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Transmission of antibiotic resistance genes, between domestic animals and humans, in a semi-rural community in Ecuador

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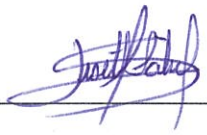
Firma del estudiante: 

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DEDICATORIA

A mis padres por siempre estar junto a mí.

A Jaime, por su apoyo, paciencia y amor, por ser mi compañero incondicional.

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RESUMEN

La resistencia a antibióticos, uno de los principales problemas de salud pública a nivel mundial, es codificada por una variedad de genes que se encuentran predominantemente asociados a elementos genéticos móviles (MGEs) como plásmidos, elementos integrativos y conjugativos, islas genómicas, integrones y transposones, asociados a bacterias. En el presente estudio se caracterizó fenotípicamente y genéticamente la resistencia a antibióticos en cepas de *E. coli* comensal aisladas a partir de 64 muestras de heces de niños asintomáticos y 203 animales domésticos de la comunidad semi rural Otón de Vélez de la parroquia Yaruquí, localizada al noreste de la ciudad de Quito, en Ecuador. Se aislaron 237 cepas de *E. coli,* 34.2% multirresistentes, y 38.4% susceptibles a los 12 antibióticos evaluados. El perfil de multirresistencia más frecuente (13.6 %) incluyó resistencia a tetraciclina, sulfisoxazol, ampicilina, estreptomicina, y trimetoprim-sulfametoxazol. Se seleccionaron 25 cepas que presentaban 7 diferentes perfiles de multirresistencia y se procedió a identificar los aislados mediante secuenciamiento de múltiples loci (MLST) y secuenciamiento del genoma completo (WGS). Identificamos 18 tipos de secuencias MLST (STs), 30 variantes alélicas de genes de resistencia a antibiótico, 22 plásmidos distintos, y 17 tipos de replicones en las bacterias transconjugadas. Estos hallazgos indican que las cepas comensales de *E. coli* (de humanos y animales domésticos) que presentan similar patrón de resistencia antibióticos en una comunidad, son muy diversas y portan diversos genes de resistencia en distintos plásmidos.

Palabras clave: *E. coli*, resistencia a antibióticos, transmisión no clonal, elementos genéticos móviles (MGEs), animales domésticos, comunidad semi rural, Ecuador.

ABSTRACT

Antibiotics resistance, one of the major public health problem worldwide, is encoded by a variety of genes that are predominantly associated with mobile genetic elements (MGEs) such as plasmids, integrative and conjugative elements, genomic islands, integrons, and transposons associated with bacteria. In the present study, phenotypic and genetic resistance profiles were determined in 64 stool samples from asymptomatic children and 203 stool sample from domestic animals of the semi-rural community Otón de Vélez of the Yaruquí parish, located in the northeast of Quito, Ecuador. We isolated 237 *E. coli* strains, 34.2 % multi-resistant, and 38.4 % susceptible to 12 evaluated antibiotics. The most frequent multiresistance profile (13.6%) included resistance to tetracycline, sulfisoxazole, ampicillin, streptomycin, and trimethoprim-sulfamethoxazole. We selected 25 strains, with 7 different multi-resistant profiles and whole genome sequencing (WGS) was performed. We identified 18 sequences types (STs) of MLST, 30 different allelic variants of antibiotic resistance genes, 22 different plasmids, and 17 replicon types in transconjugated bacteria. These findings indicate that commensal strains of *E. coli* (from humans and domestic animals) that have a similar antibiotic resistance profile in a community, are very diverse and carry different resistance genes in different plasmids.

Key words: *E. coli*, antibiotic resistance, non-clonal transmission, mobile genetic elements (MGEs), domestic animals, semi-rural community, Ecuador.

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PART I

GENERAL INTRODUCTION

Around 12 million people die annually by infectious diseases worldwide; this is exacerbated by the emergence of antibiotic resistance (AR), which reflects the ability of bacteria for developing mechanisms that allow these microorganisms to evade the lethal action of antibiotics [1], and has become one of the main problems of global public health. The presence of resistant bacteria causes changes in protocols for disease management, increased treatment costs and, in extreme cases the use of last-line therapies especially in regions with non-optimal health conditions [2; 3].

Emergence of Antibiotics Resistance

Emergence of antibiotic resistance among pathogens such as *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae*, and *Enterobacter* spp., are a global threat for hospitals [4]. Increasing antibiotic resistance in Gram-negative bacilli is a major concern because of limited therapeutic options [5]. High rates of resistance have been reported even in *Escherichia coli*, a Gram-negative bacteria from the intestinal tract of warm-blooded animals including humans [6,7].

E. coli is the predominant facultative anaerobic commensal which benefit from the stable environment and nutrients provided by the host, while preventing colonization by pathogens [8]. However, the commensal gut microbiota is a reservoir of antibiotic resistance genes and plays an important role in the spread of antibiotic resistance [9,10].

It is thought that antibiotic resistance originates in commensal microbiota and then is transferred to pathogens [10]. In fact, two pathways are known for the appearance and propagation of resistance in pathogenic bacteria: direct selection of resistant

mutants within the pathogenic bacteria during an infection, and selection of resistant bacteria within the commensal microbiota followed by horizontal gene transfer (HGT) resistance to pathogenic strains, the last being the most important in the current AR pandemic [10].

The HGT resistance from commensal to pathogenic bacteria was already described in 1959 [10], and although AR and its propagation by the HGT are old processes, the speed, as well as the range and number of strains in which they occur, has increased significantly over the last decades due to selective pressure by antibiotic use [11].

Horizontal Gene Transfer

The HGT contributes greatly in the evolution of bacterial species allowing the acquisition of genes that can play an important role in the adaptation to different environments or stressful situations [12,13]. Studies have shown that about 75% of genes in a genome have been acquired through HGT during evolution [14]. This process can be carried out by three main mechanisms such as conjugation, transduction and transformation. Conjugation is the most studied mechanism and consists in the transfer of different sizes DNA fragments. Cell-cell contact is necessary in conjugation process, for this, one of the bacterial cells extends a pili towards the other cell to form the conjugation tube that will allow the transfer of plasmid DNA [15] or chromosomic DNA in case of strains showing high frequency recombination (HFR) [16].

Transduction involves transfer of DNA via bacteriophage, which can package a portion of the host chromosome (donor), into the phage head and transfer genetic material to another bacterial cell (recipient) [15]. Third mechanism is transformation, whereby bacterial cells, in a state of competence, uptake short fragments of naked DNA from the environment and incorporate it into their genomes or re-circularize (in case of plasmid DNA) [11].

Plasmid and others Mobile Genetic Elements transmission

Most bacteria have two separate genetic systems such as chromosomal DNA and extra-chromosomal DNA. Extra-chromosomal DNA could be plasmids or phages; plasmids are circular DNA with their own replicon. Plasmids and phages can act as mobile genetic element (MGE) with the ability of self-replication and transfer from one organism to another. Essential genetic information is stored in chromosomal DNA, while genetic material for additional features such as catabolism of unusual carbon sources, degradation of complex organic matter, virulence, resistance to antibiotics, pesticides, and heavy metals are found in the extra-chromosomal DNA, then plasmids are considered accessory genetic element [17]. Plasmids can act as effective HGT vectors, during conjugation a plasmid promotes its transfer and / or that of co-resident plasmids to another bacterial cell [18] carrying smaller mobile elements such as transposons [17].

MGEs are important "vehicles" of diverse genes in the microbial genetic pool. Many genetic elements are considered MGEs: plasmids, integrative and conjugative elements (ICEs), genomic island (GIs), integrons and transposons. MGEs can be transferred by conjugation to a new host and in certain cases could be integrated into

the bacterial chromosome forming islands [19,20,21]. A striking example of bacterial adaptive evolution, driven by plasmids (and other MGEs) transmission is antibiotic resistance, which has spawned multiple hospital-level studies over the last decades, and is currently under study at the community level [20].

Escherichia coli is very important in this context; even though it only represents 0.1 % of the microbiota [22], it is the most abundant facultative anaerobe (most bacteria in microbiota are anaerobes a die very fast in open environment) [23]. Therefore *E. coli* is probably the intestinal commensal with the highest transmission rates among warm blooded animals [16,23].

