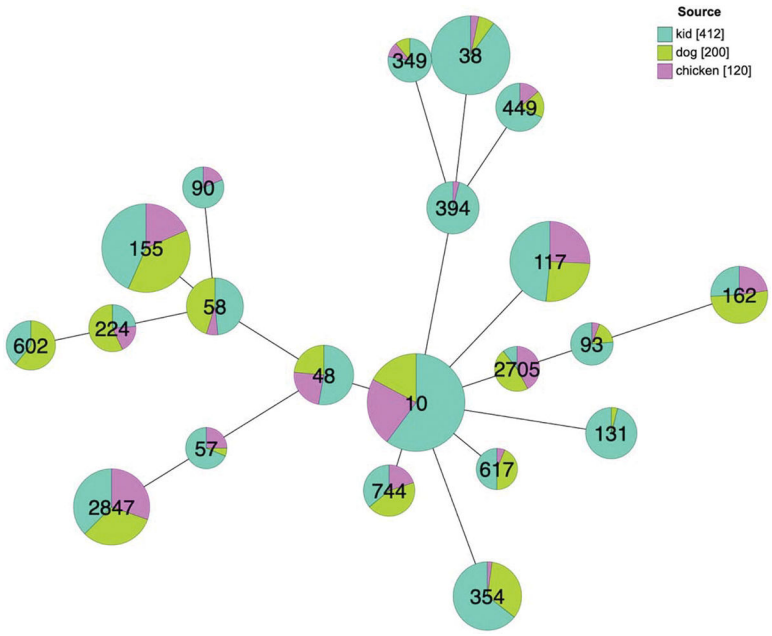

Fig. 2.

Minimal spanning tree of *E. coli* isolates collected in this study based on seven-gene MLST, colored by host source (chicken = backyard chicken, dog = canine, and kid = human). Sequence types (STs) with less than 15 isolates were excluded. STs are noted within each circle. The top 21 most frequently occurring STs are depicted.

