UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Posgrados

Analysis of mobile genetic elements and resistance genes found in commensal *Escherichia coli* from the gut of children and animals from a semi-rural community close to Quito

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DEDICATORIA

Dedico este trabajo a mi familia, a mis padres y esposo que me apoyaron durante estos 2 años de la maestría, no solo a nivel emocional con sus consejos constantes sino también no descuidando mi negocio, mi laboratorio por el cuál intento ser mejor cada día, y al cual me dedicaré por el resto de mi vida.

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RESUMEN

Un problema que amenaza la salud humana hoy en día es el desarrollo de bacterias multiresistentes o "súper-bacterias", las cuales se pueden encontrar en el tracto gastrointestinal de animales y humanos como microbiota comensal. El uso de antimicrobianos en la crianza de animales es difícil de controlar y contribuye al incremento de resistencia a antibióticos en humanos. Hoy en día está demostrado que la interacción entre animales y humanos lleva a éstos a compartir parte de su microbiota y en ella diferentes genes de resistencia que son pasados a través de los elementos genéticos móviles (MGE). Nosotros investigamos la presencia de genes de resistencia encontrados en bacterias comensales intestinales de animales y humanos de las cuales se obtuvo 56 cepas de *Escherichia coli* comensal de humanos y animales domésticos las mismas que fueron sometidas a antibiograma, análisis genético (genes cromosómicos y plasmídicos). De las 56 cepas, 44 fueron de humanos y 12 de animales. El patrón de resistencia antibiótica incluyó: tetraciclina (58,9%), cefalotina (55,4%), sulfisoxazol y ampicilina (46,4%) y trimetoprim/sulfametoxazol (39,3%). Se obtuvo 18 transconjugados cuyos replicones fueron FII y FIB en aislados de humanos y FII y X_2 en aislados de animales domésticos. Los replicones más comunes en 8 cepas seleccionadas (de animales y humanos) fueron FII18, FIA6, y FIB1. Mientras que los variantes alélicos de resistencia a antibióticos encontrados en aislados de humanos y animales fueron: *blaTEM-1B, qnrB19, mph(A), sul2 y fosA.*

Palabras clave: *Escherichia coli,* humanos, animales, Transferencia horizontal de genes, elementos genéticos móviles, genes de resistencia, plásmidos.

ABSTRACT

A problem that threatens human health today is the development of multiresistant bacteria or "super-bacteria", which can be found in the gastrointestinal tract of animals and humans as a commensal microbiota. The use of antimicrobials in animal husbandry is difficult to control and leads to an increase in antibiotic resistance. Nowadays it is demonstrated that the interaction between animals and humans leads them to share part of their microbiota and different resistance genes that are passed through the mobile genetic elements (MGE). We investigated the presence of resistance genes found in commensal intestinal microbiota of animals and humans from which 56 strains of commensal *Escherichia coli* were obtained and were subjected to antibiogram and genetic analysis (chromosomal and plasmid genes). From the 56 strains, 44 were from humans and 12 from animals. The antibiotic resistance pattern included: tetracycline (58.9%), cephalothin (55.4%), sulfisoxazole and ampicillin (46.4%) and trimethoprim / sulfamethoxazole (39.3%). We obtained 18 transconjugates whose replicons were FII and FIB in human isolates and FII and X2 in isolates of domestic animals. While the allelic variants of antibiotic resistance genes present in humans and domestic animals were: *blaTEM-1B, qnrB19, mph* (A), *sul2* and *fosA.*

Key words: Escherichia coli, humans, animals, horizontal gene transfer, mobile genetic elements (MGE), resistance genes, plasmids.

CONTENT

ÍNDICE DE TABLAS

ÍNDICE DE FIGURAS

ÍNDICE DE ANEXOS

INTRODUCTION

The Impact of Antibiotic Resistance in Human Medicine

The first WHO global reports on the impact of antibiotic resistance in human medicine reveals that this serious threat is no longer a forecast for the future but a reality in all regions of the world affecting anyone of any age in any country. In the absence of urgent and coordinated actions by many stakeholders, the world is heading for a post-antibiotic era in which common infections and minor injuries that have been treatable for decades will once again be life-threatening (Thomas, 2014).

Antibiotic resistance has an important impact in mortality and costs are estimated by many health authorities and scientists around the world like the Center for Disease Control and Prevention (CDC) who estimated that in United States each year more than two million people have antibiotic-resistant infections. It results in numerous deaths (about 23, 000 people), being the cost of these infections over $55 billion per year (which represent costs for direct healthcare $20 billion and society costs for lost productivity $35 billion). Similarly in Europe the number of deaths was around 25,000 people, and the cost around this health problem was estimated to be over 1.5 billion euros. The bacteria most frequently found in such cases were: *Staphylococcus aureus*, *Streptococcus pneumoniae, Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with the most frequent multidrug-resistant pattern (Prestinaci, *et al.*, 2015).

In Ecuador, there was a report of a Colistin resistance antibiotic gen mcr-1, which was the first clinical isolate of *Escherichia coli* (ST-609) from a male teenager with appendicitis which harboured mcr-1 and *bla*CTX-M-55 genes, the presence of commensal *E. coli* with the gene mcr-1 is an alert to the presence of antibiotic resistance genes in the region, most supported by the idea of been a resistance of the last line of drugs which are available for several treatment in Gram negative bacteria with multi drug resistant patterns. (Ortega-Paredes, Barba, & Zurita, 2016)

The cost of the antibiotic resistance over the economy is difficult to determine, because there are several factors that must be taken to provide a real account like: direct healthcare cost, society cost (deaths and loss of productivity), antibiotics cost (third-line drugs are more expensive), use of specialized equipment for treatment, large periods of hospitalization and treatments that needs isolation (Prestinaci, *et al.*, 2015).

Role of antibiotic resistance in domestic animals

An important factor which threatens human health is the increasing number of resistant strains to antibiotics and the transmission of resistance genes between humans and animal bacteria. The use and misuse of antimicrobial drugs for treatment and animal husbandry is partially responsible for the antibiotic resistance crisis (Ranjbar & Sami, 2017).

Improvement in animal growth with the help of antibiotics was described in 1940s (Moore, *et al.*, 1946) and years later the addition of growth promoting antibiotics (GPAs) became very common (Graham, *et al.*, 2007). In 2007, researchers compared chickens growth with and without antibiotics on their diet, making a summary of the economical effects of removing GPAs from broiler feed (Graham, *et al.*, 2007). The indiscriminate use of antibiotics in animals is due to permissive legislation for antibiotic commercialization, low responsibility of professionals and lack of knowledge of many people raising food animals (Ramírez, 2009). Poultry production is expanding in developed nations with thousands of birds raised in limited spaces and feed with antibiotics to prevent infections or as growth promotors. In this setings it is found a higher frequency of antimicrobial resistance compared with birds who are not exposed to antibiotics (Moser, *et al.*, 2017). Many people are in close contact with domesticated animals (and animal waste) and there is evidence that the microbiota from animals and humans could be shared, creating the opportunity for antibiotic resistance transfer between species (Guard, *et al.*, 2017).

Escherichia coli and the gut microbiota of humans and animals

Most of people think that in a fetus the gut is sterile, however around 2011, Indira Mysorekar and colleagues started questioning this idea; with a study of placental tissues samples collected from women giving birth at Barnes-Jewish Hospital, specimens were visualized under microscope, and found bacteria in nearly one-third of them (Cao, Colin Macones, & Mysorekar, 2016). Latetly after birth bacteria such as Enterobacteriaceae and Bifidobacteriaceae invade the intestine and its composition thereafter depends on the diet (breast feed or formula), environment, microbiota of the other members of the household, etc (Mountzouris, *et al.*, 2002). Commensal bacteria found in the gut belong to two important groups, the anaerobic genera like *Bifidobacterium*, *Clostridium*, *Bacteroides*, *Eubacterium (*the majority) and the aerobic genera like *Escherichia, Enterococcus, Streptococcus, Klebsiella* (O'Hara & Shanahan, 2006).

In a study of microbial diversity of intestinal microbiota in human infants, it is shown that it trends for its nutritional modulation, the evolutionary and phylogenetic relationships between microorganisms, which in turn confers protection against several infections and diseases. There is evidence of the influence of nutrition and the indigenous microbiota over immune response and host defense (Mountzouris, *et al.*, 2002).

Culture-based studies have provided limited knowledge about human gut composition, but nowadays, high throughput sequencing and molecular phylogenetic has created a better and more detailed inventory of human microbiota (Chow, *et al.*, 2010). For instance, culture independent studies allowed to detect changes in the taxonomic composition of the microbiota of preterm infants due to hospitalization, artificial ventilation, nutrition, or antibiotic treatment (Drell, *et al.*, 2014). Gut microbiota from animals could be modified due to diet, age, genetic background, shared microbiota from the mother and also exposure to antibiotics during their life and also there are found *Escherichia coli* in most of the isolates (Mosites, *et al.*, 2017).

Escherichia coli have been divided into four phylogenetic groups: A, B1, B2 and D (Clermont, *et al.*, 2000). *E. coli* is a facultative anaerobic, non-sporulating gram negative rod member of the Enterobacteriaceae family, which grows in the intestine of humans and other warm blooded animals (Conway & Cohen, 2015).