The antibiotic resistance pool (resistome) in the intestine increases as a result of exposure to antibiotics, and domestic animals are very important because consumption of antimicrobial agents by domestic animals (clinical therapy or food supplement) may even exceed consumption in humans [24]. Therefore, *E. coli* from healthy individuals in a community, outside hospitals, harbor a resistance genes and are considered a suitable population to study the possible transfer of resistome from domestic animals to humans or vice versa [25].

Plasmids transmission from *E. coli* in domestic animals to *E. coli* in human

Antibiotic resistant *E. coli* strains from domestic animals can infect or reach the human population through animal food products, by direct contact or through the environment; antibiotic resistant strains may persist in humans for a period of at least 2 weeks, without any antimicrobial selective pressure [26]. In addition, the same AR genes have been identified in domestic animals and humans, suggesting inter-species

transmission, and most likely horizontal gene transfer, between human bacteria and animal bacteria [16,26,27].

In this sense, HGT through plasmids from animal *E. coli* strains to human *E. coli* strains has received much attention, for example, it has been found significantly more resistant *E. coli* in fecal stool of swine farmers than in feces of people who did not maintain contact with these animals. This difference has been attributed to the transfer of AR genes mediated by plasmids from pigs to humans [28,29]. Likewise, broiler chickens and their products were identified as a potential source for plasmid-mediated spread of AR genes to human bacteria [30,31]. In addition, multi-resistant (including extended-spectrum beta-lactamases ESBLs) *E. coli* were isolated from humans and dogs (from a village near Taï National Park in Ivory Coast), although AR genes and replicons seemed phylogenetically related between isolates from different animal species, bacterial isolates weren't [32]. These data suggest that although antibiotic resistant *E. coli* strains from domestic animal origin do not prosper in the human gastrointestinal tract, they can transfer their AR genes to human *E. coli* strains by plasmids and possibly other MGEs during the short period of time remaining in the intestine. However, despite the studies conducted on *E. coli* plasmids transmission between animal and human microbial communities, the way in which plasmids behave in natural environments has been poorly characterized [19]; there are many gaps in the understanding of transference of plasmid-mediated antibiotic resistance, i. e. if the resistance to antibiotics observed within a community is determined by different plasmids or a type of dominant plasmid [26,33].

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PART II

SCIENTIFIC PAPER

Transmission of antibiotic resistance genes, between domestic animals and humans, in a semi-rural community in Ecuador

AUTHORS

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Key words: *E. coli*, antibiotic resistance, non-clonal transmission, mobile genetic elements, domestic animals, semi-rural community, Ecuador.

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INTRODUCTION

Antibiotic Resistance (AR), one of the major public health problems worldwide, occurs when bacteria evade the action of antibiotics that are used to prevent and treat bacterial infections [1]. AR causes changes increased mortality, prolonged hospital stay, higher medical costs and changes in protocols for disease management. This problem is exacerbated by the misuse and overuse of antimicrobial agents, as there is no adequate system for the treatment of infectious diseases, and in several places, there is no control over the sale of antibiotics [2]. The use of antimicrobial agents in domestic animals for clinical therapy or food supplement may even exceed consumption in humans [3]. This raises particular concern and it has intensified the risk for spread of resistant bacteria, since the same classes of antibiotics are used both humans and domestic animals [4].

AR is coded by several genes involved in biological mechanisms that modify or degrade antibiotics, expulse antibiotics or code for alternative targets of antibiotics [5]; these genes are predominantly found in mobile genetic elements (MGEs) such as plasmids, integrative and conjugative elements (ICEs), genomic island (GIs), integrons and transposons which are horizontally transmitted between bacterial cells [6,7]. In the last years MGEs associated with AR genes have been the focus of research since recent studies have shown the non-clonal transmission of resistance genes between domestic animal bacteria and human bacteria [8].

In this sense, the commensal gut microbiota of both humans and domestic animals have been focus of interest since they play an important role in the spread of antibiotic resistance and it is considered a reservoir of AR gene. It is thought that AR originates in

commensal microbiota and then is transferred, through horizontal transfer of genes (HTG), to pathogens [9,10].

Escherichia coli is especially important in this regard because it is probably the intestinal commensal with greater rates of transmission because it is the most abundant facultative anaerobe of the intestines of warm blooded animals [11,12].

In spite of the existence of several studies about multidrug resistant hospital strains [13] we know a little about transmission at the community level. Also, transmission of AR genes between domestic animals and humans are mostly focused in particularly dangerous AR genes and not the most common AR genes; successful (numerically dominant) bacterial clones or plasmids (or other MGEs) may be crucial for the dissemination of dangerous AR genes [3]. In this study we conducted DNA sequencing of chromosomes and plasmids of commensal *E. coli* (from the same community), from domestic animals and humans, which showed similar antibiotic resistance profiles. To increase the possibilities of detecting potential transmission events, all isolates were obtained from the same rural community during the same period of time.

MATERIALS AND METHODS

Study Area

The study was carried out in the semi-rural community of Otón de Vélez, in the parish of Yaruquí located at an altitude of 2,527 meters above sea level, northeast of the city of Quito. Sixty-five households were recruited randomly, where we got 64 stool samples from asymptomatic children, and 203 samples from 12 species of domestic animals.

Sample Collection

Fecal samples were obtained from children (up to 5 years old) and from domestic animals living in the children's household, from June to August 2014. Stool samples from the children were collected by their parents previous signing an informed consent. Animal fecal swabs or fecal matter were obtained depending whether the animal was free to move or caged. The samples were transported in a cooler (around 4 °C) to the laboratory.

E. coli Isolation

Samples were cultured in MacConkey Lactose Agar medium at 37 °C for 18 hours, after which five lactose positive colonies were transferred to Chromocult® Coliform Agar for the identification of *Escherichia coli,* through its β-D-glucoronidase activity. Isolates were preserved at -80 °C in Brain Heart Infusion (BHI) Broth with 20% glycerol.

Antibiotic Susceptibility Testing

Each isolate frozen at -80 °C was reactivated on nutrient agar at 37 °C for 18 hours for evaluation of antibiotic susceptibility by disk diffusion method using Mueller-Hinton agar plates according to the resistance or susceptibility interpretation criteria from Clinical and Laboratory Standards Institute (CLSI) 2015 guidelines. Evaluated antibiotics were: amoxicillin-clavulanic acid (AMC; 20/10 µg), ampicillin (AM, 10 µg), cefotaxime (CTX, 30 µg), cephalothin (CF, 30 µg), chloramphenicol (C; 30 µg), ciprofloxacin (CIP; 5 µg), gentamicin (CN; 10 µg), imipenem (IPM; 10 µg), streptomycin (S; 10 µg), sulfisoxazole (G; 250 µg), tetracycline (TE; 30 µg) and trimethoprim-sulfamethoxazole (SXT; 1.25 / 23.75 µg) [14].

Bacterial Conjugation

After antibiotic susceptibility determination, we selected 25 isolates, grouped into 7 phenotypic profiles of multi-resistance (resistance to 3 or more antibiotics). These isolates were used as donor strains for the conjugation assay, with strains *E. coli* J53, resistant to sodium azide, *E. coli* TOP10 (Invitrogen, Carlsbad, USA) resistant to rifampicin and *E. coli* TOP10 resistant to nalidixic acid, as receptors. Selection of mutant *E. coli* TOP10 resistant to rifampicin and nalidixic acid was performed as previously described [15]. The selection of transconjugates was performed on nutrient agar supplemented with tetracycline (15 µg/mL) and sodium azide (200 µg/mL) [16], rifampicin (100 µg/mL) or nalidixic acid (30 µg/mL) according to the recipient strain used [15]. The transconjugates were evaluated for the 12 antibiotics tested by the disk diffusion method and to determine the acquired resistance. Finally, the transconjugates were stored as described before.

DNA Extraction

Total DNA, from 25 selected isolates (isolates from domestic animals and humans which showed similar antibiotic resistance profiles) obtained from fecal samples, was extracted using the DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations. Plasmid DNA from the transconjugates was extracted using QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations.

