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Evidencia de transmisión de genes de resistencia a antibióticos entre bacterias intestinales aviares y humanas

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HOJA DE APROBACION DE TESIS

Evidencia de transmisión de genes de resistencia a antibióticos entre bacterias intestinales aviares y humanas

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Dedication

I dedicate this work to my mother, Alejandra. Also dedicate this thesis to Gabriel Trueba and his research team, who work tirelessly with all the ethics that requires scientific work.

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Abstract

Commensal *Escherichia coli* was isolated from humans and chickens located in remote communities in the northern Coast of Ecuador. Isolates were subjected to antibiotic susceptibility tests and screened for the presence of *qnrB* gene by polymerase chain reaction (PCR). Phylogenetic analysis showed that *qnrB* genes circulating in *E. coli* from these remote communities, hospital isolates and chicken isolates from an industrial operation suggested that there is a dominant *qnrB* allele in Ecuador. The present results suggest that *qnrB* genes may have been crossing from humans to chickens or vice versa.

Resúmen

Escherichia coli comensal fue aislada de humanos y aves de corral de comunidades remotas localizadas en la costa norte de Ecuador. Los aislamientos fueron sometidos a pruebas de susceptibilidad antibiótica y detección del gen *qnrB* por reacción en cadena de la polimerasa (PCR).El análisis filogenético demostró que los genes *qnrB* que circulan en *E. coli* de estas comunidades remotas están más estrechamente relacionados con los genes *qnrB* obtenidos de un aislado clínico de *E. coli* obtenido de un hospital de Ecuador que a un gen homólogo obtenido de una granja avícola industrial ecuatoriana. Secuencias de DNA de genes *qnrB* sugieren que existe un alelo *qnrB* dominante en Ecuador, que parece estar compartido por *E. coli* de humanos y de aves de corral.

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GENERAL INTRODUCTION

Human society is threatened with the possibility of returning to the pre-antibiotic era due to the increasing antibiotic resistance in microorganisms. Unfortunately infections caused by resistant organisms do not respond to a standard treatment, which prolongs the duration of the disease, increases the cost of the treatment, and the most worrying aspect is that this increases the risk of death (WHO, 2011). One of the examples of this problem is the current antibiotic resistance observed in *Shigella*. Ciprofloxacin, a quinolone, is the only antibiotic currently recommended by World Health Organization(WHO/OPS) for the treatment of bloody diarrhea caused by *Shigella* (WHO, 2011), because many strains of this organism have acquired resistance to other antibiotics. Shigellosis is a disease that affects mainly children; however quinolones are toxic in children; however they are the population most vulnerable to contracting this infection.

Agricultural use of antibiotics has been blamed for some of the antibiotic resistance found currently in human pathogens. WHO/OPS indicates that about half of the current production of antibiotics is used in the livestock to promote growth, to prevent infections and the treat sick animals; one of the key factors that favor the emergence of antimicrobial resistance is the inadequate and irrational use of drugs, especially in livestock (WHO, 2011). One of the main instances was the role that avoparcin (glycopeptide antibiotic used as growth promoter in food animals) played in the selection of resistant strains of *Enterococcus* and

dissemination of genes conferring resistance to vancomycin to pathogenic bacteria (French, 1998; Johnsen *et al.*, 2011). Resistance to other antimicrobials such as amoxicillin, ampicillin, bacitracin, cloxacillin, erythromycin, gentamycin, lincomycin, penicillin, polymyxin, spectinomycin, tetracycline,etc, have been influenced by food animal production (Mathew *et al.*, 2007; Hawkey *et al.*, 2009)

Quinolones are one of the last resources left for medical doctors to treat diseases caused particularly by Gram-negative bacteria; new studies have shown that bacterial resistance to quinolone is rising, not just by chromosomal mutations but by resistance genes transferable by plasmids from one bacterium to another.

The finding of plasmid-mediated quinolone resistance (PMQR) in 1998 by Martínez-Martínez, helped to explain how some independent clones of initially susceptible wild-type Enterobacteriaceae reached to develop high-level quinolone resistance. (Martínez-Martínez *et al.*, 1998)

RESISTANCE TO QUINOLONE

Nalidixic acid, a synthetic antimicrobial drug, was the first quinolone introduced into clinical use in 1962. In 1980s appeared ciprofloxacin, a fluoroquinolone with wider spectrum antibacterial activity particularly against gram-negative bacteria (Strahilevitz*et al.*, 2009)