In epidemiologic studies of bacterial infections is recognized that an individual may be colonized with more than one distinct *Escherichia coli* strain at any moment and time (Lautenbach, *et al.*, 2008). Successful colonization of an *E. coli* clone depends primarily on its ability to compete for nutrients in the lumen of the intestine where will be eliminated by the feces and the cycle will begin again this process is called: "The circle of colonization" and "the extra-intestinal survival", this circle occurs equally in commensal and pathogenic *E. coli* (Conway & Cohen, 2015).

In the stomach of humans and animals *E. coli* is exposed to acid, and then it reaches the colon where is thought to multiply logarithmically (Conway & Cohen, 2015). The mucus secreted by the intestine cells of animals and humans brings nutrients to *E. coli* niche; *E. coli* has adapted to use sugars like gluconate and other polysaccharides found in the host; the strains that colonize during infancy, are originated from the contamination with the feces of the mother in the moment of labor or from the manipulations from the staff of the hospital (Tenaillon, *et al.*, 2010). Non-pathogenic *Escherichia coli* normally found in the host's gut is considered commensal bacteria as they are part of the normal intestinal microbiota in both humans and warm-blooded animals; the host and the bacteria benefit from this interaction, in one case protecting against pathogen colonization and on the other case suppling of nutrients necessary to grow and disseminate (Picard, *et al.*, 1999).

Intestinal infection occurs when a virulent clone introduced to the intestine and surpasses the defense barrier formed by commensal bacteria (Picard, *et al.*, 1999). Natural selection is involved in the structure and composition of the microbiota; bacterial density increases or decreases depending on the surrounding environment like stomach, bile or pancreatic acids reaching about an amount estimated of 10^{11} – 10^{12} UFC/ml of bacteria in the colon, 10^{5} – 10^{6} UFC/ml in jejunum/ileum, 10^{3} – 10^{4} UFC/ml in the stomach and duodenum (Tlaskalová-Hogenová, *et al.*, 2011).

Escherichia coli as well as other bacteria have a fast mechanism of replication (division occurs every 30 minutes) producing large populations of Gram negative rods where we could find a mutant cell which is rare and altered by a specific process. During the process of DNA replication alterations on the nucleotide sequence could occur which are called “mutations”; these errors or mutations could benefit, in most cases could be slightly detrimental or in some cases could disrupt the sequence code of a protein causing deleterious effects, loss of competitiveness or even death (Alberts, *et al.*, 2002). *Escherichia coli* genome evolution requires these errors, however some genes coding for optimized proteins are highly conserved which means that they are not easy to be changed or altered, as an example is the 16S ribosomal RNA subunit (Alberts, *et al.*, 2002).

Genetic information of *Escherichia coli* and mechanism of horizontal gene transfer.

Due to its wide spread disemination, *Escherichia coli* is an important bacteria in terms of antibiotic resistance (Carlet, 2012). Genomic information in bacteria can change or innovate which are: mutation, duplication of genes, segment shuffling, horizontal transfer (Alberts, *et al.*, 2002).

The mechanisms for horizontal acquisition of resistance are: conjugation, transduction and natural transformation (Tenaillon, *et al.*, 2010). During conjugation, a donor bacterium connects a transient pilus to a recipient and transfers plasmids, integrative and conjugative elements, or also chromosomal DNA (in high recombination frequency strains) (Konraimann & Wagner, 2014). Conjugation requires cell to cell contact where the ssDNA is transported from a donor to a recipient cell and depends on a machinery of transfer called type IV secretion systems (T4SS) and additional proteins which mobilized plasmids or genomic islands; in *E. coli* ssDNA which is generated in the donor cell starts to separate from the dsDNA strand at the termed site *oriT* also

called origin of transfer (Konraimann & Wagner, 2014). At this stage, DNA transfer complex (Dtr) is composed by a relaxase/helicase and auxiliary proteins and is usually docked and interacts with the proteins of the T4SS system. This T4SS complex has ATPases, translocase proteins, core proteins and pilus proteins and adhesins. To start with the translocation of the ssDNA a mating between two bacteria has to happen, which includes initial contact with the pilus, F conjugation with the retraction of the pilus and finally the formation of contact zones. For a successful gene transfer, *tra* genes which are composed by Dtr and T4SS proteins are in charge of turning on the system at a specific moment, positive feedback which involves de Dtr complex and the assembly of T4SS apparatus. And finally the stimuli will turn off, when the *tra* genes are switched off as the negative feedback (Konraimann & Wagner, 2014). Some plasmids encode mechanisms which destroy the progeny which have not received the plasmid (toxin-anti-toxin systems). Plasmids have the ability to retain resistance genes and transfer them to different strains (Carattoli A., 2009).

In transduction, phages (or bacterial viruses) are the carriers of transducing DNA between bacteria. In generalized transduction, phages can take bacterial DNA incorporating accidentally random pieces of the bacteria genome inside the phage. In specialized transduction the phage takes up accidentally the DNA of the bacteria which is near the integration site (Carlet, 2012).

Transformation is as mechanism in which foreign DNA from an external source is introduced in other bacteria making the conversion of one genotype into another; it was first discovered in 1928 by Frederik Griffith in *Streptococcus pneumoniae* giving the elucidation of molecular nature of the genes. The principle of transformation starts with an exogenous DNA which will be incorporated into another bacterial chromosome by a mechanism called “breakage-and-insertion” similar to Hfr × F− crosses of conjugation, but with the difference that in conjugation DNA is transferred through close contact, while, in transformation, isolated pieces of exogenous DNA are taken up by a cell not in close contact necessary. Transformation with plasmids is important because bacteria are used for both storing and replicating plasmids (Griffiths, *et al.*, 2000).

The transposable elements or transposons which are jumping short sections of DNA that move within the genome creating a cut and paste mechanism in which they could insert new and foreign DNA into virus of plasmids (Muñoz López & García Pérez, 2010).

Antibiotics and resistance genes

For the past years, antibiotics have been used for the treatment or prevention of infections in humans and animals. The first antimicrobial discovered by Fleming in 1929 was penicillin an

active β-lactam which binds to the penicillin binding proteins or PBPs to inhibit the synthesis of peptidoglycan. β-Lactams as bactericide presents low toxicity for humans or animals and slow time action; despite of the fact, is the treatment of choice in many infections because this antibiotic interferes with the cross link structure of peptidoglycan avoiding transpeptidation which make the lysis of the cytoplasm or increase the osmotic pressure (Suárez & Gudiol, 2009).

Other antibiotics are sulfonamides which inhibit the synthesis of folic acid; aminoglycosides which inhibit protein synthesis; quinolones and rifampicin which interact with the synthesis of DNA and RNA respectively, etc (Van Hoek, *et al*., 2011). Sulfonamides are synthetic drugs which in combination with trimethoprim make this drug synergetic to bactericidal effects, inhibiting 2 enzymes dihydropteroate synthase and dihydrofolate reductase respectivelly (Van Hoek, *et al*., 2011).

Aminoglycosides are bactericide antibiotics produced by soil Actinobacteria such as *Streptomyces* and *Micromonospora;* they are very active against Enterobacteriaceae, with a rapid killing action (Vakulenko & Mobashery, 2003). Streptomycin was isolated from *Streptomyces griseus*, is an aminocyclitol aminoglycoside which inhibits bacterial growth by intercating with the 30S small ribosomal subunit resulting in bacterial dead (Kikuvi, *et al.*, 2007).

Chloramphenicol was obtained from *Streptomyces venezuelae*, is a potent inhibitor of the synthesis of proteins with affinity to peptidyltransferasa of 50S subunit of the 70S ribosomal RNA and prevents the elongation of the peptide's chain. Quinolones and flouroquinolones inhibit DNA gyrases (GyrA and GyrB subunits), and the topoisomerase IV (encoded by the genes *parC* and *parE*) (Kikuvi, *et al.*, 2007).

Fosfomycin was isolated from different *Streptomyces* species, is a broad spectrum antibiotic for gram negative and positive bacteria; derived from phosphonic acid which inhibit the cell wall synthesis and permeability of bacteria (Alrowais, *et al.*, 2015). Tetracycline was isolated from *Streptomyces aureofaciens,* is a broad spectrum antibiotic which inhibit microbial growth by interacting with the ribosomes interfering with the synthesis of the proteins, also depends on efflux pumps or enzime inactivation (Chopra & Roberts, 2001).

Intrinsic antibiotic resistance is caused by physicochemical characteristics in the bacteria which are no subject natural selection; these mechanisms include: exclusion of drug molecules by constraints of the envelope of the bacteria, generally by the outer membrane, the lipopolysaccharide and porin constituents. For instance, glycopeptides are large molecules which cannot penetrate the outer membrane of Gram negatives or do not affect *Mycoplasma* which does not have peptidoglycan (Brown & Wright, 2016); *Enterococci* species are intrinsically resistant to cephalosporins because the drug binds to peptidoglycan binding protein PBP that this

bacterium lacks, *Klebsiella* species intrinsically resist to ampicillin because of its production of beta-lactamases that could destroy the drug before it reaches the PBP targets (Wolf, 2017).