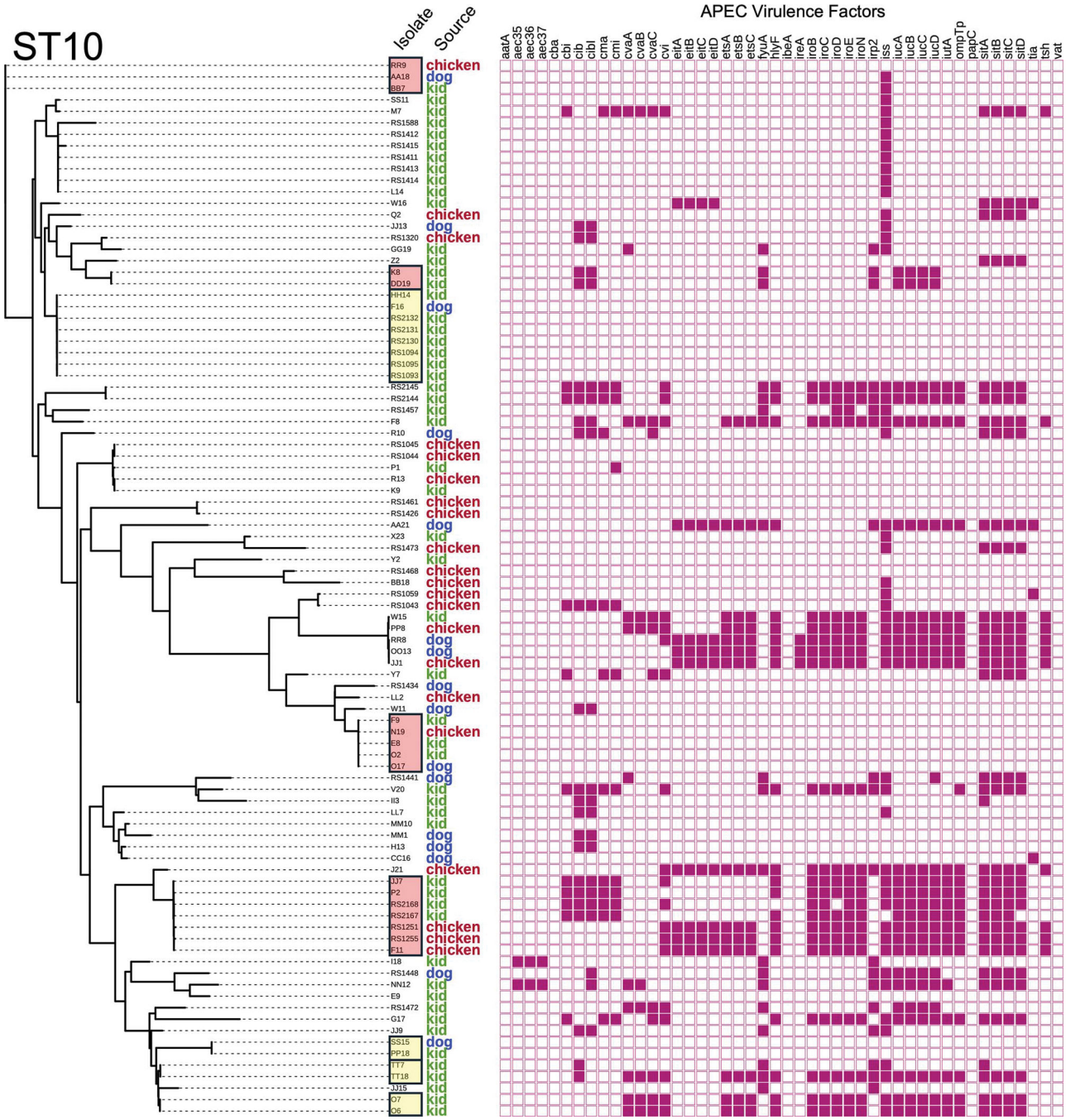

Fig. 3.

Phylogenetic tree depicting inferred relationships between ST10 isolates from different host sources. Host source is indicated as kid (child), chicken (backyard chicken), or dog (household canine). Presence of APEC virulence genes is depicted for each isolate with a filled box indicating presence of the gene. Isolate clusters are designated by a surrounding black box, with same colored denoting isolates belonging to the same cluster. Clusters were defined as isolates less than 25 SNPs different from one another irrespective of virulence gene profile, or less than 50 SNPs different from one another but containing identical

virulence gene profiles. Clusters are colored in boxes based on if they contained isolates from different households at different timepoints (red), different households from the same timepoints (yellow), or the same household at the same timepoint (green).

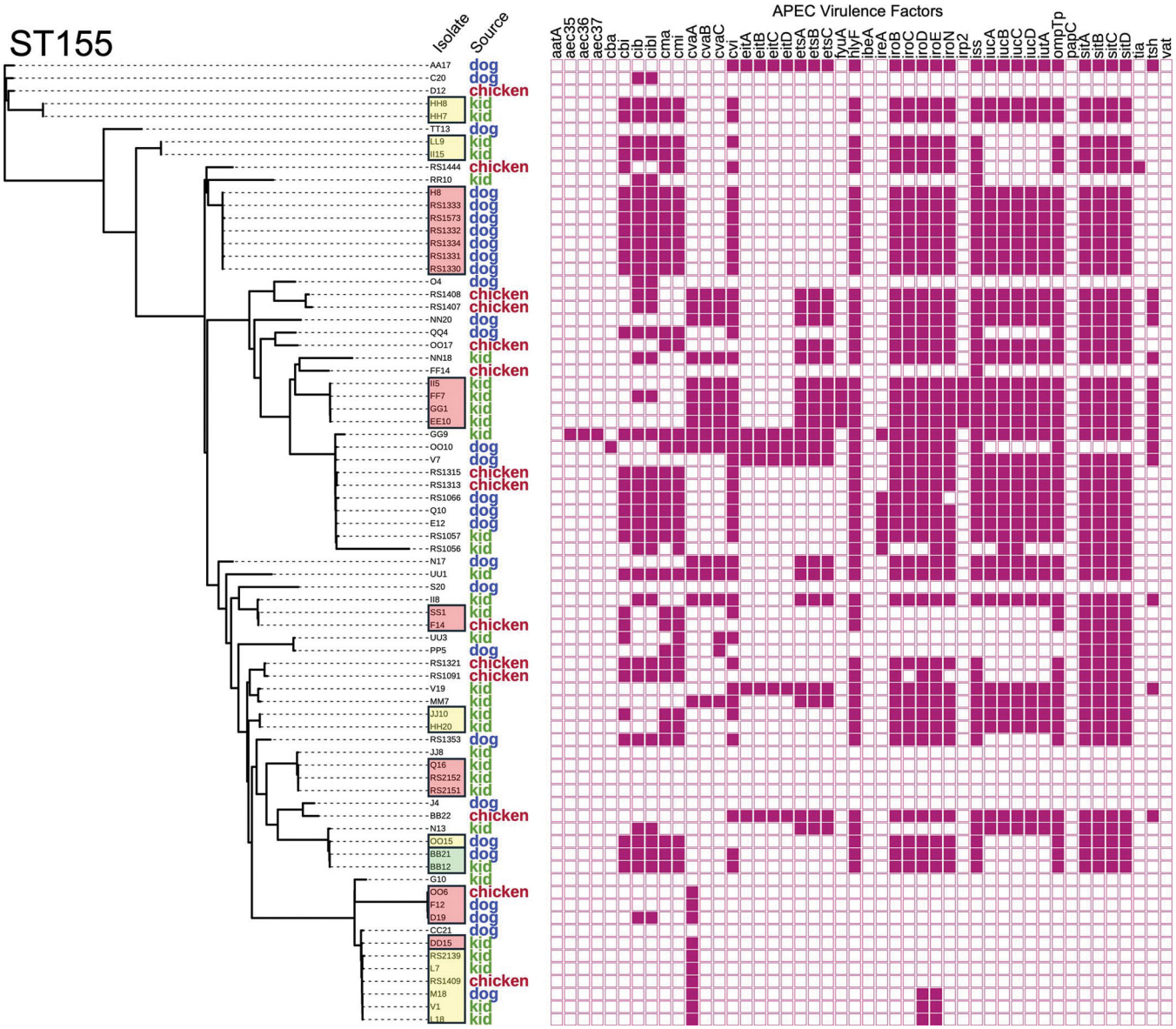

Fig. 4.