Replicon Typing

Replicon typing of transconjugates was carried out from plasmid DNA, using PCR-based replicon typing kit (PBRT Kit; DIATHEVA, Cartoceto, Italy) following the manufacturer's instructions [5].

DNA Sequencing

Whole genome sequencing (WGS) was performed from total DNA by the Mid Central Research & Outreach Center of the University of Minnesota.

Data Analysis

Assembling reads into contigs were performed using Velvet 1.2.10 with E. coli O157:H7 as reference genome obtained in the GenBank database [17], then contigs obtained were annotated with RAST [18]. Resistance genes (ResFinder 2.1), plasmids (PlasmidFinder 1.3), plasmid typing (pMLST; pMLST 1.4), and Multilocus sequence typing profiles, (MLST; MLST 1.8) were obtained based on WGS from Center for Genomic Epidemiology [19]. Sequence types (STs) and resistance genes phylogeny was

reconstructed by building neighbor joining phylogenetic using MEGA7. WGS aligments were performed using Mauve [20, 21]. Phenotypic antibiotic resistance frequency differences and antibiotic resistance genes frequency differences between human and animal origins of isolates were tested by chi-square analysis. Venn diagrams were performed using Microsoft Office Power Point 2017, and statistical analyses were performed using Microsoft Office Excel 2017.

Ethical Considerations

The study protocol was approved by the Institutional Animal Care and Use Committee at the George Washington University (IACUC#A296), as well as the Bioethics Committee at the Universidad San Francisco de Quito (#2014-135M) and the George Washington University Committee on Human Research Institutional Review Board (IRB#101355).

RESULTS

We analyzed 237 *E. coli* isolates, 63 obtained from children and 174 from domestic animals; 46 (19.4 %) of these were resistant to only one antibiotic; 19 (8.0%) to two antibiotics, and 81 (34.2%) were multidrug resistant (three or more antibiotics); while 91 (38.4 %) were susceptible to twelve antibiotics evaluated.

Antibiotic Resistance in Humans and Domestic Animals

Isolates obtained from children showed more resistance to tetracycline (50.8 %) followed by sulfisoxazole (49.2 %) and ampicillin (49.2 %). The highest percentages of resistance in isolates obtained from animals were to tetracycline (39.7%), sulfisoxazole (24.1%) and cephalothin (23%). The most frequent antibiotic resistance profile (13.6%), found in multi-resistant isolates, included resistance to tetracycline, sulfisoxazole, ampicillin, streptomycin and trimethoprim-sulfamethoxazole; the majority of these isolates belonged to humans (63.6%).

Resistance distribution for antibiotics, showed association with children for ampicillin, amoxicillin-clavulanate, streptomycin, sulfisoxazole and trimethoprim-sulfamethoxazole, as there were independent for cephalotine, cefotaxime, ciprofloxacin, chloramphenicol, gentamicin and tetracycline (Table 1).

Bacterial Conjugation

For bacterial conjugation assay, we selected 25 isolates, 15 from children and 10 from domestic animals, grouped into 7 phenotypic patterns of multi-resistance. We obtained 32 transconjugates, from 24 of the 25 selected isolates; 23 of them

transferred their complete resistance patterns to the receptor bacteria, 7 isolates showed both and total transference of antibiotic resistance patterns, and 1 isolate only transferred a partial resistance profile (Table 2).

Isolate Genotyping

Based on the analysis of the MLST of the selected 25 isolates of the adk, *fumC, gyrB, icd, mdh, purA* and *recA* genes, we identified 18 different STs, 7 STs were found in isolates from domestic animals only (STNEW1, ST189, ST48, ST101, STNEW2, ST394 and ST155) and 10 STs in isolates from children (ST157, ST349, ST4577, ST226, STNEW3, ST2952, ST131, STNEW4, ST3075 and ST1196); ST10 was present in isolates from both sources (Figures 1), however whole genome sequencing data indicated that they were not clonal (data not shown).

Among animal isolates, an isolate from guinea pig and another from chicken, belonged to ST189 and an isolate from chicken and another from pig belonged to STNEW1, however extended MLST (genes *dinB, icdA, pabB, polB, putP, trpA, trpB* and *uidA*) showed that only isolates belonged to STNEW1 were similar, ST305; while isolates belonged to ST189 were different, STNEW5 and STNEW6. Additionally, WGS showed that none of the isolates were clonal. On the other hand, 3 isolates from humans belonged to ST226 were similar too, ST681 (Table 3). Three human isolates and one from cat belonged to ST10, however whole genome sequencing showed that only the two human isolates shared a recent common ancestor (Figure A1). Additional extended MLST analysis of the of ST10 isolates showed that 3 of them belonged to ST2

and 1 to ST767 (Table 3). All other strains were classified in different ST from 7 genes MLST.

Plasmid Genotyping

We found 17 replicon types in transconjugates; 7 (X3, FIC, I1γ, W, X2, B/O, and K) only from human isolates, I1α only from 1 animal isolates, and 9 (L, P, FIIS, FII, FIA, A/C, γ, I2, and FIB) from both (Figure 2). Most common replicons were FII and FIIS with 23 (92%) and 22 (88 %) respectively (Table 4).

Whole genome sequencing of 25 selected isolates showed 22 replicons, 4 replicons were found only in animal isolates (IncN3, IncFIA(HI1), IncFIB(K) and Col156); 7 replicons only in human isolates (IncFII(pSE11), IncFII(pCoo), IncFIC(FII), IncFIB(pLF82), Col(MG828), IncFIA y p0111) and 11 in isolates from both sources (IncFII(pRSB107), IncI1, IncQ1, IncFII(29), IncY, IncFII, IncB/O/K/Z, IncI2, IncFIB(AP001918), IncFII(pHN7A8) and Col(BS512)) (Figure 3, Table 4). Also 28 F plasmids were characterized by pMLST; 8 of them (FII64, FIB27, FI43, FIA13, FII34, FII48, FIB25 and FIB24) came from animal samples, 15 (FIB11, FI33, FI79, FIB28, FII10, FIA2, FIB20, FII16, FII6, FIA1, FII17, FIB1, FIB29, NEW1 and NEW2) from human samples, while 5 (FII43, FII11, FII29, FII1 and FIB54) were identified in both sources (Figure 4). We found no association between replicons and AR patterns (Table 4).

Analysis of Antibiotic Resistance Genes

Whole genome sequence of the 25 selected isolates showed 30 allelic variants of antibiotic resistance genes: bla_{CMY-2}, *catA1, mef, tetM, dfrA1* and *aadA24* were found

only in animal isolates; *dfrA5, dfrA7, dfrA17, aadA5* and *mphA* only in human isolates and bla_{TEM-1B}, *dfrA8, dfrA12, dfrA14, dfrA15, qnrB19, strA, strB, tetA, tetB, sul1, sul2, sul3, floR, aadA1, aadA2, cmlA1, lnuF* and *fosA* in both sources (Figure 5, Table 5).

Resistance genes distribution, showed association with domestic animals for *dfrA14*, as there were independent for *aadA1, aadA2,* bla_{TEM-1B}, *cmlA1, dfrA12, dfrA15, dfrA8, floR, fosA, lnuF, qnrB19, strA, strB, sul1, sul2, sul3, tetA* and *tetB* (Table 6).

Phylogenetic analysis of most common genes showed that *tetA, tetB* and *dfrA8* are identical; as *aadA1, strA, strB* and *sul2* sequences showed SNPs, clustered independently from strain origin (Figure 6).

DISCUSSION

In this study we investigated whether numerically dominant commensal *E. coli* (from humans and domestic animals) or their MGEs played a role in the transmission of the most common antibiotic resistance in a semi-rural community. Antibiotic susceptibility testing showed association with human isolates for ampicillin, amoxicillin-clavulanate, streptomycin, sulfisoxazole and trimethoprim-sulfamethoxazole, while no association was found for animal isolates (Table 1). Molecular analysis of the isolates (MLST and WGS) showed that numerically dominant and antibiotic resistant strains of commensal *E. coli* in humans or domestic animals are not shared neither were they shared among other animal species (Table 3).