Bacterial mechanisms of resistance are caused by lack of penetration, efflux pumps, enzyme inactivation by antibiotic degradation or antibiotic modification (acetylation, adenylation, glucosylation and phosphorylation), target modification (i.e. methylation of ribosomal RNA), target mutations or deletion, target overproduction by increased transcription or multiplication of the genes and drug sequestration by specific biding proteins (Rhodes & Schweizer, 2016).

In 1940, the first bacterial enzyme that could destroy penicillin was discovered by Abraham and Chain; the enzyme was AmpC β-lactamase (gene bla_{CMY}) found in *E. coli*. Currrently the most common mechanism of antimicrobial resistance to β-lactam antibiotics are β-lactamases (bla_{TEM-1}, bla_{TEM-2}, and bla_{SHV-1}). Evolution due to antibiotic pressure has resulted in extended spectrum β-lactamases (ESBLs) which can hydrolyze a wide range for β-lactam antibiotics (Suárez & Gudiol, 2009) (Van Hoek, *et al.*, 2011).

Some aminoglycoside's resistance genes cause ribosomal alterations like: *armA (*aminoglycoside resistance methylase) gene, is a "novel plasmid-borne 16S rRNA methyltransferase" that confers high-level of resistance to 4,6-disubstituted deoxystreptamines and fortimicin including the antibiotics: gentamicin, arbekacin, amikacin, kanamycin and tobramycin, and could be transferred between *E. coli* by conjugation (González-Zorn, *et al.*, 2005); initially found in *Klebsiella pneumoniae,* detected in many isolated bacteria like: *E. coli, Citrobacter freundii, Enterobacter cloacae, Salmonella enterica, Shigella flexneri* associated with bla(CTX-M-3) on the plasmid IncL/M which confers the extended-spectrum beta-lactamase resistance (Galimand, *et al.*, 2005), armA gene has spread in bacteria of diverse origin, present in human and animal isolates within a novel transposon composite and encodes a protein similar to 16S rRNA methylases found in actinomycetes which produce aminoglycosides (González-Zorn, *et al.*, 2005). other genes modify the aminoglycosides and they are classified according to the mechanism of antibiotic modification, the enzymes are classified in: ACC Acetyltransferases (AAC(1), AAC(2'), AAC(3) and AAC(6')); ANT Adenyltransferases (ANT(2"), ANT(3"), ANT(4'), ANT(6), and ANT(9)); and APH Phosphotransferases (APH(2"), APH(3'), APH(3"), APH(4), APH(6), APH(7"), and APH(9)) each one with their specific gene for example *aac(1)* (Vakulenko & Mobashery, 2003).

Mutations in the *rpsL* gene which encode a polypeptide S12 generate resistance to streptomycin, Enterobacteriacea as a result of a transposition with the transposon Tn5393 found in plasmids (Pezzella, *et al.*, 2004) (Springer, *et al.*, 2001).

Resistance to chloramphenicol occurs also by modification of the antibiotic molecule; an acetyltransferase or CATs which is coded by the genes *catA* and *catB*. (Kikuvi, *et al.*, 2007) Other genes involved in chloramphenicol resistance encode efflux pumps systems, such as *cmlA* and *floR* (Kikuvi, *et al.*, 2007) (Van Hoek, *et al.*, 2011).

Resistance to quinolones occurs by mutation in these chromosomal genes encoding the A and B subunits of the protein targets: gyrA, gyrB, parC and parE (Fàbrega, *et al.*, 2009). Plasmid genes also confer parcial resistance to quinolones: *qnrA* , *qnrB* , *qnrC* , *qnrD* , and *qnrS,* which encodes a pentapeptide and blocks the action of quinolones over DNA gyrase and topoisomerase IV (Jacoby, 2005) (Van Hoek, *et al.*, 2011).

Resistance to sulfonamides could be coded by genes *sul1, sul2, sul3;* they are carried in class 1 integrons which are carried by plasmids (Sköld, 2000) (Van Hoek, *et al.*, 2011). Resistance genes for trimethoprim are *dfrA*,*dfrB*, *dfrK* and *dfrA27* and the newest reported DHFR gene which inhibits the enzyme dihydrofolate reductase among Gram negatives (Huovinen, *et al.*, 1995) (Van Hoek, *et al.*, 2011).

Resistance to Tetracycline, is coded by twenty nine different tetracycline resistance genes or called "*tet"* genes and three oxytetracycline resistance or "*otr"* genes with no inherent difference between them. All the *tet* efflux genes found in Gram positive and Gram negative bacteria, code for membrane associated proteins which export tetracycline from the cell, and reduces the intracellular drug concentration protecting the ribosomes inside the cell; some of these genes are *tet*(P), *tetA*(P) and *otr*(C) (Chopra & Roberts, 2001). Other *tet* genes like *tet*(M), *tet*(O), *tetP*(B), *tet*(Q), *tet*(S), *tet*(T), *tet*(W) and *otr*(A), which protects the ribosome of the bacterial from binding to the antibiotic tetracycline. The presence of *tet* and *otr* genes with efflux or ribosomal protection mechanisms of resistance is similar with the hypothesis of lateral gene transfer from the tetracycline producing *Streptomycetes* to other bacteria (Chopra & Roberts, 2001).

Fosfomycin resistance mechanisms described in *Escherichia coli* include target modification of the gene *mur*A, expression of enzymes which degradate the antibiotic, reduce uptake, and rescue of the biogenesis pathway of "UDP-MurNAc" which is a pentapeptide of l-alanine ligase and alanyl-tRNA synthetase genes overexpressed in Escherichia coli (Bouhss, *et al.*, 2001). *FosA* a glutathione S-transferase causes enzymatic resistance to fosfomycin and several *Escherichia coli* lineages carrying *FosA* also produce ESBLs. Sequenced genes which are encode by glutathione S-transferase variants are *fosA*, *fosA2*, *fosA3*, *fosA4*, *fosA5*, and *fosC2* (Benzerara, *et al.*, 2017) (Alrowais*, et al.*, 2015).

OBJECTIVE

- Analyze the diversity of antimicrobial resistance genes found in *Escherichia coli* strains from the population of children and animal of Yaruqui-Centro, Quito Ecuador.

MATERIALS AND METHODS

Time and study location

Samples were collected from the community of Yaruqui Centro in Quito Ecuador during February to August 2015, fresh fecal samples from children from 0 to 5 years old (with informed consent from parents). Domestic animal fecal samples (approved by the Institute for Animal Care and Use Committee at the George Washington University (IACUC#A296)) were collected from the floor. Samples were transported to the USFQ Microbiology Laboratory immediately in a cooler at 4ºC; as soon as they arrived samples were cultured and stored frozen at -80ºC in 1.5 ml aliquots (Sarzosa, 2016).

Microbiological Identification and isolation.

Fecal samples (n=56) were cultured in MacConkey Agar, after 24h incubation at 37ºC were selected lactose fermenting colonies. Antibiotic susceptibility test was carried out using Mueller Hinton for 24h incubation at 37ºC with the Kirby Bauer method following CLSI instructions (described in the next step). At the same time the susceptibility test was done, a biochemical reaction in different culture media for identification of bacteria was performed, to help identify by genera and specie the bacteria found in fecal samples: *Escherichia coli*, lately confirmed by DNA sequencing and MLST.

Antibiotic Susceptibility Test.

The antibiotic susceptibility of *E. coli* was measured using disk diffusion method assessed by Kirby-Bauer method according to Clinical and Laboratory Standards Institute (CLSI 2016). 12 different antibiotic disks vials were used: Amoxicillin-Clavulanic acid [AMC], Ampicillin [AM], Streptomycin [S], Gentamicin [CN], Trimethoprim-sulfamethoxazole [SXT], Ciprofloxacin [CIP], Chloramphenicol [C], Cephalothin [KF], Cefotaxime [CTX], Tetracycline [TE], Sulfisoxazole [G] and Imipenem [IPM]. The interpretive criteria or cut-off values to determine susceptible or resistance strains were determined by the measurement of diameters of the halos and the interpretation was taken from CLSI 2016 guidelines (CLSI, 2016).

Mutant Selection

For the selection of transconjugants, we selected a mutant strain from *E. coli* One Shot™ TOP10 (lac-, Strep^R) Invitrogen (Carlsbad, USA). One Shot™ TOP10 *E. coli* was inoculated in 100 ml of Brain Heart Infusion BHI liquid media and incubated at 37°C for 24h. After 24 hours another 100 mls of BHI liquid media containing 50 ug/ml of Nalidixic Acid [NA] to reach a final concentration of 25 ug/ml was added to the culture and incubated with no bacteria at 37ºC for 24h. After incubation, the volume of BHI with 50 ug/ml of Nalidixic Acid was added to the culture media with One Shot™ TOP10 *E. coli.* The mixture was incubated at 37°C for additional 24h. Later, 200 µL of the mixture was transferred to a MacConkey Lactose Agar plate with 25 ug/ml of Nalidixic Acid, and incubated at 37°C for 24h. A colony was selected and named Ec10Na^R.