Phylogenetic tree depicting inferred relationships between ST155 isolates from different host sources. Host source is indicated as kid (child), chicken (backyard chicken), or dog (household canine). Presence of APEC virulence genes is depicted for each isolate with a filled box indicating presence of the gene. Isolate clusters are designated by a surrounding black box, with same colored denoting isolates belonging to the same cluster. Clusters were defined as isolates less than 25 SNPs different from one another irrespective of virulence gene profile, or less than 50 SNPs different from one another but containing identical virulence gene profiles. Clusters are colored in boxes based on if they contained isolates from different households at different timepoints (red), different households from the same timepoints (yellow), or the same household at the same timepoint (green).

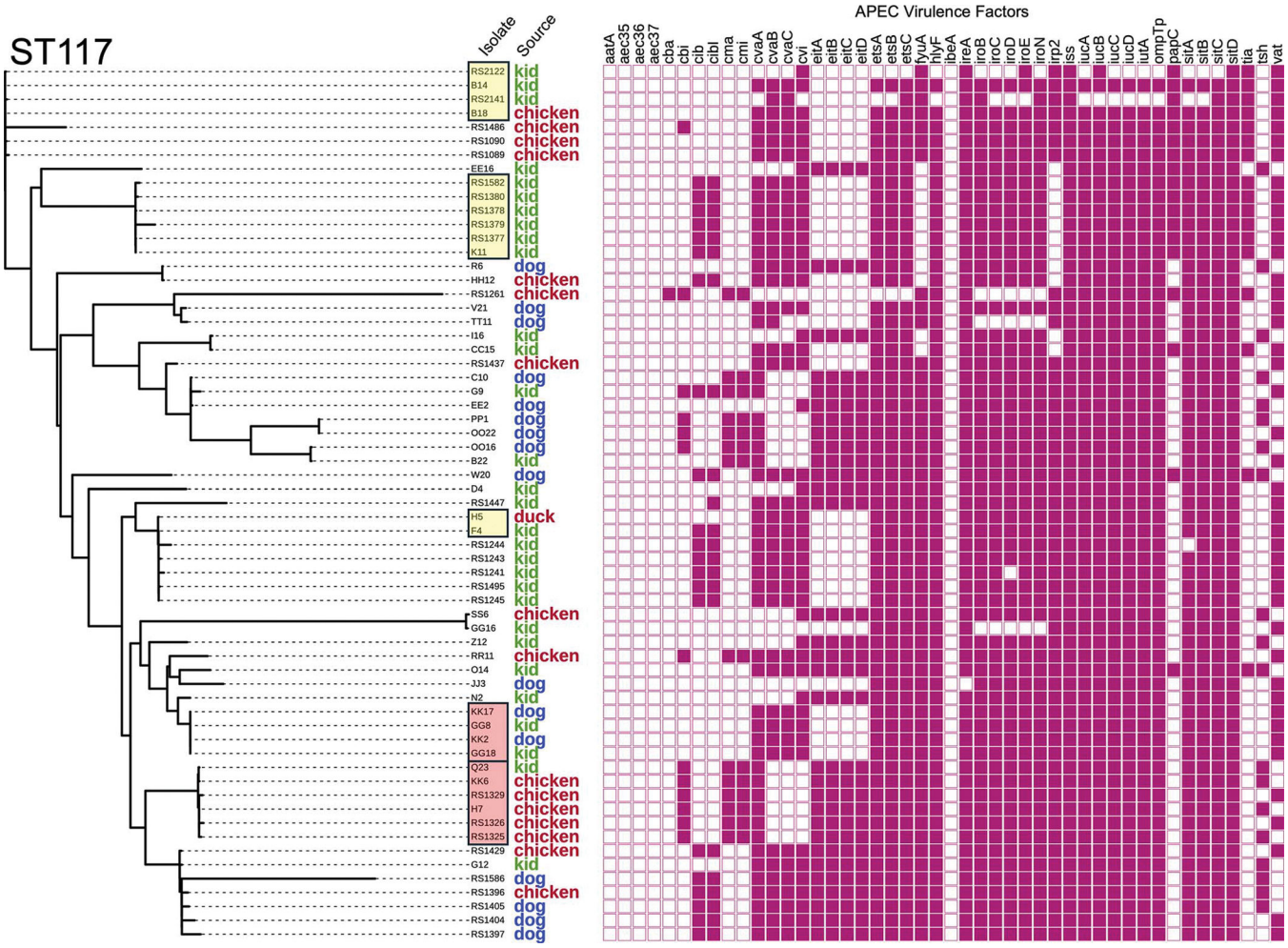

Fig. 5.