Previous studies have found that some numerically dominant *E. coli* strains from domestic animals and humans could be shared in same household [22,23,24]. The reason for this discrepancy may be the reliance, in previous studies, on MLST to detect clonal transmission [25,26,27] which seem inadequate based on our data and previous reports [28,29]. Another reason may be that we selected individuals in a community instead of individuals in the same household.

We showed evidence that human and domestic animal strains share same replicons and pMLST profiles: IncFII, IncFII(29), IncFII(pRSB107), IncFII(pHN7A8), IncFIB(AP001918), IncI1, IncI2, IncQ1, IncY, IncB/O/K/Z, Col(BS512), and FII1, FII11, FII29, FII43, FIB54, suggesting that plasmids were shared numerically dominant and antibiotic resistant *E. coli* from humans and domestic animals in this community. Similarly, allelic variants of some antibiotic resistance genes were identical in isolates from humans and domestic animals (Figure 6) suggesting that MGEs like transposons, integrons may be involved in the mobility of AR genes between *E. coli*

from domestic animals and humans [30,31]. Our results seem to confirm the notion that resistome is transferred, from domestic animals to humans by horizontal gene transfer and not by colonization of antibiotic resistance clones from domestic animals [31].

CONCLUSION

Overall, our findings demonstrate a high presence of numerically dominant *E. coli* strains with multidrug resistance, from domestic animals and humans of a semi-rural community, with high evidence of non-clonal resistance gene transmission between domestic animals and humans of a semi-rural community. We found evidence of horizontal transmission of AR genes between humans and domestic animals, suggesting that MGEs are important mechanisms of AR genes transmission.

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PART III

TABLES AND FIGURES

Table 1. Frequency and percentage of Antibiotic Resistance in semi-rural community of Otón de Vélez

Antibiotic	Human (n=63)	Animals (n=174)	Chi-square analysis	*P-value
AMC	6 (9,52%)	4 (2,30%)	5,970	0,015
AM	31 (49,21%)	35 (20,11%)	19,480	<0,001
CTX	4 (6,35%)	10 (5,75%)	0,030	0,862
CF	20 (31,75%)	40 (22,99%)	1,876	0,171
C	6 (9,52%)	32 (18,39%)	2,701	0,100
CIP	4 (6,35%)	16 (9,20%)	0,485	0,486
CN	2 (3,17%)	2 (1,15%)	1,143	0,285
IPM	0 (0,00%)	2 (1,15%)	-	-
S	26 (41,27%)	31 (17,82%)	13,929	<0,001
G	31 (49,21%)	42 (24,14%)	13,637	<0,001
TE	32 (50,79%)	69 (39,66%)	2,347	0,126
SXT	27 (42,86%)	36 (20,69%)	11,646	<0,001

*Chi square test was used to the comparison between human and domestic animals (p≤0.05).

Table 2. Antibiotic Resistance profile of isolates and transconjugates

Isolate ID	Origin	Isolate AR profile	Transconjugate AR profile
47	Human	TE-G-AM-CF-SXT-S	TE-G-AM-CF-SXT-S
52	Human	TE-G-AM-CF-SXT-S	TE-G-AM-CF-SXT-S
145	Human	TE-G-AM-CF-SXT-S	TE-G-AM-CF-SXT-S
157	Human	TE-G-AM-CF-SXT-S	TE-G-AM-CF-SXT-S
159	Human	*TE-G-AM-CF-SXT-S-C-CTX-AMC	TE-G-AM-CF-SXT-S-C-CTX-AMC
159	Human	*TE-G-AM-CF-SXT-S-C-CTX-AMC	AM-AMC-S-TE
211	Chicken	*TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP	TE-G-SXT-S
191	Pig	*TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP
58	Chicken	**TE-G-AM-SXT-C-CIP	No conjugated
132	Chicken	**TE-G-AM-SXT-C	TE-G-AM-SXT-C
19	Dog	TE-G-AM-SXT-S	TE-G-AM-SXT-S
44	Cat	TE-G-AM-SXT-S	TE-G-AM-SXT-S
90	Pig	TE-G-AM-SXT-S	TE-G-AM-SXT-S
90	Pig	TE-G-AM-SXT-S	TE-G-AM-S
113	Human	TE-G-AM-SXT-S	TE-G-AM-SXT-S
113	Human	TE-G-AM-SXT-S	TE-G-AM-S
169	Human	TE-G-AM-SXT-S	TE-G-AM-SXT-S
200	Human	TE-G-AM-SXT-S	TE-G-AM-SXT-S
202	Human	TE-G-AM-SXT-S	TE-G-AM-SXT-S
203	Human	TE-G-AM-SXT-S	TE-G-AM-SXT-S
203	Human	TE-G-AM-SXT-S	TE-G-AM-S
212	Chicken	TE-G-AM-SXT-S	TE-G-AM-SXT-S
233	Human	TE-G-AM-SXT-S	TE-G-AM-SXT-S
233	Human	TE-G-AM-SXT-S	TE-G-AM-S
71	Child	TE-G-SXT	TE-G-SXT
253	Human	TE-G-SXT	TE-G-SXT
50	Guinea pig	***TE-G-SXT-S-CIP	TE-G-SXT-S
226	Chicken	***TE-G-SXT-S	TE-G-SXT-S
241	Human	***TE-G-SXT-S	TE-G-SXT-S
241	Human	***TE-G-SXT-S	TE-G-S
102	Human	TE-G-AM-CF-SXT-S-C-CIP	TE-G-AM-CF-SXT-S-C-CIP

Most isolates transferred resistance patterns, some transferred complete and partial patterns and, one only a partial pattern.
*,**,***Antibiotic resistance patterns differing only in CIP were considered as a single group due to this resistance is associated with chromosomal elements, however in some isolates it was transferred into transconjugates.

Table 3. Probability of clonal isolates based on MLST and WGS

Isolate ID	Origin	ST (7 genes)	ST (8 genes)	WGS	Antibiotic Resistance Profile
58	Chicken	ST189	STNew5	No clone	TE-G-AM-SXT-C-CIP
50	Guinea pig	ST189	STNew6	No clone	TE-G-SXT-S-CIP
211	Chicken	STNew1	ST305	No clone	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP
191	Pig	STNew1	ST305	No clone	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP
157	Human	ST226	ST681	No clone	TE-G-AM-CF-SXT-S
159	Human	ST226	ST681	No clone	TE-G-AM-CF-SXT-S-C-CTX-AMC
113	Human	ST226	ST681	No clone	TE-G-AM-SXT-S
44	Cat	ST10	ST2	No clone	TE-G-AM-SXT-S
200	Human	ST10	ST767	No clone	TE-G-AM-SXT-S
203	Human	ST10	ST2	No clone	TE-G-AM-SXT-S
241	Human	ST10	ST2	No clone	TE-G-SXT-S

WGS showed no clonality for isolates grouped in the same ST, obtained by 7 or 8 genes analysis; despite in some cases they shared similar AR profiles.