The same procedure was repeated to select another mutant strain but Rifampicin resistant with One Shot™ TOP10 *E. coli*. A second mutant was selected and named: Ec10Ra^R (Segovia Limaico, 2008).

Conjugation.

Eighteen strains (16 human isolates and 2 animal isolates) were selected for their similar resistance pattern and were subjected to conjugation with One shot TOP 10 *Escherichia coli*, or any of the mutants Ec10Na^R or Ec10Ra^R, depending to the antibiotics that they were susceptible or resistant. The process of conjugation in which a donor cell (Yaruquí *E. coli* strains) transfer resistance plasmids to a recipient cell (One shot TOP 10 *Escherichia coli*, Ec10Na^R, Ec10Ra^R) was done by mixing in a proportion of 1:1 of a 10 mL tube of Tryptic Soy Broth liquid medium with a donor inoculated bacteria from Yaruquí strains with 10 mL tube of Tryptic Soy Broth liquid medium with a recipient inoculated bacteria (One shot TOP 10 *Escherichia coli*, Ec10Na^R, Ec10Ra^R) both previously incubated at 37ºC for 24h. Transconjugants were selected transferring 200 µL of the mixture to a MacConkey agar plate which had a concentration of antibiotics like: streptomycin, tetracycline, nalidixic acid, rifampicin, cephalothin, depending which recipient we chose. Antimicrobial susceptibility of transconjugants was confirmed in Mueller Hinton using disk diffusion method by Kirby-Bauer and according Clinical and Laboratory Standards Institute (CLSI 2016).

Replicon typing analysis of the transconjugated.

The 18 selected strains that were conjugated resulted in 18 transconjugated strains that were used to analyze by the replicon using PBRT kit DIATHEVA (Fano PU, Italy) (https://www.diatheva.com). Bateria were grown in MacConkey media to confirm the Lactose negative pattern. And 3 to 5 strains were selected for DNA extraction using DNAzole ThermoFisher Scientific (Carlsbad, USA). 2 µL of the extracted DNA or positive controls and negative controls (*E. coli* K12 with no antibiotic resistance) were taken to the mix reaction. Replicons were amplified using of 28 primers, each of them represent an especific amplicon length (bp), performed by 8 multiplex. For each PCR master mix, there were added 23,8 µL of the mix solution plus 0,2 µL of DNA polymerase Invitrogen (Carlsbad, USA); each PCR mix solution of 600 µL included specific levels of primers, dNTPs, Mg^{+2}, PCR water non specified by the manufacturer because it was a pre-fabricated kit. The PCR parameters were: 1 cycle of 95°C for 10min; 25-30 cycles of: 95°C for 60sec, 60°C for 30sec, 72°C for 60sec; and last 1 cycle of 72°C for 5min. It was cooled down to 4°C. The final volume of the reaction was 26 µL.

We detected 25 replicons: HI1, HI2, I1, I2, X1, X2, L/M, N, FIA, FIB, FIC, FII, FIIS, FIIK, W, Y, P, A/C, T, K, U, R, B/O, HIB-M and FIB-M, which are the most representative of a major plasmid incompatibility groups and replicase genes which were identified on resistance plasmids circulating among Enterobacteriaceae; positive controls for each multiplex were also included in every reaction. PCR reaction was perfomed following specifications described by (Carattoli, *et al.*, 2005).

Later, the amplified products of the multiplex (5 µL) were separated by electrophoresis with agarose gel 2.5% using ethidium bromide and a DNA standar specific ladder of low range (100-1000 bp). The PCR products obtained were run in the gel as long as all amplicons had generated (aproximately 35 min), visualized under the transilluminator of UV.

DNA extraction and whole genome sequencing.

A subset of 8 strains isolated from Yaruquí in 2005 were subjected to DNA extraction using DNeasy Blood & Tissue of the QIAGEN (Texas, USA) and plasmid DNA was obtained from corresponding transconjugants using Plasmid QIAprep Spin Miniprep QIAGEN (Texas, USA) in both cases we followed instructions from the manufacturer. The chromosomic and plasmid DNA were sent to University of Minnesota, Saint Paul Office: 1971 Commonwealth Ave., 205 Veterinary Science Saint Paul, MN 55108, USA. DNA sequences were obtained using Illumina miSeq. Paired reads were assembled using Velvet 1.2.1, genes were annotated using Patric 3.4.12, MLST of total genome sequences bacteria was performed in the Center for Genomic

Epidemiology server according to: (Larsen, *et al.*, 2012) PlasmidFinder and pMLST was performed in the Center for Genomic Epidemiology server according to: (Carattoli, *et al.*, 2014) Antimicrobial Resistance genes finder was performed in the Center of Genomic Epidemiology server based on the parameter of ResFinder 3.0 which identifies acquired antimicrobial resistance genes but also chromosomal mutations in total or partial sequenced isolates of bacteria (Zankari, *et al.*, 2012).

Data Analysis.

Chi square test was performed using IBM SPSS Statistic 20. For tables and graphs Microsoft Office Excel 2010 (Redmond, WA, USA)

RESULTS

Participants.

From the 56 strains that were obtained 44 (78,6%) of them corresponded to bacteria isolated from human feces (children), while the remaining 12 (21,4%) corresponded to bacterial isolated from domestic animal feces; 12 of the isolated strains from domestic animal feces of the community of Yaruquí: 7 (12%) belonged to *Canis lupus familiaris* (dog), 2 (4%) to *Gallus gallus domesticus* (rooster), 1 (2%) to *Felis silvestrus catus* (cat), 1 (2%) to *Ovis orientalis aries* (sheep) and the last one corresponded to 1 (2%) *Anser anser domesticus* (Goose).

Antibiotic resistance profiles

The 56 strains showed resistance to: Amoxicillin - Clavulanic acid [AMC], 21 (37,5%), Ampicillin [AM], 26 (46,4%); Streptomycin [S], 18 (32,1%); Gentamicin [CN], 7 (12,5%); Trimethoprim-sulfamethoxazole [SXT], 22 (39,3%); Ciprofloxacin [CIP], 3 (5,4%); Chloramphenicol [C], 5 (8,9%); Cephalothin [KF], 31 (55,4%); Cefotaxime [CTX], 7 (12,5%); Tetracycline [TE], 33 (58,9%) and Sulfisoxazole [G], 26 (46,4%).

Eighteen isolates had similar pattern of resistance: ampicillin, trimethoprim-sulfamethoxazole, cephalotin, tetracycline and sulfisoxazole, 16 of them corresponded to isolates from children and 2 corresponded to isolates from domestic animals (1 dog and 1 goose).

The transconjugates from the 18 strains showed a resistance pattern to ampicillin, trimethoprim-sulfamethoxazole, cephalotin and tetracycline and in fewer cases resistance to gentamicin and cefotaxime. (Appendix 1)

The rate of resistance in *E. coli* from human and domestic animals was different for all antibiotics: Resistance to amoxicillin - clavulanic acid was 38,6% for human and 33,3% for animals isolates; streptomycin 36,4% in human and 16,7% in animals isolates; gentamicin 13,6% for humans and 8,3% in animals isolates; trimethoprim-sulfamethoxazole 40,9% for humans and 33,3% for animals isolates; ciprofloxacin 6,8% for humans and there were no percentage of resistance in animals isolates; chloramphenicol 6,8% for humans and higher percentage was showed in animals isolates with 16,7%; cephalotin 52,3% for humans and 66,7% in animal isolates; cefotaxime 13,6% for humans and a lower percentage in animals isolates with 8,3%; tetracycline 61,4% for humans and 50,0% for animal isolates; sulfisoxazole 50,0% for humans and 33,3%

for animals isolates. The difference in the resistance patterns found in human and animal strains had no statistical significance ($p>0.05$) (Table 2)

Isolates obtained from children showed more resistance to Tetracycline (61,4%) followed by Cephalothin (52,3%) and Ampicillin (52,3%). The highest percentages of resistance in isolates obtained from animals were Cephalothin (66,7%) and Tetracycline (50,0%). The most frequent pattern of antibiotic resistance profile is: resistance to tetracycline, sulfisoxazole, ampicillin, streptomycin, trimethoprim-sulfamethoxazole and cephalothin; the majority of these isolates belonged to humans. (Table 2)

The dog and the goose cohabitated on the same household, shared some resistance pattern to: amoxicillin / clavulanic acid, ampicillin, streptomycin, cephalothin, trimethoprim sulfamethoxazole, tetracycline and sulfisoxazole, except in the antibiotic chloramphenicol, gentamicin and cefotaxime which was presented only in the dog. The fact that the dog and the goose belonged to the same household has no statistical significance in contrast to the shared antibiotic resistance genes.

Genotyping

Based on the mutilocus sequence typing (MLST) from eight of the fifty six samples, with the combination of the genes: *adk, fumC, gyrB, icd, mdh, purA* and *recA*, we conclude that each of the eight sequenced samples have a different sequence type (ST): 941, 1485, Unknown1, Unknown2, 117, 5019, 120 and Unknown3.