Phylogenetic tree depicting inferred relationships between ST117 isolates from different host sources. Host source is indicated as kid (child), chicken (backyard chicken), or dog (household canine). Presence of APEC virulence genes is depicted for each isolate with a filled box indicating presence of the gene. Isolate clusters are designated by a surrounding black box, with same colored denoting isolates belonging to the same cluster. Clusters were defined as isolates less than 25 SNPs different from one another irrespective of virulence gene profile, or less than 50 SNPs different from one another but containing identical virulence gene profiles. Clusters are colored in boxes based on if they contained isolates from different households at different timepoints (red), different households from the same timepoints (yellow), or the same household at the same timepoint (green).

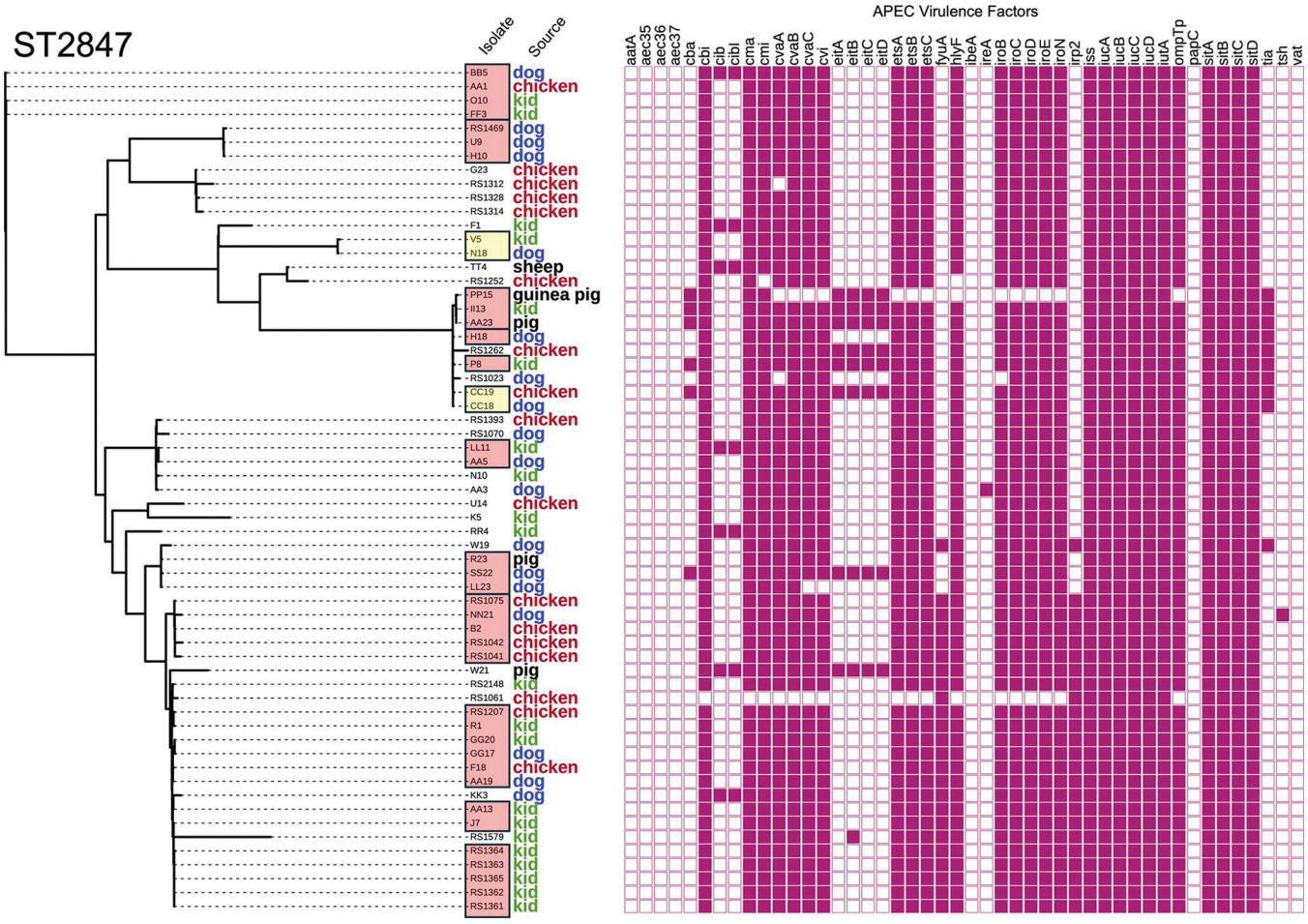

Fig. 6.

Phylogenetic tree depicting inferred relationships between ST2847 isolates from different host sources. Host source is indicated as kid (child), chicken (backyard chicken), or dog (household canine). Presence of APEC virulence genes is depicted for each isolate with a filled box indicating presence of the gene. Isolate clusters are designated by a surrounding black box, with same colored denoting isolates belonging to the same cluster. Clusters were defined as isolates less than 25 SNPs different from one another irrespective of virulence gene profile, or less than 50 SNPs different from one another but containing identical virulence gene profiles. Clusters are colored in boxes based on if they contained isolates from different households at different timepoints (red), different households from the same timepoints (yellow), or the same household at the same timepoint (green).