Table 4. Plasmid Genotyping and Antibiotic Resistance profile of isolates and transconjugates

Isolate ID	Origin	Plasmids	pMLST	Replicons	Transconjugate AR profile	Isolate AR profile
47	Human	IncFII(pRSB107), IncFIB(AP001918)	FII43, FIB11	L, P, X3, FIIS, FIC, FII	TE-G-AM-CF-SXT-S	TE-G-AM-CF-SXT-S
52	Human	IncFII(pHN7A8), IncFII, IncQ1	FI33, FIB29	P, I1Y, FIIS, FIC, FII	TE-G-AM-CF-SXT-S	TE-G-AM-CF-SXT-S
145	Human	IncFIB(pLF82), IncFII(pHN7A8)	FII11	FIA, W, A/C, FIIS, X2, FII	TE-G-AM-CF-SXT-S	TE-G-AM-CF-SXT-S
157	Human	IncFII(pSE11), IncFIB(AP001918), IncFII, Col(MG828)	FI79, FIB28	P, A/C, FIIS, X2, FII	TE-G-AM-CF-SXT-S	TE-G-AM-CF-SXT-S
159	Human	IncFII(pSE11), IncFIB(AP001918), IncFII, Col(MG828)	FI79, FIB28	P, A/C, FIIS, X2, FII	TE-G-AM-CF-SXT-S-C-CTX-AMC	TE-G-AM-CF-SXT-S-C-CTX-AMC
159	Human	IncFII(pSE11), IncFIB(AP001918), IncFII, Col(MG828)	FI79, FIB28	A/C, FIIS, X2, FII	AM-AMC-S-TE	TE-G-AM-CF-SXT-S-C-CTX-AMC
211	Chicken	IncB/O/K/Z, IncFII, IncFIB(AP001918), IncQ1	FII64, FIB27	FIB, L, P, FIIS, FII	*No conjugated	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP
211	Chicken	IncB/O/K/Z, IncFII, IncFIB(AP001918), IncQ1	FII64, FIB27	FIB, FIIS, FII	TE-G-SXT-S	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP
191	Pig	IncFIB(AP001918), IncFII, IncB/O/K/Z, IncQ1	FII64, FIB27	FIB, L, P, A/C, FIIS, FII	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP
58	Chicken	IncI2, IncY	FI43	I2, L, A/C, FIIS, FII	**No conjugated	TE-G-AM-SXT-C-CIP
132	Chicken	IncFII(29), IncFIB(K), IncFIA(HI1)	FII29, FIA13	L	TE-G-AM-SXT-C	TE-G-AM-SXT-C
19	Dog	IncFII, IncN3	FII34	L, FIIS, Y, FII	TE-G-AM-SXT-S	TE-G-AM-SXT-S
44	Cat	IncFIB(AP001918), IncFII(pRSB107)	FII1, FIB54	L, FIIS, Y, FII	TE-G-AM-SXT-S	TE-G-AM-SXT-S
90	Pig	IncFII(pRSB107), IncFIB(AP001918), IncI1	FII48, FIB 25	L, FIIS, Y, FII	TE-G-AM-SXT-S	TE-G-AM-SXT-S
90	Pig	IncFII(pRSB107), IncFIB(AP001918), IncI1	FII48, FIB 25	I2, FIB, L, P, FIIS, Y, FII	TE-G-AM-S	TE-G-AM-SXT-S
113	Human	IncFIB(AP001918), IncFII(pRSB107), IncFII(29)	FII1, FIB54	I2, FIB, P, FIIS, Y, FII	TE-G-AM-SXT-S	TE-G-AM-SXT-S
113	Human	IncFIB(AP001918), IncFII(pRSB107), IncFII(29)	FII1, FIB54	I2, FIIS, FII	TE-G-AM-S	TE-G-AM-SXT-S
169	Human	IncFIB(AP001918), IncFII, IncFII(pRSB107)	FII1, FIB54	FIIS, FII	TE-G-AM-SXT-S	TE-G-AM-SXT-S
200	Human	IncFIB(AP001918), IncFIA, IncFII(pCoo)	FII10, FIA2, FIB20	FIB, FIA, A/C, FIIS, FII	TE-G-AM-SXT-S	TE-G-AM-SXT-S
202	Human	IncQ1, IncFII(pCoo)	FII16	FIIS, FII	TE-G-AM-SXT-S	TE-G-AM-SXT-S
203	Human	IncFII(pRSB107), IncB/O/K/Z, IncI2	FII6	FIIS, FII	TE-G-AM-SXT-S	TE-G-AM-SXT-S
203	Human	IncFII(pRSB107), IncB/O/K/Z, IncI2	FII6	I2, L, FIIS, FIC, FII	TE-G-AM-S	TE-G-AM-SXT-S
212	Chicken	IncFII(pHN7A8), IncFII, Col(BS512)	FII11	I1α, FIB, FIA, P, FIIS, FII	TE-G-AM-SXT-S	TE-G-AM-SXT-S
233	Human	IncFIB(AP001918), IncFII, IncFIC(FII), IncFIA, IncFII(pRSB107), IncI1, Col(BS512)	FII1, FIA1, FIB1	I2, FIIS, FIC, FII	TE-G-AM-SXT-S	TE-G-AM-SXT-S
233	Human	IncFIB(AP001918), IncFII, IncFIC(FII), IncFIA, IncFII(pRSB107), IncI1, Col(BS512)	FII1, FIA1, FIB1	FIIS, FIC, FII	TE-G-AM-S	TE-G-AM-SXT-S
71	Child	p0111	NEW1	I1Y, FIIS, FII	TE-G-SXT	TE-G-SXT
253	Human	IncFII(pCoo), IncY, IncB/O/K/Z	FII43, FIB24	B/O, I1Y, A/C, FIIS, FIC, FII	TE-G-SXT	TE-G-SXT
50	Guinea pig	IncFIB(AP001918), IncI1, IncI2, Col156	FII17	I1α, FIB, P, FII	TE-G-SXT-S	TE-G-SXT-S-CIP
226	Chicken	IncFII(29)	FII29	FIIS, FII	TE-G-SXT-S	TE-G-SXT-S
241	Human	IncB/O/K/Z, Col(MG828)	NEW2	P, K	TE-G-SXT-S	TE-G-SXT-S
241	Human	IncB/O/K/Z, Col(MG828)	NEW2	P, K	TE-G-S	TE-G-SXT-S
102	Human	IncFIB(AP001918), IncFII(29), p0111	FII29, FIB1	FIB, FIIS, FII	TE-G-AM-CF-SXT-S-C-CIP	TE-G-AM-CF-SXT-S-C-CIP

Plasmids and pMLST were obtained from WGS, replicons were characterized from transcojugates.

*Result of replicon typing referred to donor strain, because we obtained only a partial transconjugate. **Result of replicon typing referred to donor strain, because there were no transconjugate obtained.

Table 5. Antibiotic Resistance genes and Antibiotic Resistance profile

Isolate ID	Origin	AR genes	AR profile
47	Human	*bla*$_{TEM-1B}$, *dfrA8, qnrB19, strA, strB, tetB*	TE-G-AM-CF-SXT-S
52	Human	*bla*$_{TEM-1B}$, *dfrA7, dfrA8, strA, strB, sul1, sul2, tetA*	TE-G-AM-CF-SXT-S
145	Human	bla$_{TEM-1B}$, *dfrA8, strA, strB, sul2, tetB*	TE-G-AM-CF-SXT-S
157	Human	*aadA1, bla*$_{TEM-1B}$, *dfrA15, qnrB19, tetA*	TE-G-AM-CF-SXT-S
159	Human	*aadA1, bla*$_{TEM-1B}$, *dfrA15, qnrB19, sul1, tetA*	TE-G-AM-CF-SXT-S-C-CTX-AMC
211	Chicken	*lnuF, floR, bla*$_{CMY-2}$, *sul2, sul3, aadA1, cmlA1, catA1, strA, strB, fosA, bla*$_{TEM-1B}$, *qnrB19, tetA*	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP
191	Pig	*bla*$_{CMY-2}$, *bla*$_{TEM-1B}$, *catA1, qnrB19, strA, strB, sul2, tetA*	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP
58	Chicken	*dfrA14, strA, strB, sul2*	TE-G-AM-SXT-C-CIP
132	Chicken	*aadA1, aadA2, bla*$_{TEM-1B}$, *cmlA1, dfrA12, dfrA14, mef, qnrB19, strA, strB, sul2, sul3, tetA, tetB*	TE-G-AM-SXT-C
19	Dog	*aadA2, bla*$_{TEM-1B}$, *dfrA12, sul3, tetA, tetM*	TE-G-AM-SXT-S
44	Cat	*bla*$_{TEM-1B}$, *dfrA8, strA, strB, sul2, tetB*	TE-G-AM-SXT-S
90	Pig	*bla*$_{TEM-1B}$, *dfrA8, strA, strB, sul2, tetB*	TE-G-AM-SXT-S
113	Human	*bla*$_{TEM-1B}$, *dfrA8, strA, strB, sul2, tetA*	TE-G-AM-SXT-S
169	Human	*bla*$_{TEM-1B}$, *dfrA8, strA, strB, sul2, tetB*	TE-G-AM-SXT-S
200	Human	*aadA5, bla*$_{TEM-1B}$, *dfrA17, mphA, strA, strB, sul1, sul2, tetA*	TE-G-AM-SXT-S
202	Human	*bla*$_{TEM-1B}$, *dfrA8, strA, strB, sul2, tetA*	TE-G-AM-SXT-S
203	Human	*bla*$_{TEM-1B}$, *dfrA5, strA, strB, sul1, sul2, tetB*	TE-G-AM-SXT-S
212	Chicken	*aadA1, bla*$_{TEM-1B}$, *dfrA1, strA, strB, sul1, sul2, tetA*	TE-G-AM-SXT-S
233	Human	*bla*$_{TEM-1B}$, *dfrA8, strA, strB, sul2, tetB*	TE-G-AM-SXT-S
71	Human	*aadA5, dfrA17, qnrB19, sul2, tetA*	TE-G-SXT
253	Human	*dfrA14, strA, strB, sul2, tetA*	TE-G-SXT
50	Guinea pig	*aadA24, dfrA14, dfrA15, strA, strB, sul1, sul2*	TE-G-SXT-S-CIP
226	Chicken	*dfrA14, qnrB19, strA, strB, sul2, tetA*	TE-G-SXT-S
241	Human	*dfrA5, qnrB19, strA, strB, sul1, sul2, tetA*	TE-G-SXT-S
102	Human	*aadA1, aadA2, bla*$_{TEM-1B}$, *cmlA1, dfrA12, floR, fosA, lnuF, sul3, tetA*	TE-G-AM-CF-SXT-S-C-CIP