Plasmid Genotyping

Plasmid replicons from transconjugants showed the same type of antibiotic resistance profiles as the donor strains; replicons were: HI1, I1γ, I2, FIIK, FII, X3, X2, FIB, P, I1α, N, FIIS, FIC, K, FIA, R (Table 4). The most common replicons were FII found in all of the sequenced samples except one, and I1γ was found in most of the samples but two (Table 3). Some replicons found only in human strains: FIIK, FIB, P, FIA, R; others only in domestic animal strains: N. In both types of animals and humans transconjugated strains the replicons found were: I1α, K, X2, FII, HI1, X3, I1γ, FIIS and FIC. (Fig 1) Results of pMLST of donor strains showed that FII18 was present in the majority of the samples except 2. These pMLSTs were found only in human strains: FII1, FIA13, FIB31, FIA5, FII2, FIB10, FIB26, and FIB20. In both animals and human strains it was found: pMLSTs: FII18, FIB1, and FIA6. (Fig 2) The results of pMLST showed that one of the plasmids FII18 is in the majority of the samples except two of them. (Table 4) The most prevalent

plasmids FIB1 and FIA6 were found in four of eight sequenced samples. (Appendix 1) We also found plasmids in unique isolates obtained in the pMLST which are: FII11, FIA13, FIB31, FIA5, FIB10, FIB26, and FIB20. (Appendix 1)

Analysis of Antibiotic resistance genes

The strains that had a similar resistance pattern like: ampicillin, streptomycin, trimethoprim/sulfamethoxazole, cephalothin, tetracyclin and sulfisoxazole belonged to 2 isolates from humans that live in different houses of Yaruqui´s community. These strains shared some plasmids sequenced from WGS which type is IncFIB (AP001918), and resistant genes like *bla-TEM*-1B*, qnrB19, strA, strB, sul2*. (Appendix 1)

Most isolates shared resistance gene *blaTEM*-1B which causes resistance to beta lactams,

The next most shared genes are: *strA*, *strB* which causes resistance to streptomycin. (Appendix 1) *Sul 2* gene is in five of eight strains and *Sul 1* associated with resistance to Sulfisoxazole and sulfamethoxazole. (Appendix 1)

The gene *cepA* which belongs to a human and present a resistance pattern for Cefotaxime that confirm the ability of this gene to give resistance to cephalosporin up to fourth generation because of the presence of the enzyme Extended Spectrum Beta Lactamase (ESBL). (Table 5)

A phylogenetic tree was done by Mega (Maximum Likelihood) with the most repeated genes and showed that all of the compared sequences were identical or similar without nucleic bases that differ from each other. (Figure 4-9)

Plasmid and Antibiotic resistance profile

Not all the strains that had been sequenced have the same plasmids, the plasmid which is the most repeated identified by WGS are: IncFIB(AP001918) in all of the strains except one sample from a human isolate, followed by IncFII present in four samples from eight of the total. (Appendix 1)

Different isolates IncFIB(AP001918), IncFII, IncI2, IncFIC(FII), IncQ1, IncFIB(K), IncFII(Yp), IncR, ColRNAI, Col(MP18), Col156, IncFII(pHN7A8), IncI1, IncN, IncFIA, IncFII(pRSB107), IncX4, Col(MG828), Col(BS512) seemed associated with different Antibiotic Resistance patterns (Appendix 1).

The transconjugates of the isolates carrying these plasmids showed different and uncomplete resistance this could explain that there is not only one plasmid which is transmitted.

Antibiotic resistance genes found in humans and animals

The antibiotic resistance genes found only in human isolates were: *dfr*A8, *dfr*A17, *dfr*A18, *str*A, *tet*(B), *dfr*A7, *dfr*A14, *cep*A, *mph*(A), *tet*(Q), *aac*(3)-Iid, *bla*CTX-M-3, aadA5, the antibiotic resistance genes found only in animals were blaACT-14, aadA2, *aac*(3)-Via, *aad*A1, *bla*CTX-M-55, *cm*lA1, *dfr*A1, *flo*R, *sul*3. And antibiotic resistance genes found in both were *bla*TEM-1B, *qnr*B19, *str*B, *sul*2, *tet*(A), *fos*A, *sul*1. (Table 5) (Fig 3) Between the results of antibiotic resistance in humans and animals from Yaruquí Centro there was no statistical significance. (p>0.05). In the replicon typing assay, a statistical significance was found in the replicon "N" with a p value of 0.004, only present in animals. Also a statistical significance was found in the presence of the gene *fosA* with a *P* value of 0.04 present in 1 human and the two studied animals.

DISCUSSION

In this study we atempted to determine the potential transfer of antibiotic resistance genes from domestic animals to humans. Based on the mutilocus sequence typing (MLST) we conclude that none of the individuals sampled in this study was colonized by the same antibiotic resistance *E. coli* clone (Appendix 1). The scientific literature indicate that there is a potential spread of antibiotic resistant clones between humans and animals, and this could happen because of direct contact with animals or ingestion of contaminated food (Alonso, *et al.*, 2017). The uncontrolled use of antibiotics in poultry and livestock production, and animal's fecal management systems as well as close contact with animal may favor the selection of antimicrobial-resistant bacteria and transmission from domestic animals to humans (Alonso, *et al.*, 2017). Additionally, international livestock and derived meat trade is leading to an emergence in the dissemination of resistant strains and genetic determinants. Resistance to antimicrobial families is co-selected and disseminated not only by clonal spread but also horizontally via plasmids carrying genes (Alonso, *et al.*, 2017).

The presence of these allelic variants of antibiotic resistance resistance genes *bla*TEM-1B, *qnr*B19, *str*B, *sul*2, *tet*(A), *fos*A, *sul*1 in *E. coli* from domestic animals and humans suggests potential migration of these genes between domestic animals and humans. The antibiotic resistance genes found only in human isolates were: *dfr*A8, *dfr*A17, *dfr*A18, *str*A, *tet*(B), *dfr*A7, *dfr*A14, *cep*A, *mph*(A), *tet*(Q), *aac*(3)-Iid, *bla*CTX-M-3, *aad*A5. The antibiotic resistance genes found only in animals were *bla*ACT-14, *aad*A2, *aac*(3)-Via, *aad*A1, *bla*CTX-M-55, *cml*A1, *dfr*A1, *flo*R, *sul*3. There were no statistically significant differences in resistance genes or phenotypic resistrance between isolates from domestic animals and humans. Our results are in agreement with previous reports showing evidence of horizontal gene transfer between bacteria in domestic animals and bacteria in humans (Sheppard, *et al*., 2016).

Replicon typing analysis and plasmid sequencing suggested that several distinct plasmids carrying different assortment of replicons and antibiotic resistance genes circulate in this community and the most common replicons found in bacteria from domestic animals and humans were I1α, K, X2, FII, HI1, X3, I1γ, FIIS and FIC. The results of pMLST present in animals and humans were: FII18, FIB1, and FIA6, which could also suggest migration of plasmid by horizontal transfer between human and domestic animal strains and vice versa. The most prevalent replicon which was able to conjugate was FII with 87.5% and FIB with 62.5%. While the most prevalent replicon in bacteria from domestic animals was X_2 and also FII

representing the 50% of the animal cases. Also no statistically significant (*p* value >0.05) showed association of replicons with either domestic animals and humans. The replicon "N" was found only in *E. coli* from domestic animals, but these observations were not statistically significant (*p*=0.54), however this lack of statistic association may be due to low number of animal isolates. The presence of similar replicons in human and animal bacteria suggests the transmition of plasmids between species.

E. coli from chickens in Colombia showed various incompatibility groups : IncI1, IncK, IncF, IncHI2, IncA/C and IncB/O and harboured ESBL/AmpC genes. However, they also showed association of blaCMY-2 and blaSHV-12 with IncI1 plasmids (Castellanos, *et al.,* 2017). The features associated to replicon typing, plasmids, STs and its interactions with *E. coli* hosts are of further interest to assess the factors influencing the successful spread of resistance genes between domestic animals and humans. In a minireview by Carattoli in 2009 showed that in Perú the presence of the enzyme CTX-M2 is associated with the replicon A/C, FVII, NT, found in *E. coli* isolates from human sources, also the enzyme CTX-M-14 with the replicon I1 found in *E. coli* and other enzymes like CTX-M-15 with the most common replicons FII, FIA, FIB. The gene CTX-M-24 with the replicon I1 and CTX-M-56 with the replicon A/C (Carattoli A., 2009). Interestingly, CTX-M-14 gene which is a member of the linage of ESBLs a CTX-M-type β-lactamases had exhibited a dramatic dissemination in the past decade and with a difference in a sinlge nucleotide mutation is similar to CTX-M-2 found also in Bolivia which suggest an evolution of resistance in local regions of Latin America (Pallecchi , *et al.*, 2007). Also Carattoli supported the evidence of the rapid dissemination of genetically unrelated *E. coli* which produced CTX-M-14, with the blaCTX-M-14 gene related with a common plasmid from the IncK group observed in human *E. coli* isolates found in Spain and France, suggesting an epidemic diffusion in Europe (Carattoli A. , *et al.*, 2009).