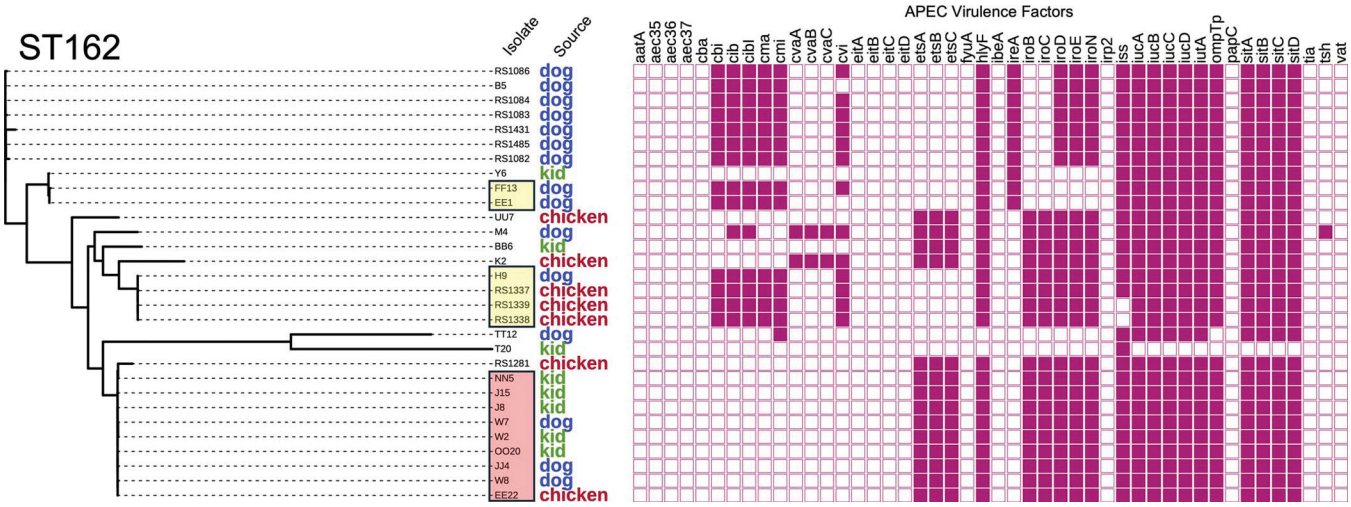

Fig. 7.

Phylogenetic tree depicting inferred relationships between ST162 isolates from different host sources. Host source is indicated as kid (child), chicken (backyard chicken), or dog (household canine). Presence of APEC virulence genes is depicted for each isolate with a filled box indicating presence of the gene. Isolate clusters are designated by a surrounding black box, with same colored denoting isolates belonging to the same cluster. Clusters were defined as isolates less than 25 SNPs different from one another irrespective of virulence gene profile, or less than 50 SNPs different from one another but containing identical virulence gene profiles. Clusters are colored in boxes based on if they contained isolates from different households at different timepoints (red), different households from the same timepoints (yellow), or the same household at the same timepoint (green).