Table 6. Frequency and percentage of Antibiotic Resistance gens in semi-rural community of Otón de Vélez

Resistance genes	Human (n=15)	Animals (n=10)	Chi-square analysis	*P-value
aadA1	3 (20,00%)	3 (30,00%)	0,329	0,566
aadA2	1 (6,67%)	2 (20,00%)	1,010	0,315
aadA24	0 (0,00%)	1 (10,00%)	-	-
aadA5	2 (13,33%)	0 (0,00%)	-	-
bla_{CMY-2}	0 (0,00%)	2 (20,00%)	-	-
bla_{TEM-1B}	12 (80,00%)	7 (70,00%)	0,329	0,566
catA1	0 (0,00%)	2 (20,00%)	-	-
cmlA1	1 (6,67%)	2 (20,00%)	1,010	0,315
dfrA1	0 (0,00%)	1 (10,00%)	-	-
dfrA12	1 (6,67%)	2 (20,00%)	1,010	0,315
dfrA14	1 (6,67%)	4 (40,00%)	4,167	0,041
dfrA15	2 (13,33%)	1 (10,00%)	0,063	0,802
dfrA17	2 (13,33%)	0 (0,00%)	-	-
dfrA5	2 (13,33%)	0 (0,00%)	-	-
dfrA7	1 (6,67%)	0 (0,00%)	-	-
dfrA8	7 (46,67%)	20 (20,00%)	1,852	0,174
floR	1 (6,67%)	10 (10,00%)	0,091	0,763
fosA	1 (6,67%)	10 (10,00%)	0,091	0,763
lnuF	1 (6,67%)	10 (10,00%)	0,091	0,763
mef	0 (0,00%)	10 (10,00%)	-	-
mph(A)	1 (6,67%)	0 (0,00%)	-	-
qnrB19	5 (33,33%)	40 (40,00%)	0,116	0,734
strA	11(73,33%)	90 (90,00%)	1,042	0,307
strB	11 (73,33%)	90 (90,00%)	1,042	0,307
sul1	5 (33,33%)	20 (20,00%)	0,529	0,467
sul2	11 (73,33%)	90 (90,00%)	1,042	0,307
sul3	1 (6,67%)	30 (30,00%)	2,431	0,119
tet(A)	10 (66,67%)	60 (60,00%)	0,116	0,734
tet(B)	5 (33,33%)	30 (30,00%)	0,031	0,861
tet(M)	0 (0,00%)	10 (10,00%)	-	-

*Chi square test was used to the comparison between human and domestic animals ($p \leq 0.05$).