Quinolone resistance has been associated mainly with chromosomal mutations in the gyrase gene (*gyrA* and *gyrB*) or topoisomerase IV (*parC* and *parE*) gene (Allesiani *et al.*, 2009; Strahilevitz *et al.*, 2009; Hawkey *et al.*, 2009). More recently, plasmid mediated quinolone resistance is associated to *qnr* genes (*qnrA, qnrB, qnrS, qnrC, qnrD, Aac(6´)-Ib-cr* and *QepA*) (Robicsek *et al.*, 2006; Jacoby *et al.*, 2008; Wang M*et al.*, 2009; Cavaco *et al.*, 2009a; Cavaco *et al.*, 2009b) which are transferred trough conjugation (Kuo *et al.*, 2009; Ma *et al.*, 2009; Wu *et al.*, 2007; Huang *et al.*, 2009; García-Fernández *et al.*, 2009; Zhou *et al.*, 2011). The gene was the most common plasmid mediated quinolone resistance gene (Kim *et al.*, 2009). These genes code for peptides that prevent quinolone from interacting with DNA gyrase and topoisomerase IV (Robicsek *et al.*, 2006; Strahilevitz *et al.*, 2009; Xiong *et al.*, 2011).

It is thought that the use of fluoroquinolones in food-producing animals has contributed to the problem of quinolone resistance in human pathogens such as *Escherichia coli* , *Enterobacter* spp. , *Klebsiella* spp., *Citrobacter* sp, *Salmonella* sp,

Campylobacter sp (Nelson *et al*., 2007; Yue *et al*., 2008; Thorsteinsdottir *et al*., 2010; Xia *et al*., 2010). Plasmid mediated quinolone resistance in human pathogens has been associated with food-producing animals (McDermott *et al*., 2001; Johnson *et al*., 2006; Mathew *et al*., 2007; Huang *et al*., 2009; Xia *et al*., 2010). Plasmid mediated quinolone resistance is also increasing in chicken *E. coli* in some parts of the world (Huang *et al*., 2009: Riccobono *et al*., 2011).

Chromosomal quinolone resistance occurs by mutations in gyrase and/or topoisomerase genes. The frequency at which this resistance occurs around the world, due to spontaneous mutations in these genes, is unusual genetic events (with a frequency of 10^{-14} to 10^{-16}). Plasmid mediated *qnr* resistance is less effective in the reduction of susceptibility to quinolones and it may be the first event in the process of gaining resistance, setting the stage for the chromosomal mutations (Strahilevits *et al*., 2009).

The discovery of PMQR in the late 1990s was made accidentally (Martínez-Martínez *et al*., 1998), while studying pMG252, a plasmid from a multiresistant strain of *Klebsiella pneumonia* isolated in 1994 from a patients urine specimen in Alabama. A quinolone was included as a control in a study of the ability of this plasmid to increase resistance to β-lactam antibiotics in porin-deficient strains of *Klebsiella pneumoniae*. Researchers found that there was an increase of 4- to 16-fold in the minimum inhibitory concentration (MIC) to quinolone. They then

observed that in an *Escherichia coli* strain with intact porins the addition of pMG252 increased the quinolone MICs between 8- and 64-fold. The protein was named Qnr, for quinolone resistance (GenBank accession number AY070235) and now called QnrA1 (Strahilevitz *et al.*, 2009). Eight years later, Jacoby and colleagues while investigating qnrA in strains of *K. pneumonia* from India, found that several could transfer low-level quinolone resistance, but were negative by PCR for *qnrA*(Jacoby *et al.*, 2006). The PMQR gene coded for a 214 or 226 amino-acid protein and was termed *qnrB1* (GenBank accession number DQ351241).

From 1998 to 2011, a wide range of variants of *qnrB* have been found, wider than the *qnrA* and *qnrS* counterparts.Some allele of *qnrB* are*: qnrB2* (Jacoby *et al.*, 2006), *qnrB3* and *qnrB4* (Robicsek *et al.*, 2006), *qnrB5* (Gay *et al.*, 2006); *qnrB13*, *qnrB14*, and *qnrB15* (Tamang *et al.*, 2008) and *qnrB19* describe by Jacoby in 2008 (Jacoby *et al.*, 2008).The QnrB1 protein shares 43% and 44% amino acid identities with QnrA and QnrS, respectively (Jacoby *et al.*, 2006). The *qnr* families (such as *qnrA*, *qnrB*, *qnrS*, *qnrC* or *qnrD*) are defined by a 30% of difference in nucleotide or derived amino acid sequences; and within each family, *qnr* alleles differ in one or more amino acids (Jacoby *et al.*, 2008). Two of the five known *qnr* gene families (*qnrC* and *qnrD*) were first reported in 2009 (Wang *et al.*, 2009).