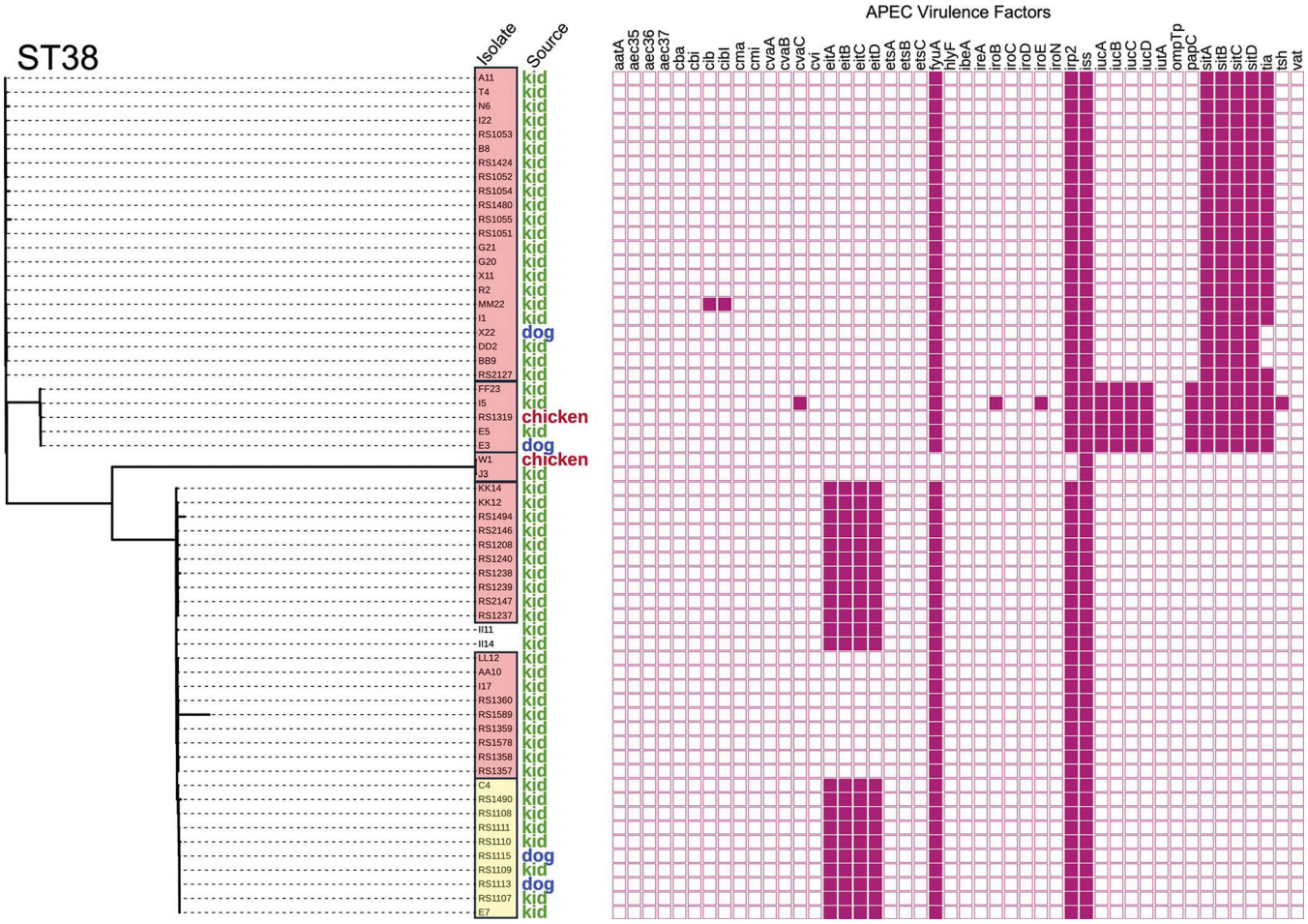

Fig. 8.

Phylogenetic tree depicting inferred relationships between ST38 isolates from different host sources. Host source is indicated as kid (child), chicken (backyard chicken), or dog (household canine). Presence of APEC virulence genes is depicted for each isolate with a filled box indicating presence of the gene. Isolate clusters are designated by a surrounding black box, with same colored denoting isolates belonging to the same cluster. Clusters were defined as isolates less than 25 SNPs different from one another irrespective of virulence gene profile, or less than 50 SNPs different from one another but containing identical virulence gene profiles. Clusters are colored in boxes based on if they contained isolates from different households at different timepoints (red), different households from the same timepoints (yellow), or the same household at the same timepoint (green).