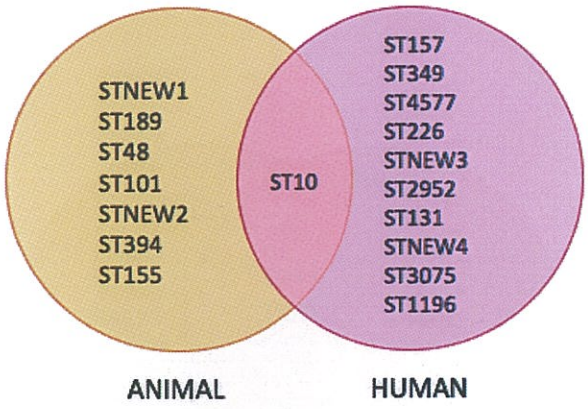

Figure 1. Sequence Types (STs) based on the origin of the isolate

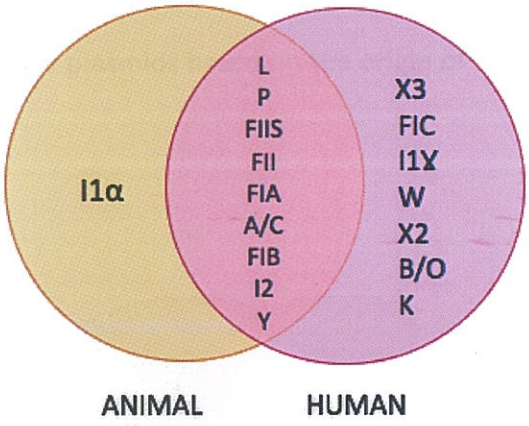

Figure 2. Replicons based on the origin of the isolate

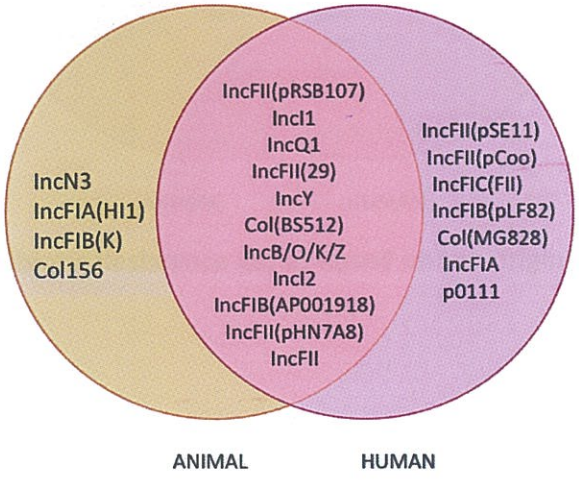

Figure 3. Plasmids based on the origin of the isolate

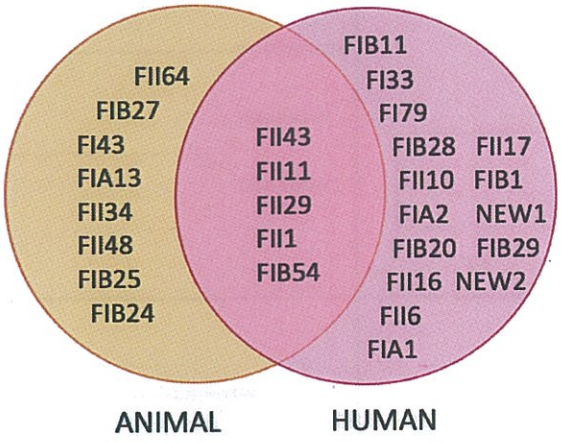

Figure 4. F plasmids based on the origin of the isolate

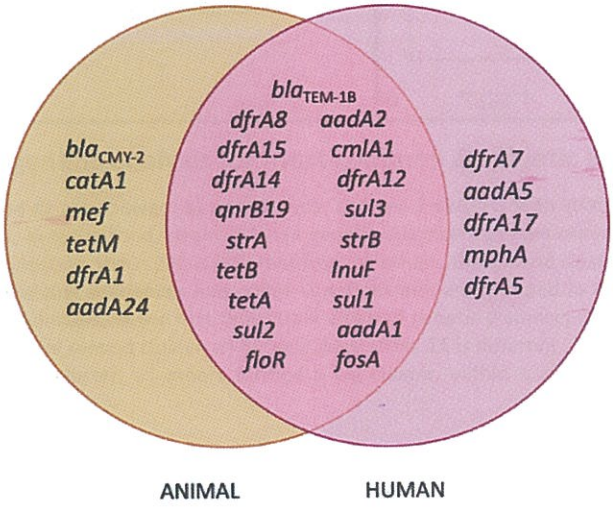

Figure 5. Antibiotic Resistance genes based on the origin of the isolate

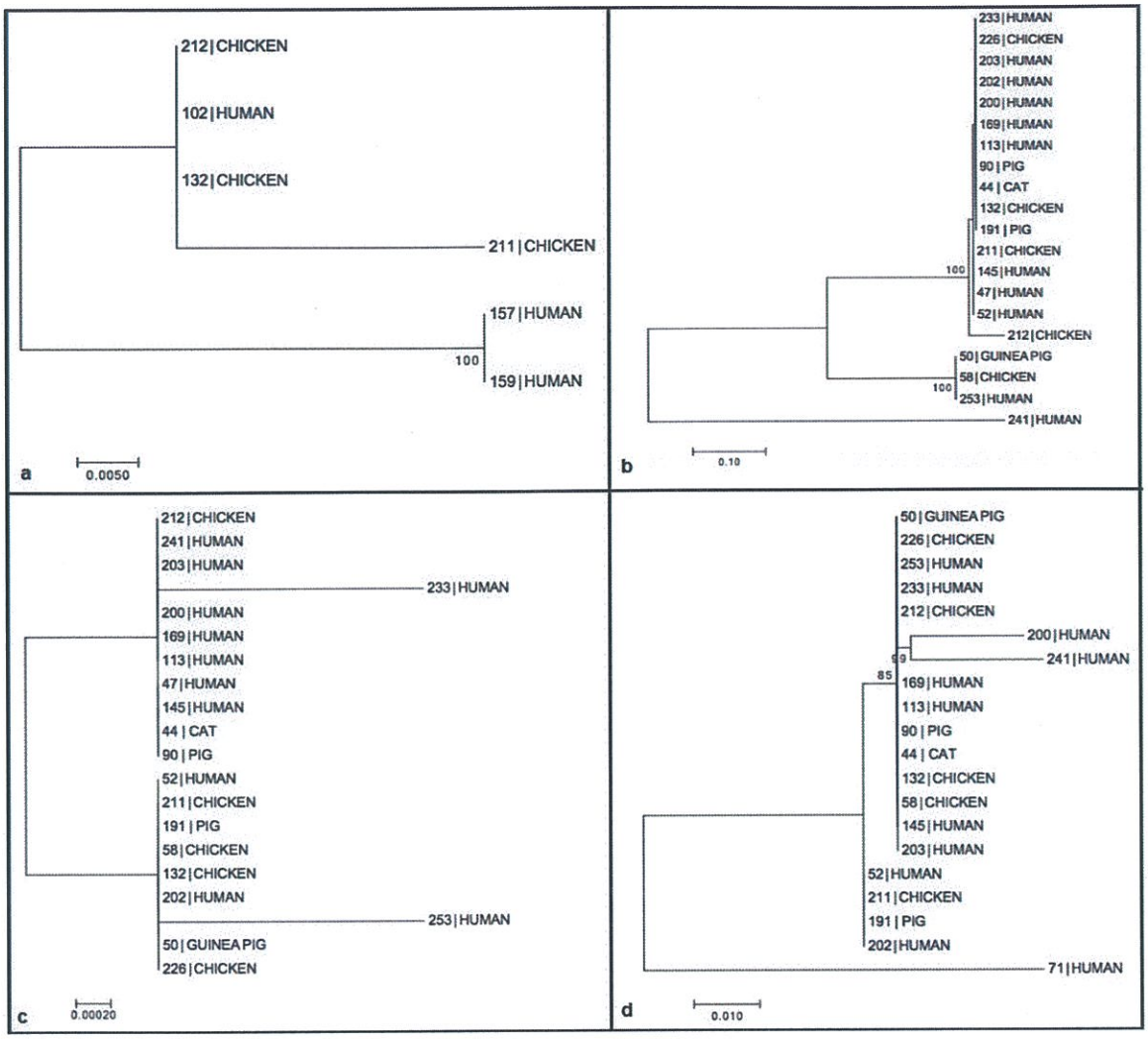

Figure 6. Phylogenetic analysis for most common Antibiotic Resistance genes

For each isolate, origin and ID are showed. **a)** *aadA1* gene, showed 2 clusters with strains 157 and 159 in the first and 212,102, 132 and 211, in the second, strain 211 had genetic distance from the others in the cluster, **b)** for *strA* gene, showed three main clusters, strain 241 is different from all others, the second cluster formed by strains 50, 58 and 253 are identical; remaining sequences had minor variations and are grouped in the third cluster, **c)** for *strB* gene there were two main clusters, in the first only strain 233 had genetic distance, in the same way strain 253 is the only one different from the second cluster, **d)** for *sul2* gene strain 71 is different , in a second clusters, strain 52, 211, 191 and 202 were in a cluster, all others formed a big cluster, within it strains 200 and 241 had genetic distance.

PART IV

ANNEXES

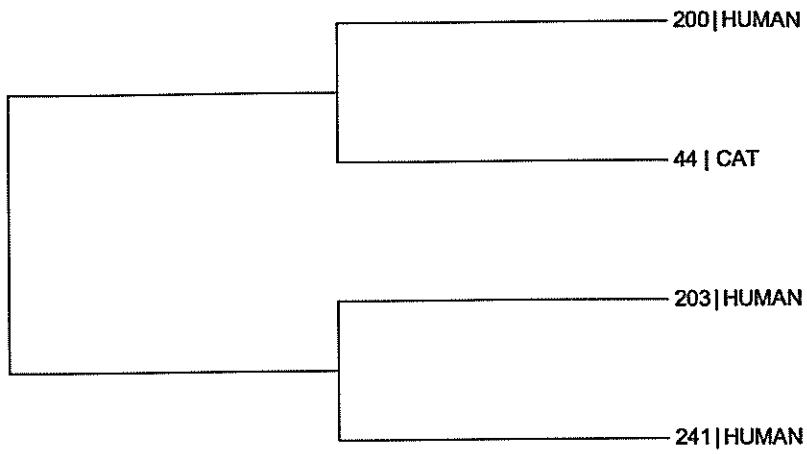

Figure A 1. Phylogenetic tree of isolates belonged to ST10, based on extended MLST analysis

Table A 1. Genetic characteristics and Antibiotic Resistance profile of *E. coli* isolates of humans and domestic animals of semi-rural community of Otón de Vélez