As a conclusion we can say that horizontal transfer of antibiotic resistance genes between bacteria in domestic animals and bacteria in humans is probably occurring, however crosscolonization of antibiotic resistant *E. coli* strains is probably less common (in the community of Yaruquí).

Most of the antibiotic resistance genes were transferred by conjugation (*in vitro* experiments) which may suggest that conjugation is an important mechanism of horizontal transfer of antibitic resistant genes.In most of the cases the resistance antibiotica fenotype pattern coincide with the gene present in the bacteria genotype.

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TABLES AND FIGURES

Table 1. Antibiotic Resistance prevalence in the population

Origin	*Number of samples*	*Number of Isolates Resistant to Antibiotics (%)*											
		AMC	**AM**	**S**	**CN**	**SXT**	**CIP**	**C**	**CF**	**CTX**	**TE**	**G**	**IPM**
Human	44	17 (38,6)	23 (52,3)	16 (36,4)	6 (13,6)	18 (40,9)	3 (6,8)	3 (6,8)	23 (52,3)	6 (13,6)	27 (61,4)	22 (50,0)	0 (0)
Domestic animals	12	4 (33,3)	3 (25,0)	2 (16,7)	1 (8,3)	4 (33,3)	0 (0)	2 (16,7)	8 (66,7)	1 (8,3)	6 (50,0)	4 (33,3)	0 (0)
Total of Samples	56	21 (37,5)	26 (46,4)	18 (32,1)	7 (12,5)	22 (39,3)	3 (5,4)	5 (8,9)	31 (55,4)	7 (12,5)	33 (58,9)	26 (46,4)	0 (0)

Table 2. Antibiotic resistance in humans and animals from Yaruqui Centro

Antibiotic Resistance	*Homo sapiens N (%)*	*Animal N (%)*	*P value. X2*
Amoxicillin/clavulanic acid			
Resistance	17 (38,6)	4 (33,3)	0,74
Ampicilin			
Resistance	23 (52,3)	3 (25)	0,09
Streptomycin			
Resistance	16 (36,4)	2 (16,7)	0,2
Gentamicin			
Resistance	6 (13,6)	1 (8,3)	0,62
Trimethoprim/sulfamethoxazole			
Resistance	18 (40,9)	4 (33,3)	0,6
Ciprofloxacin			
Resistance	3 (6,8)	0	0,35
Chloramphenicol			
Resistance	3 (6,8)	2 (16,7)	0,29
Cephalothin			
Resistance	23 (52,3)	8 (66,7)	0,37
Cefotaxime			
Resistance	6 (13,6)	1 (8,3)	0,62
Tetracycline			
Resistance	27 (61,4)	6 (50)	0,47
Sulfisoxazole			
Resistance	22 (50)	4 (33,3)	0,31
Imipenem			
Resistance	0	0	0

Table 3. Replicon typing of 18 transconjugants, 16 from Humans and 2 from Animals

Replicon typing	*Homo sapiens N (%)*	*Animal N (%)*	*P value. X2*
HI1			
Present	1 (6.2)	1 (50.0)	0.06
I1α			
Present	1 (6.2)	1 (50.0)	0.06
I2			
Present	1 (6.2)	0	0.72
N			
Present	0	1 (50.0)	**0.004**
X3			
Present	2 (12.5)	1 (50.0)	0.18
I1γ			
Present	8 (50.0)	1 (50.0)	1.00
FIIS			
Present	8 (50.0)	1 (50.0)	1.00
X2			
Present	8 (50.0)	2 (100.0)	0.18
FIC			
Present	2 (12.5)	1 (50.0)	0.18
K			
Present	3 (18.8)	1 (50.0)	0.32
FII			
Present	14 (87.5)	2 (100)	0.60
FIB			
Present	10 (62.5)	0	0.09
P			
Present	4 (25.0)	0	0.42
FIIK			
Present	2 (12.5)	0	0.60
FIA			
Present	3 (18.8)	0	0.50
R			
Present	2 (12.5)	0	0.60

Table 4. pMLST of the sequenced strains

pMLST	Homo sapiens N (%)	Animal N (%)	P value. X2
FII11			
Present	1 (16.7)	0	0.54
FIA13			
Present	1 (16.7)	0	0.54
FIB31			
Present	1 (16.7)	0	0.54
FII18			
Present	4 (66.7)	2 (100)	0.35
FIA5			
Present	1 (16.7)	0	0.54
FIB1			
Present	2 (33.3)	2 (100)	0.1
FII2			
Present	1 (16.7)	0	0.54
FIA6			
Present	3 (50.0)	1 (50.0)	1.00
FIB10			
Present	1 (16.7)	0	0.54
FIB20			
Present	1 (16.7)	0	0.54
FIB26			
Present	1 (16.7)	0	0.54

Table 5. Resistance genes in humans and animals

Resistance genes	*Homo sapiens N (%)*	*Animal N (%)*	*P value. X2*
blaTEM-1B			
Present	5 (83.3)	2 (100)	0.54
blaACT-14			
Present	0	1 (50.0)	0.06
blaCTX-M-55			
Present	0	1 (50.0)	0.06
blaCTX-M-3			
Present	1 (16.7)	0	0.54
dfrA1			
Present	0	1 (50.0)	0.06
dfrA7			
Present	2 (33.3)	0	0.35
dfrA8			
Present	1 (16.7)	0	0.54
dfrA14			
Present	1 (16.7)	0	0.54
dfrA17			
Present	3 (50.0)	0	0.21
dfrA18			
Present	1 (16.7)	0	0.54
qnrB19			
Present	4 (66.7)	1 (50.0)	0.67
strA			
Present	3 (50.0)	0	0.21
strB			
Present	3 (50.0)	1 (50.0)	1.00
sul1			
Present	2 (33.3)	1 (50.0)	0.67
sul2			
Present	3 (50.0)	2 (100)	0.21
sul3			
Present	0	1 (50.0)	0.06
tet(A)			
Present	2 (33.3)	1 (50.0)	0.67
tet(B)			
Present	2 (33.3)	0	0.35
tet(Q)			
Present	1 (16.7)	0	0.54
fosA			

Present	1 (16.7)	2 (100)	**0.04**
cepA			
Present	1 (16.7)	0	0.54
mph(A)			
Present	4 (66.7)	0	0.1
aadA1			
Present	0	1 (50.0)	0.06
aadA2			
Present	0	1 (50.0)	0.06
aadA5			
Present	1 (16.7)	0	0.54
aac(3)-Via			
Present	0	1 (50.0)	0.06
aac(3)-lid			
Present	2 (33.3)	0	0.35
cmlA1			
Present	0	1 (50.0)	0.06
floR			
Present	0	1 (50.0)	0.06

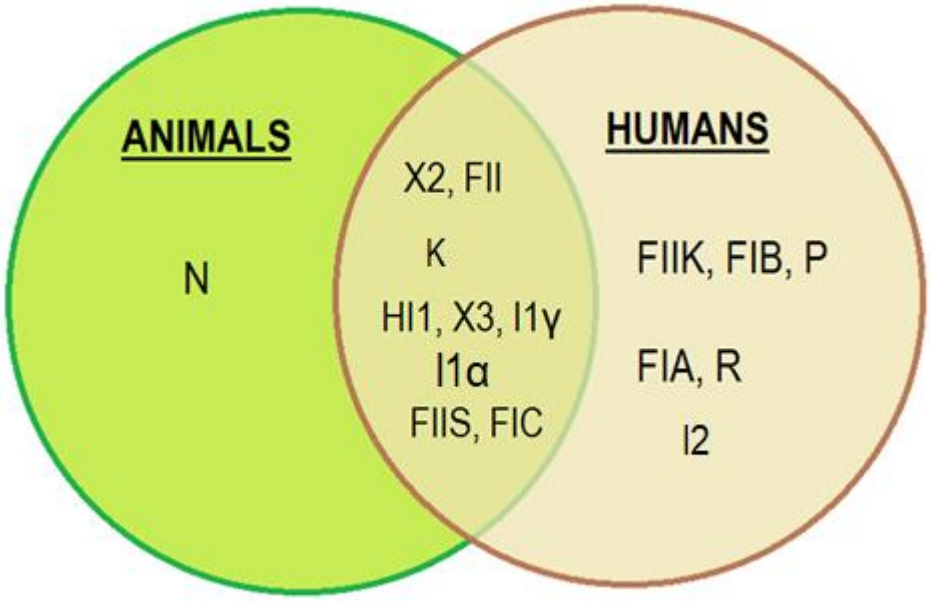

Figure 1. Venn´s Diagram: Results of the analysis of replicon typing showing prevalence in human and animal strains separately and some shared replicons between them.