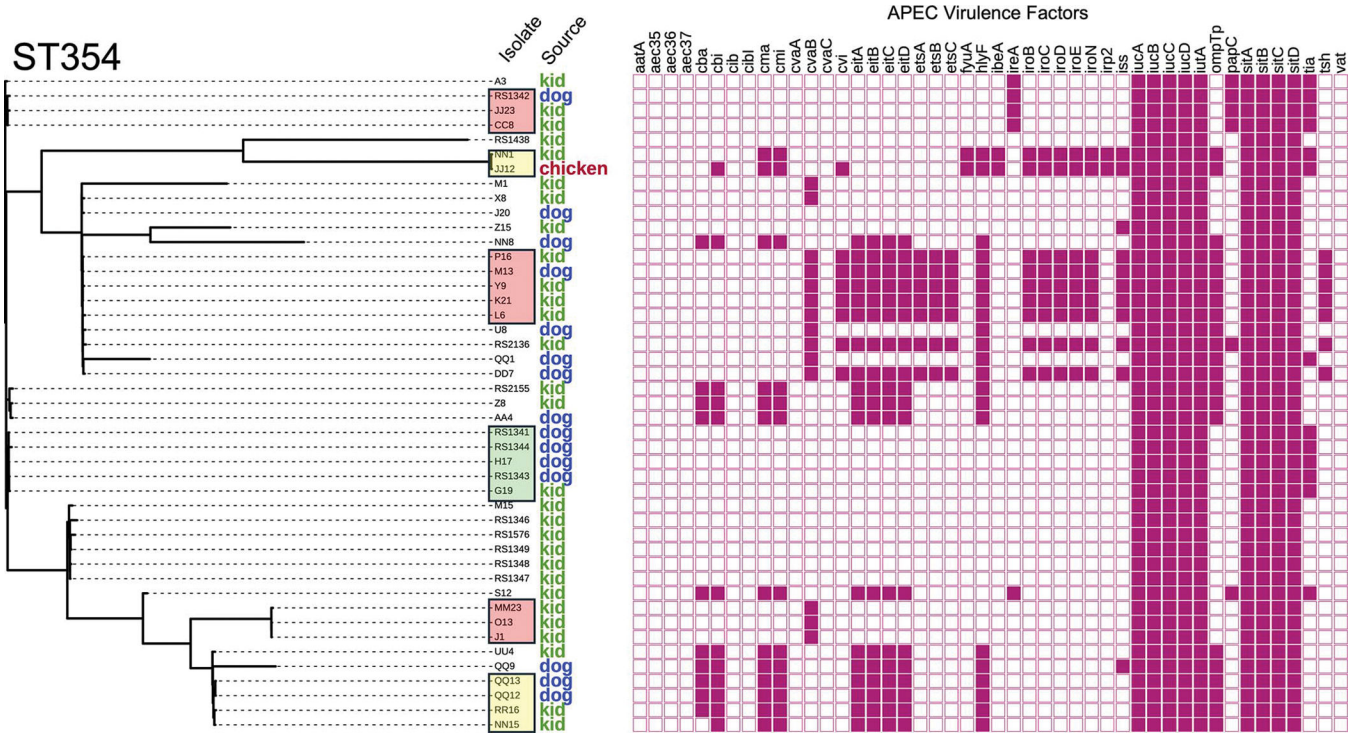

Fig. 9.

Phylogenetic tree depicting inferred relationships between ST354 isolates from different host sources. Host source is indicated as kid (child), chicken (backyard chicken), or dog (household canine). Presence of APEC virulence genes is depicted for each isolate with a filled box indicating presence of the gene. Isolate clusters are designated by a surrounding black box, with same colored denoting isolates belonging to the same cluster. Clusters were defined as isolates less than 25 SNPs different from one another irrespective of virulence gene profile, or less than 50 SNPs different from one another but containing identical virulence gene profiles. Clusters are colored in boxes based on if they contained isolates from different households at different timepoints (red), different households from the same timepoints (yellow), or the same household at the same timepoint (green).

Table 1.

Genes with significantly different prevalence among different host sources (chicken = backyard chicken, dog = canine, and child = human) based on Fisher's exact tests with Benjamini-Hochberg adjusted *P* values.

Gene	Trait type	Chicken (%)	Dog (%)	Child (%)	Adjusted *P* value
bla$_{\text{CTX-M-15}}$	AMR	2.7^{a}	1.3^{a}	12.6^{b}	2.4×10^{-12}
floR	AMR	49.5^{b}	44.6^{b}	27.9^{a}	4.2×10^{-10}
bla$_{\text{CTX-M-27}}$	AMR	0.0^{a}	1.3^{a}	7.0^{b}	1.3×10^{-7}
bla$_{\text{CTX-M-65}}$	AMR	20.3^{b}	25.7^{b}	12.0^{a}	8.4×10^{-7}
sul3	AMR	27.9^{ab}	35.4^{b}	20.6^{a}	1.0×10^{-5}
bla$_{\text{CTX-M-130}}$	AMR	4.5^{b}	2.1^{b}	0.1^{a}	2.9×10^{-5}
aph(4)-Ia	AMR	11.3^{b}	14.7^{b}	5.9a	5.8×10^{-5}
bla$_{\text{OXA-1}}$	AMR	1.4^{ab}	0.0^{a}	4.0^{b}	5.8×10^{-5}
aac(6′)-Ib-cr	AMR	0.0^{a}	0.0^{a}	3.1^{b}	8.7×10^{-5}
fosA3	AMR	41.9^{b}	46.2^{b}	32.7^{a}	1.8×10^{-4}
*sul2*_3	AMR	2.3^{a}	11.3^{b}	10.8^{b}	2.2×10^{-4}
aac(3)-IVa	AMR	10.4^{ab}	13.9^{b}	5.9^{a}	2.7×10^{-4}
tet(A)	AMR	67.1^{b}	58.0^{ab}	51.4^{a}	5.9×10^{-4}
*cmlA1*_1	AMR	15.8^{b}	18.6^{b}	9.9^{a}	6.0×10^{-4}
bla$_{\text{CTX-M-3}}$	AMR	0.5^{a}	6.6^{b}	5.7^{b}	1.1×10^{-3}
dfrA15	AMR	4.5^{b}	0.3^{a}	1.1^{a}	1.3×10^{-3}
aadA2	AMR	24.8^{b}	24.1^{b}	15.7^{a}	1.3×10^{-3}
bla$_{\text{CTX-M-55}}$	AMR	36.5^{b}	36.7^{b}	26.9^{a}	2.2×10^{-3}
bla$_{\text{CTX-M-14b}}$	AMR	0.5^{a}	0.5^{a}	3.4^{b}	3.1×10^{-3}
ant(3′′)-Ia	AMR	38.3^{ab}	42.5^{b}	31.7^{a}	4.3×10^{-3}
aph(3′)-IIa	AMR	5.9^{a}	11.5^{b}	5.8^{a}	8.1×10^{-3}
tet(B)	AMR	13.5^{a}	23.9^{b}	17.0^{a}	8.8×10^{-3}
*cmlA1*_2	AMR	3.6^{b}	0.3^{a}	1.1^{a}	9.1×10^{-3}
fosA7	AMR	3.6^{ab}	4.7^{b}	1.6^{a}	0.02
dfrA5	AMR	0.0^{a}	2.9^{b}	3.1^{b}	0.02
mph(A)_2	AMR	9.0^{a}	11.0^{ab}	15.8^{b}	0.03