Isolate ID	Origin	AR profile	ST	Plasmids	pMLST F	AR genes	Transconjugate 1	Replicons	Transconjugate 2	Replicons
47	Human	TE-G-AM-CF-SXT-S	ST517	IncFII(pRSB107), IncFIB(AP001918)	FII43, FIB11	*bla_{TEM-1B}, dfrA8, qnrB19, strA, strB, tetB*	TE-G-AM-CF-SXT-S	L, P, X3, FIIS, FIC, FII	-	-
52	Human	TE-G-AM-CF-SXT-S	ST349	IncFII(pHN7A8), IncFII, IncQ1	FI33, FIB29	*bla_{TEM-1B}, dfrA7, dfrA8, strA, strB, sul1, sul2, tetA*	TE-G-AM-CF-SXT-S	P, I1Ɣ, FIIS, FIC, FII	-	-
145	Human	TE-G-AM-CF-SXT-S	ST4577	IncFIB(pLF82), IncFII(pHN7A8)	FII11	*bla_{TEM-1B}, dfrA8, strA, strB, sul2, tetB*	TE-G-AM-CF-SXT-S	FIA, W, A/C, FIIS, X2, FII	-	-
157	Human	TE-G-AM-CF-SXT-S	ST226	IncFII(pSE11), IncFIB(AP001918), IncFII, Col(MG828)	FI79, FIB28	*aadA1, bla_{TEM-1B}, dfrA15, qnrB19, tetA*	TE-G-AM-CF-SXT-S	P, A/C, FIIS, X2, FII	-	-
159	Human	TE-G-AM-CF-SXT-S-C-CTX-AMC	ST226	IncFII(pSE11), IncFIB(AP001918), IncFII, Col(MG828)	FI79, FIB28	*aadA1, bla_{TEM-1B}, dfrA15, qnrB19, sul1, tetA*	TE-G-AM-CF-SXT-S-C-CTX-AMC	P, A/C, FIIS, X2, FII	AM-AMC-S-TE	A/C, FIIS, X2, FII
211	Chicken	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP	STnew1	IncB/O/K/Z, IncFII, IncFIB(AP001918), IncQ1	FII64, FIB27	*Inu(F), floR, bla_{CMY-2}, sul2, sul3, aadA1, cmlA1, catA1, strA, strB, fosA, bla_{TEM-1B}, qnrB19, tetA*	-	FIB, L, P, FIIS, FII	TE-G-SXT-S	FIB, FIIS, FII
191	Pig	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP	STnew1	IncFIB(AP001918), IncFII, IncB/O/K/Z, IncQ1	FII64, FIB27	*bla_{CMY-2}, bla_{TEM-1B}, catA1, qnrB19, strA, strB, sul2, tetA*	TE-G-AM-CF-SXT-S-C-CTX-AMC-CIP	FIB, L, P, A/C, FIIS, FII	-	-

continue...

Table A 1. Genetic characteristics and Antibiotic Resistance profile of *E. coli* isolates of humans and domestic animals of semi-rural community of Otón de Vélez

Isolate ID	Origin	AR profile	ST	Plasmids	pMLST F	AR genes	Transconjugate 1	Replicons	Transconjugate 2	Replicons
58	Chicken	TE-G-AM-SXT-C-CIP	ST189	IncI2, IncY	FI43	*dfrA14, strA, strB, sul2*	-	I2, I, A/C, FIIS, FII	-	-
132	Chicken	TE-G-AM-SXT-C	ST48	IncFII(29), IncFIB(K), IncFIA(HI1)	FII29, FIA13	*aadA1, aadA2, bla_{TEM-1B}, cmlA1, dfrA12, dfrA14, mef, qnrB19, strA, strB, sul2, sul3, tetA, tetB*	TE-G-AM-SXT-C	I	-	-
19	Dog	TE-G-AM-SXT-S	ST101	IncFII, IncN3	FII34	*aadA2, bla_{TEM-1B}, dfrA12, sul3, tetA, tetM*	TE-G-AM-SXT-S	I, FIIS, Y, FII	-	-
44	Cat	TE-G-AM-SXT-S	ST10	IncFIB(AP001918), IncFII(pRSB107)	FII1, FIB54	*bla_{TEM-1B}, dfrA8, sul2, strA, strB, tetB*	TE-G-AM-SXT-S	I, FIIS, Y, FII	-	-
90	Pig	TE-G-AM-SXT-S	STnew2	IncFII(pRSB107), IncFIB(AP001918), IncI1	FII48, FIB 25	*bla_{TEM-1B}, dfrA8, strA, strB, sul2, tetB*	TE-G-AM-SXT-S	I, FIIS, Y, FII	TE-G-AM-S	I2, FIB, I, P, FIIS, Y, FII
113	Human	TE-G-AM-SXT-S	ST226	IncFIB(AP001918), IncFII(pRSB107), IncFII(29)	FII1, FIB54	*bla_{TEM-1B}, dfrA8, strA, strB, sul2, tetA*	TE-G-AM-SXT-S	I2, FIB, P, FIIS, Y, FII	TE-G-AM-S	I2, FIIS, FII
169	Human	TE-G-AM-SXT-S	STnew3	IncFIB(AP001918), IncFII, IncFII(pRSB107)	FII1, FIB54	*bla_{TEM-1B}, dfrA8, strA, strB, sul2, tetB*	TE-G-AM-SXT-S	FIIS, FII	-	-
200	Human	TE-G-AM-SXT-S	ST10	IncFIB(AP001918), IncFIA, IncFII(pCoo)	FII10, FIA2, FIB20	*aadA5, bla_{TEM-1B}, dfrA17, mphA, strA, strB, sul1, sul2, tetA*	TE-G-AM-SXT-S	FIB, FIA, A/C, FIIS, FII	-	-

continue...

Table A 1. Genetic characteristics and Antibiotic Resistance profile of *E. coli* isolates of humans and domestic animals of semi-rural community of Otón de Vélez

Isolate ID	Origin	AR profile	ST	Plasmids	pMLST F	AR genes	Transconjugate 1	Replicons	Transconjugate 2	Replicons
202	Human	TE-G-AM-SXT-S	ST2952	IncQ1, IncFII(pCoo)	FII16	*bla_{TEM-1B}, dfrA8, strA, strB, sul2, tetA*	TE-G-AM-SXT-S	FIIS, FII	-	-
203	Human	TE-G-AM-SXT-S	ST10	IncFII(pRSB107), IncB/O/K/Z, IncI2	FII6	*bla_{TEM-1B}, dfrA5, strA, strB, sul1, sul2, tetB*	TE-G-AM-SXT-S	FIIS, FII	TE-G-AM-S	I2, I, FIIS, FIC, FII
212	Chicken	TE-G-AM-SXT-S	ST394	IncFII(pHN7A8), IncFII, Col(BS512)	FII11	*aadA1, bla_{TEM-1B}, dfrA1, strA, strB, sul1, sul2, tetA*	TE-G-AM-SXT-S	I1α, FIB, FIA, P, FIIS, FII	-	-
233	Human	TE-G-AM-SXT-S	ST131	IncFIB(AP001918), IncFII, IncFIC(FII), IncFIA, IncFII(pRSB107), IncI1, Col(BS512)	FII1, FIA1, FIB1	*bla_{TEM-1B}, dfrA8, strA, strB, sul2, tetB*	TE-G-AM-SXT-S	I2, FIIS, FIC, FII	TE-G-AM-S	FIIS, FIC, FII
71	Human	TE-G-SXT	STnew4	p0111	Unknown	*aadA5, dfrA17, qnrB19, sul2, tetA*	TE-G-SXT	I1Y, FIIS, FII	-	-
253	Child	TE-G-SXT	ST3075	IncFII(pCoo), IncY, IncB/O/K/Z	FII43, FIB24	*dfrA14, strA, strB, sul2, tetA*	TE-G-SXT	B/O, I1Y, A/C, FIIS, FIC, FII	-	-
50	Guinea pig	TE-G-SXT-S-CIP	ST189	IncFIB(AP001918), IncI1, IncI2, Col156	FII17	*aadA24, dfrA14, dfrA15, strA, strB, sul1, sul2*	TE-G-SXT-S	I1α, FIB, P, FII	-	-
226	Chicken	TE-G-SXT-S	ST155	IncFII(29)	FII29	*dfrA14, qnrB19, strA, strB, sul2, tetA*	TE-G-SXT-S	FIIS, FII	-	-
241	Human	TE-G-SXT-S	ST10	IncB/O/K/Z, Col(MG828)	Unknown	*dfrA5, qnrB19, strA, strB, sul1, sul2, tetA*	TE-G-SXT-S	P, K	TE-G-S	P, K

continue...

Table A 1. Genetic characteristics and Antibiotic Resistance profile of *E. coli* isolates of humans and domestic animals of semi-rural community of Otón de Vélez

Isolate ID	Origin	AR profile	ST	Plasmids	pMLST F	AR genes	Transconjugate 1	Replicons	Transconjugate 2	Replicons
102	Human	TE-G-AM-CF-SXT-S-C-CIP	ST1196	IncFIB(AP001918), IncFII(29), p0111	FII29, FIB1	*aadA1, aadA2,* bla_{TEM-1B}*, cmlA1, dfrA12, floR, fosA, lnuF, sul3, tetA*	TE-G-AM-CF-SXT-S-C-CIP	FIB, FIIS, FII	-	-