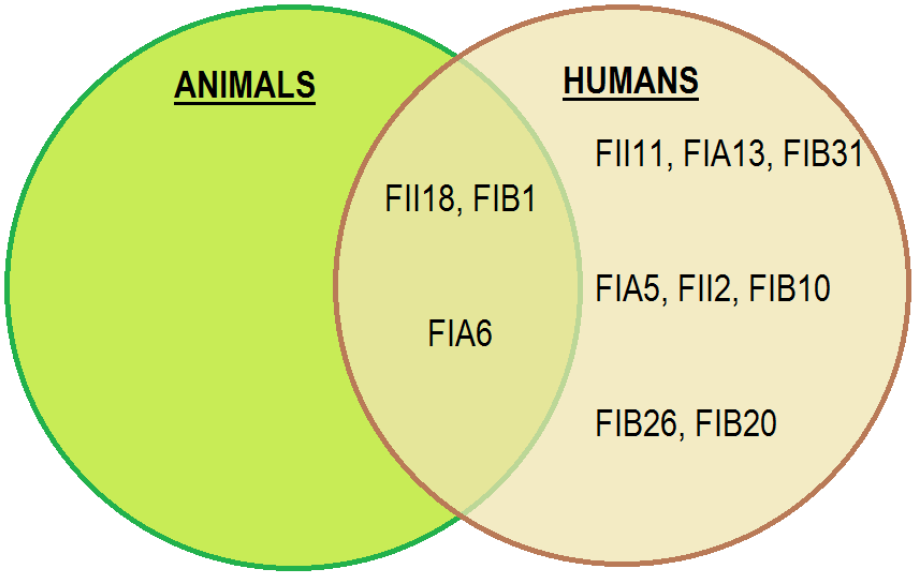

Figure 2. Venn´s Diagram: Results of pMLST found in strains of humans and animals

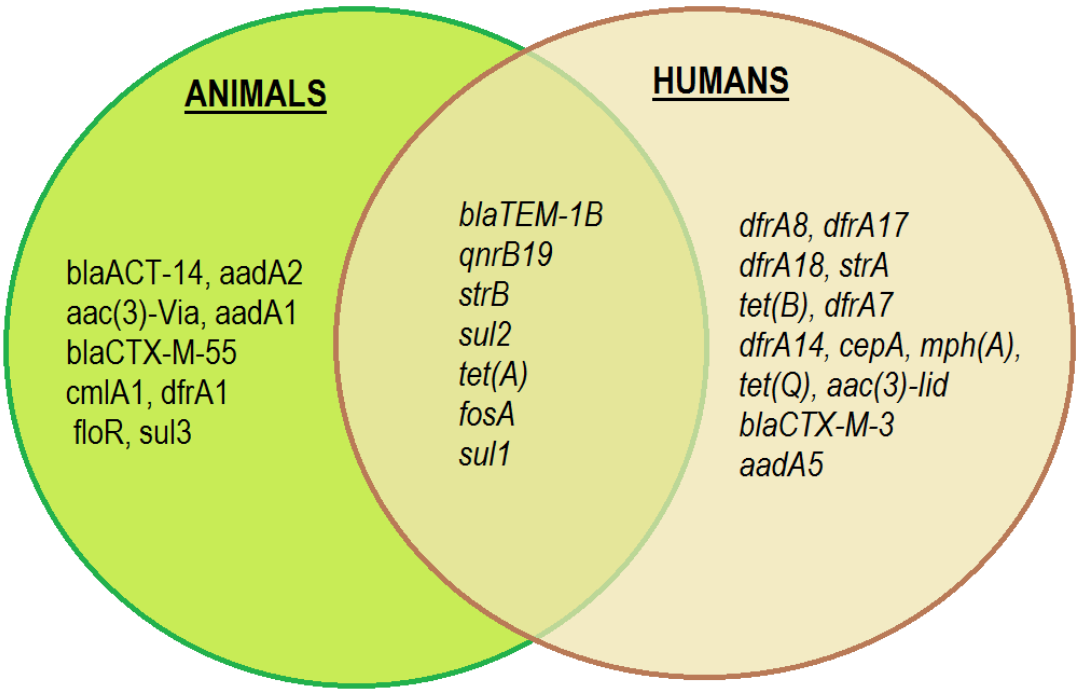

Figure 3. Venn´s Diagram: gene resistance in strains of humans and animals

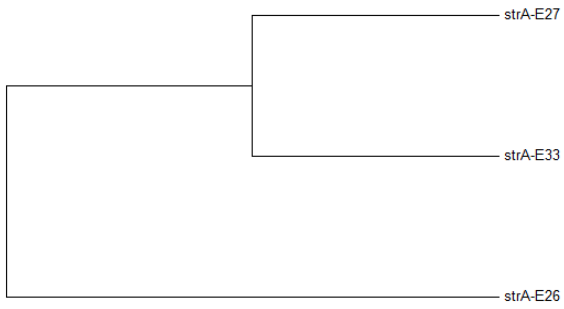

Figure 4. Phylogenetic tree: (Maximum Likelyhood) of the gene *strA* found in 3 different human strains. Bootstrap <9.

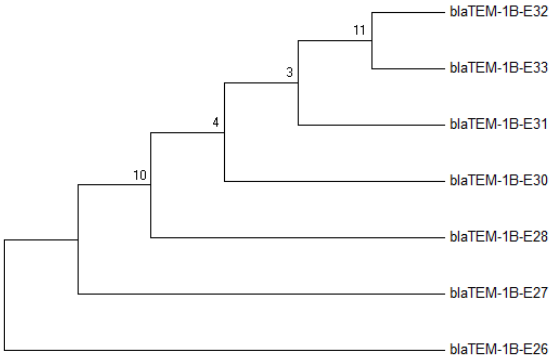

Figure 5. Phylogenetic tree (Maximum Likelyhood) of the gene *bla*TEM-1B found in 7 different strains: 2 belong to animals (a goose and a dog), and the rest from human isolates. Bootstrap value 11.

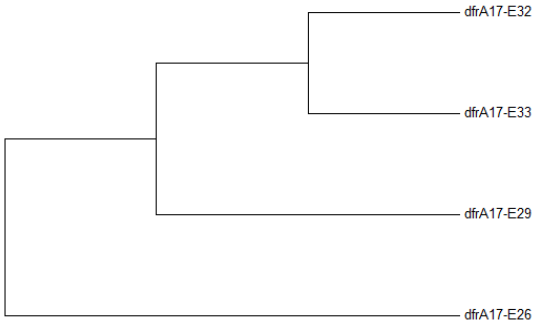

Figure 6. Phylogenetic tree: (Maximum Likelyhood) of the gene *dfrA*17 found in 4 different strains which belonged to humans isolates. Bootstrap < 9

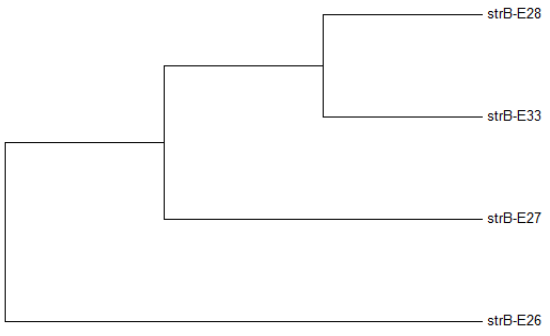

Figure 7. Phylogenetic tree: (Maximum Likelyhood) of the gene *strB* found in 4 different strains, three belong to human isolates and one from animal. Bootstrap < 9

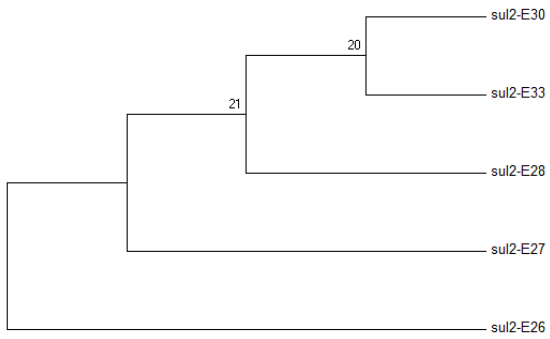

Figure 8. Phylogenetic tree (Maximum Likelyhood) of the gene *sul*2 found in 5 different strains: 3 belonged to human isolates and 2 from animal. Bootstrap value 20.

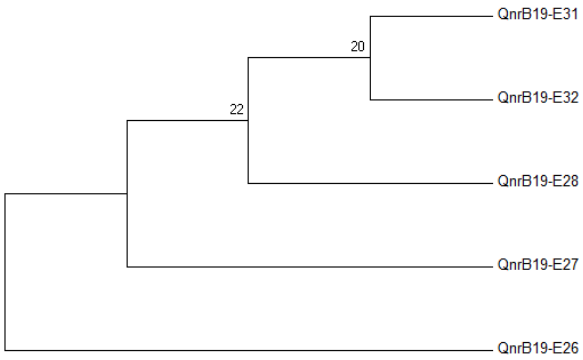

Figure 9. Phylogenetic tree (Maximum Likelyhood) of the gene *QnrB*19 found in 5 different strains, which belonged from human isolates. Bootstrap value 20.