Gene	Trait type	Chicken (%)	Dog (%)	Child (%)	Adjusted *P* value
aph(3′)-Ia	AMR	3.6[a]	9.7[b]	8.5[b]	0.04
qnrB19	AMR	28.8[ab]	28.3[b]	21.7[a]	0.04
dfrA12	AMR	13.1[ab]	14.7[b]	9.3[a]	0.04
aatA	APEC	60.4[b]	53.3[b]	37.2[a]	5.9×10^{-10}
cbi	APEC	35.6[c]	26.8[b]	17.9[a]	2.0×10^{-6}
iip2	APEC	26.1[a]	21.5[a]	36.4[b]	8.1×10^{-6}
fyuA	APEC	27.5[a]	22.0[a]	36.8[b]	1.4×10^{-5}
cmi	APEC	35.1[b]	31.8[b]	20.9[a]	3.2×10^{-5}
iroN	APEC	50.5[b]	47.5[b]	35.1[a]	3.4×10^{-5}
iroC	APEC	50.0[b]	48.0[b]	35.2[a]	3.4×10^{-5}
cma	APEC	33.3[b]	29.1[b]	19.2[a]	3.6×10^{-5}
iroB	APEC	50.9[b]	46.5[b]	36.2[a]	2.3×10^{-4}
ompTp	APEC	54.5[b]	51.4[b]	40.4[a]	2.3×10^{-4}
iroD	APEC	52.3[b]	49.3[b]	38.3[a]	2.6×10^{-4}
hlyF	APEC	55.0[b]	51.7[b]	40.9[a]	2.8×10^{-4}
iroE	APEC	53.6[b]	49.9[b]	39.4[a]	3.1×10^{-4}
cibI	APEC	22.5[ab]	29.7[b]	18.5[a]	7.2×10^{-4}
cib	APEC	28.4[ab]	34.4[b]	23.1[a]	1.3×10^{-3}
iucA	APEC	56.8[b]	53.3[b]	43.9[a]	1.5×10^{-3}
etsC	APEC	44.1[b]	38.8[b]	31.0[a]	1.7×10^{-3}
etsA	APEC	43.7[b]	38.3[b]	30.8[a]	2.2×10^{-3}
etsB	APEC	42.8[b]	38.3[b]	30.5[a]	2.9×10^{-3}
cvaA	APEC	36.5[b]	29.4[b]	24.0[a]	3.4×10^{-3}
ireA	APEC	14.4[ab]	17.3[b]	10.1[a]	7.7×10^{-3}
tsh	APEC	26.6[b]	19.7[ab]	16.6[a]	0.01
cvi	APEC	40.5[b]	40.4[b]	32.3[a]	0.02
afaC	Pathogenicity	0.0[a]	1.3[a]	9.6[b]	7.9×10^{-12}
afaD	Pathogenicity	0.0[a]	0.3[a]	3.8[b]	5.5×10^{-5}
espB	Pathogenicity	0.5[ab]	0.3[a]	2.3[b]	0.03
IncFII_1	Plasmid	4.1[a]	11.5[b]	23.5[c]	2.2×10^{-12}

Gene	Trait type	Chicken (%)	Dog (%)	Child (%)	Adjusted *P* value
IncX1_4	Plasmid	3.2^{ab}	6.6^{b}	1.3^{a}	1.4×10^{-4}
IncB/O/K/Z_2	Plasmid	5.4^{a}	5.5^{a}	12.4^{b}	3.4×10^{-4}
IncFII(pHN7A8)_1_pHN7A8	Plasmid	35.1^{b}	36.0^{b}	25.2^{a}	8.8×10^{-4}
p0111_1	Plasmid	22.5^{b}	22.0^{b}	13.9^{a}	1.5×10^{-3}
IncB/O/K/Z_4	Plasmid	4.5^{b}	0.5^{a}	0.8^{a}	1.6×10^{-3}
IncX4_2	Plasmid	0.9^{ab}	3.4^{b}	0.8^{a}	1.5×10^{-3}
IncI_Gamma_1	Plasmid	10.8^{ab}	15.5^{b}	9.0^{a}	0.02
IncN_1	Plasmid	23.4^{b}	22.0^{b}	15.9^{a}	0.02
IncFII(pRSB107)	Plasmid	2.3^{ab}	1.3^{a}	4.6^{b}	0.02
IncHI1B(CIT)_1_pNDM-CIT	Plasmid	5.4^{b}	1.3^{a}	2.2^{a}	0.03
IncX1_1	Plasmid	16.7^{ab}	18.9^{b}	12.4^{a}	0.03
IncFIA_1	Plasmid	9.9^{a}	13.1^{ab}	17.3^{b}	0.03

Trait type indicates the database to which a gene belonged (AMR = antimicrobial resistance; APEC = APEC virulence; Pathogenicity = *E. coli* virulence factor; Plasmid = plasmid replicon).