Subsequently, two genes were found also carried on plasmids: *AAC(6_)-Ib-cr,* a variant aminoglycoside acetyltransferase capable of reducing ciprofloxacin activity (Vetting *et al.*, 2008), which may be even more prevalent than Qnr proteins, and

*qepA***,** a quinolone extrusion (Gay *et al*., 2006). Both mechanisms provide the low-level quinolone resistance shown in vitro to facilitate the emergence of higher-level resistance in the presence of quinolones at therapeutic levels (Strahilevitz *et al*., 2009; Robicsek *et al*., 2006). Thirteen years have passed since the first report of PMQR, but knowledge is still expanding quickly.

Qnr PROTEINS

To understand how Qnr work, it is necessary to understand the mechanism of action of quinolones. The two quinolone targets, the type II topoisomerase enzymes DNA gyrase and topoisomerase IV, regulate conformational changes in the topology of DNA by catalyzing the breakage and rejoining of DNA strands during cellular division. The DNA gyrase catalyze the ATP-dependent negative supercoiling of DNA, and therefore it is necessary for the initiation of DNA synthesis (Strahilevitz *et al.*, 2009). The main role of topoisomerase IV is to decatenate daughter replicons, measured in the decatenation of kinetoplast DNA. Both enzymes are heterodimers; DNA gyrase is composed of two A subunits and two B subunits, and topoisomerase IV is also an A2B2 enzyme composed of two ParC and two ParE subunits. The ParC subunit is homologous to GyrA, and ParE is homologous to GyrB. In brief, quinolones inhibit gyrase-mediated DNA supercoiling and topoisomerase IV-mediated DNA decatenation (Tran *et al.*, 2005).

Qnr proteins are part of the pentapeptide repeat family, which is defined by a series of tandem 5 amino-acid repeats. In the pentapeptide repeats, no position is completely conserved, but each of the residues of an individual pentapeptide exhibits a tendency for a restricted number of amino acids with the recurrent general motif roughly represented by the sequence A(D/N)LXX and particularly

represented by [Ser, Thr, Ala, or Val][Asp or Asn][Leu or Phe][Ser, Thr, or Arg][Gly] (Vetting *et al.*, 2006; Strahilevitz *et al.*, 2009). Qnr proteins, often have a cysteine at position *i*_2 (with position *i* representing the central amino acid of each repeat). A characteristic feature of the Qnr proteins is that they are formed by two domains of pentapeptide repeats separated by a single amino acid, usually glycine. Primary structures of QnrA, QnrB, and QnrS are similar, with nine pentapeptide repeat units connected by a single glycine, followed by a cysteine, with variable numbers of units (22 in QnrS, 28 in QnrA, and 29 inQnrB, QnrC, and QnrD) (Vetting *et al.*, 2006; Strahilevitz *et al.*, 2009).

The mechanism of the Qnr protective effect has been shown through gel retardation assays, where QnrA can bind to the DNA gyrase holoenzyme as well as to its respective subunits, GyrA and GyrB. This binding did not require the presence of the ternary complex of enzyme, DNA, and quinolone. Similar findings were also reported for QnrA and *E. coli* topoisomerase IV (Tran *et al.*, 2005). It was proposed that QnrA binds to gyrase or topoisomerase IV at a site overlapping the DNA binding site. Recent data suggests that MfpAMt (pentapeptide repeat protein identified on the chromosome of *Mycobacterium tuberculosis* having 18.9% amino acid similarity to QnrA) and QnrB4 may interact with DNA gyrases in a gyrase specific manner, so QnrB4 protected *E. coli* but not *M. tuberculosis* gyrase from the inhibitory effect of fluoroquinolone and MfpAMt protected *M. tuberculosis* but not

E. coli gyrase (Jacoby *et al*., 2008; Rodriguez-Martínez *et al*., 2006; Strahilevits *et al*., 2009).

In year 2011, Xion and colleagues have shown that, consistent with previous results from other aquatic organisms such as *Vibrio*, *Shewanella* and *Stenotrophomonas* spp., *A. hydrophila* contains a chromosomal pentapeptide repeat protein (AhQnr), qnr like protein, that in its recombinant form increases the quinolone MIC of host *E. coli* and protects *E. coli* DNA gyrase from quinolone inhibition in vitro assays of supercoiling activity. This study provides the AhQnr crystal structure, the first for any Gram-negative Qnr, where reveals two prominent loops (1 and 2) that project from the PRP structure.

Xion present a model for the AhQnr: DNA gyrase interaction where loop1 interacts with the gyraseA and loop2 with the gyraseB domains, this could be the general mechanism for the interactions of Qnr proteins with DNA gyrase in Gram-negative bacteria (Xion *et al*., 2011).