ANNEXES

Appendix 1. Recompilation of the information of the study

	Nº	Isolate ID	Origin	ST	WGS	Antibiotic Resistance profile	Plasmid	Transconjugated Replicons	pMLST	Transconjugate Antibiotic Resistance profile	Antibiotic resistance genes
1	8	8,5	Homo sapiens	ND	ND	AM, S, SXT, C, CF, TE, G	ND	FIB, FIA, FIIS, FII	ND	ND	ND
2	26	26,1	Felis silvestris catus	ND	ND	CF	ND	ND	ND	ND	ND
3	32	32,1	Ovis orientalis aries	ND	ND	AM, TE	ND	ND	ND	ND	ND
4	35	35,1	Gallus gallus domesticus	ND	ND	SXT, TE	ND	ND	ND	ND	ND
5	46	46,1	Homo sapiens	ND	ND	AM, S, SXT, TE, G	ND	ND	ND	ND	ND
6	54	54,5	Homo sapiens	**941**	No clone	AM, S, SXT, CF, TE, G	IncFIB(AP001918), **IncFII**, IncI2	HI1, X3, I1γ, FIIK, **FII**	FII11, FIA13, FIB31	AM, SXT, CF, TE	blaTEM-1B, dfrA8, dfrA17, dfrA18, qnrB19, strA, strB, sul2, tet(B)
7	74	74,1	Homo sapiens	ND	ND	SXT,TE	ND	ND	ND	ND	ND
8	76	76,1	Homo sapiens	ND	ND	NONE	ND	ND	ND	ND	ND
9	77	77,4	Homo sapiens	ND	ND	NONE	ND	ND	ND	ND	ND
10	78	78,3	Homo sapiens	ND	ND	AM, S, SXT, G	ND	ND	ND	ND	ND
11	79	79,1	Canis lupus familiaris	ND	ND	NONE	ND	ND	ND	ND	ND
12	80	80,4	Homo sapiens	**1485**	No clone	AM, S, SXT, CF, TE, G	IncFIB(AP001918), IncFIC(FII), IncQ1	X3, I1γ, FIIK	FII18, FIA5, FIB1	AM, SXT, CF, TE	blaTEM-1B, dfrA7, dfrA14, qnrB19, strA, strB, sul2, tet(A)
13	81	81,2	Canis lupus familiaris	ND	ND	NONE	ND	ND	ND	ND	ND
14	82	82,3	Homo sapiens	ND	ND	TE	ND	ND	ND	ND	ND
15	87	87,1	Goose	**Unknown1**	No clone	AMC, AM, S, SXT, CF, TE, G	IncFIB(AP001918), IncFIB(K), **IncFII**, IncFII(Yp), IncR	X2, **FII**	FII18, FIA6, FIB1	AM, SXT, CF, TE	blaACT-14, blaTEM-1B, fosA, qnrB19, strB, sul2
16	88	88,1	Homo sapiens	ND	ND	TE	ND	ND	ND	ND	ND
17	89	89,1	Homo sapiens	ND	ND	CF	ND	ND	ND	ND	ND

18	90	90,1	Homo sapiens	ND	ND	AMC, AM, S, SXT, CF, TE, G	ND	FIIS, X2, FIC, K, FII	ND	ND	ND
19	91	91,1	Homo sapiens	**Unknown2**	No clone	AMC, AM, S. SXT, CF, CTX,TE, G	**IncFII**, ColRNAI, Col(MP18), Col156	FIB, P, I1γ, X2, **FII**	FII2, FIB10	AM, SXT, CF, CTX, TE	cepA, dfrA17, mph(A), tet(Q)
20	92	92,2	Canis lupus familiaris	**117**	No clone	AMC, AM, S, CN, SXT, C, CF, CTX, TE, G	IncFIB(AP001918), **IncFII**(pHN7A8), **IncFIC**(FII), **IncI1**, **IncN**, ColRNAI	HI1, I1α, **N**, X3, **I1γ,** FIIS, X2, **FIC**, K, **FII**	FII18, FIB1	AM, CN, SXT, CF, CTX, TE	aadA2, aac(3)-Via, aadA1, blaTEM-1B, blaCTX-M-55, cmlA1, dfrA1, floR, fosA, sul1, sul2, sul3, tet(A)
21	93	93,4	Homo sapiens	ND	ND	AMC, AM, S, SXT, CF, TE, G	ND	X2, FII	ND	ND	ND
22	95	95,2	Canis lupus familiaris	ND	ND	AMC, CF	ND	ND	ND	ND	ND
23	96	96,2	Homo sapiens	ND	ND	AMC, CF	ND	ND	ND	ND	ND
24	97	97,1	Canis lupus familiaris	ND	ND	SXT, CF, TE, G	ND	ND	ND	ND	ND
25	98	98,2	Homo sapiens	ND	ND	NONE	ND	ND	ND	ND	ND
26	99	99,4	Homo sapiens	ND	ND	AMC, CF	ND	ND	ND	ND	ND
27	100	100,1	Homo sapiens	ND	ND	AMC, CF, TE	ND	ND	ND	ND	ND
28	101	101,3	Homo sapiens	ND	ND	AMC, AM, S, SXT, CF, G	ND	I1α, I2, I1γ, FIIS, R, X2, FII	ND	ND	ND
29	103	103,2	Gallus gallus domesticus	ND	ND	CF, TE	ND	ND	ND	ND	ND
30	104	104,2	Homo sapiens	ND	ND	AMC, AM, G	ND	ND	ND	ND	ND
31	105	105,4	Canis lupus familiaris	ND	ND	AMC, C, CF, G	ND	ND	ND	ND	ND
32	106	106,1	Homo sapiens	**5019**	No clone	AMC, AM, S, CN, CIP, CF, CTX, TE	IncFIB(AP001918), IncFIC(FII), IncI1	FIB, P, I1γ, FIIS, X2, FIC, FII	FII18, FIA6, FIB1	AM, CN, CF, CTX, TE	aac(3)-Iid, blaTEM-1B, blaCTX-M-3, fosA, mph(A), qnrB19, tet(A)
33	107	107,5	Homo sapiens	ND	ND	AMC, AM, SXT, TE, G	ND	ND	ND	ND	ND
34	108	108,2	Homo sapiens	ND	ND	AMC	ND	ND	ND	ND	ND
35	109	109,2	Homo sapiens	**120**	No clone	AMC, AM, S, CN, SXT, CF, CTX, TE, G	IncFIB(AP001918), IncFIA, IncFIC(FII), IncI1	FIB, FIA, I1γ, FIIS, R, FII	FII18, FIA6, FIB26	AM, SXT, CF, CTX, TE	aac(3)-Iid, aadA5, blaTEM-1B, dfrA17, mph(A), qnrB19, sul1
36	110	110,2	Homo sapiens	ND	ND	AMC, AM	ND	ND	ND	ND	ND
37	111	111,1	Homo sapiens	ND	ND	AMC, AM, S, SXT, C, CF, TE, G	ND	FIB, P, FII	ND	ND	ND

38	112	112,5	Homo sapiens	ND	ND	AMC, CF, TE	ND	ND	ND	ND	ND
39	113	113,4	Homo sapiens	ND	ND	NONE	ND	ND	ND	ND	ND
40	114	114,1	Homo sapiens	ND	ND	NONE	ND	ND	ND	ND	ND
41	115	115,2	Homo sapiens	ND	ND	CF	ND	ND	ND	ND	ND
42	116	116,2	Homo sapiens	ND	ND	AM, SXT, CF, TE, G	ND	FIB, FIIS, X2, K, FII	ND	ND	ND
43	117	117,2	Homo sapiens	ND	ND	TE, G	ND	ND	ND	ND	ND
44	118	118,1	Homo sapiens	**Unknown3**	No clone	AMC, AM, S, CN, SXT, CIP, CF, CTX, TE, G	IncFIB(AP001918), IncFII(pHN7A8), IncFII(pRSB107), IncFII, IncX4, Col(MG828), Col(BS512)	FIB, FIIS, X2, FII	FII18, FIA6, FIB20	AM, CN, SXT, CF, CTX, TE	blaTEM-1B, dfrA17, mph(A), strA, strB, sul1, sul2, tet(B)
45	119	119,4	Homo sapiens	ND	ND	NONE	ND	ND	ND	ND	ND
46	120	120,5	Homo sapiens	ND	ND	AM, S, SXT, TE, G	ND	ND	ND	ND	ND
47	130	130,2	Homo sapiens	ND	ND	AM, S, SXT, CF, TE, G	ND	FIB, I1γ, FII	ND	ND	ND
48	131	131,2	Homo sapiens	ND	ND	NONE	ND	ND	ND	ND	ND
49	132	132,3	Canis lupus familiaris	ND	ND	CF	ND	ND	ND	ND	ND
50	133	133,1	Homo sapiens	ND	ND	TE	ND	ND	ND	ND	ND
51	134	134,2	Homo sapiens	ND	ND	AM, C, TE, G	ND	ND	ND	ND	ND
52	135	135,5	Homo sapiens	ND	ND	AMC, AM, CN, CF, TE, G	ND	P	ND	ND	ND
53	136	136,5	Homo sapiens	ND	ND	TE	ND	ND	ND	ND	ND
54	139	139,4	Homo sapiens	ND	ND	AM, CN, CIP, CF, CTX, TE, G	ND	FIB, FIIS, X2, K, FII	ND	ND	ND
55	141	141,2	Homo sapiens	ND	ND	CF	ND	ND	ND	ND	ND
56	142	142,3	Homo sapiens	ND	ND	AM, S, CN, SXT, CF,TE, G	ND	FIB, FIA, I1γ, FII	ND	ND	ND