Plasmid-carried *qnrB* alleles have been shown to have upstream LexA binding sites. Quinolones, mitomycin, and possibly other DNA-damaging agents as part of the SOS response have increased the level of expression of qnrB (Wang M. *et al*., 2009). Da Re maintain that QnrB may have a native function in protection from naturally occurring DNA-damaging agents, besides some, *qnrB*-containing strain,

do not express quinolone resistance in non-inducing conditions, in other words act as silent resistance gene, however this resistance will be activated under selective antibiotic pressure (Da Re *et al.,* 2009).

Qnr PLASMIDS

The plasmids carrying the *qnr* vary in size and specificity, suggesting that the propagation of multiple plasmids has been responsible for the dissemination of this resistance worldwide. Sometimes the plasmids are so similar that a broad dissemination of the same plasmid does appear likely. Analysis of the genetic environment around the cloned *qnrB* genes showed that they were present in diverse plasmid backbones, sometimes within novel genetic contexts, but always associated with mobile or transposable elements to generate the diversity of plasmid structures seen today (Strahilevits *et al.*, 2009; Pei *et al.*, 2009)

Most *qnrB* alleles show a wide distribution, for example *qnrB1*was discovered on a 340-kb multiresistance plasmid from India (Jacoby *et al.*, 2006) and was subsequently reported in Europe and Asia (Park *et al.*, 2007; Murray *et al.*, 2008; Strahilevits *et al.*, 2009); *qnrB2*was found originally in isolates from the United States (Jacoby *et al.*, 2006) and subsequently in isolates from South Korea (Park *et al.*, 2007),The Netherlands (Veldman *et al.*, 2008), Scotland (Murray *et al.*, 2008), China (Yang *et al.*, 2008), Taiwan (Wu *et al.*, 2008), etc.

On the other hand *qnrB19* was originally found incommensal enterobacteria from healthy children living in South America (Pallecchi *et al*., 2009) and few alleles have been found worldwide. Pallecchi and colleagues observed a high prevalence (54%) of *qnrB* genes in a metagenomic analysis of commensal enterobacteria from healthy children living in Peru and Bolivia, and shown the presence of 67% of pECY6-7- and 16% of pECC14-9-like plasmids, suggesting a key role in the widespread dissemination of *qnrB* through these small plasmids (Pallecchi *et al*., 2010). The gene *qnrB19* was also reported for an *S. enteric* serovar Typhimurium strain from The Netherlands (García-Fernández *et al*., 2009), and a *K. pneumonia* isolate on an 80-kb plasmid from the United States (Strahilevits *et al*., 2009)

Some alleles of *qnrB* are associated with β-Lactamase genes, and also to the ESBLs SHV-12, TEM-52, CTXM- 3 , CTX-M-9 , CTX-M-14 , CTX-M-15 and blaVIM-1 (Yang *et al*., 2008, Tamang *et al*.,2008; Wu *et al*., 2008; Jacoby *et al*., 2008; Miró *et al*., 2010; Park *et al*., 2007; Yang *et al*., 2008; Dolejska *et a*l., 2010). The study by Kuo et al, demonstrated a close linkage between the *qnrS* gene and *bla*CTX-M-1, suggesting CTX-M and Qnr might be co-emerging in *E. coli* strains isolated from healthy chickens and pigs under selective pressure of quinolone and cephalosporine administration (Kuo *et al*, 2009). In addition, *qnrB4* alleles are closely linked to eight genes or pseudogenes unrelated to antibiotic resistance:

sapA and *sapB* which are peptide transport system permeases (Strahilevits *et al*., 2009).

The presence of several types of antibiotic resistance in the same plasmid facilitates the dissemination of multidrug-resistant *Enterobacteriaceae*, hence, the use quinolone could select not only for quinolone resistance, but also cephalosporin, aminoglycosides resistance and any other forms of resistance borne on plasmids carrying PMQR genes.

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Part II

Evidence of transmission of *qnrB*genes between commensal *E. coli* from humans and chickens

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Introduction

Agricultural use of antibiotics has been blamed for some of the antibiotic resistance found currently in human pathogens. One of the main examples of this is the role that avoparcin (glycopeptide antibiotic used as growth promoter in food animals) has been shown to have played in the selection of resistant strains of *Enterococcus* and dissemination of genes conferring resistance to vancomycin to pathogenic bacteria (French, 1998; Johnsen *et al.*, 2011). Resistance to other antimicrobials such as amoxicillin, ampicillin, bacitracin, cloxacillin, erythromycin, gentamycin, lincomycin, penicillin, polymyxin, spectinomycin, tetracycline, etc, have been influenced by food animal production (Mathew *et al.*, 2007; Hawkey *et al.*, 2009; Harada *et al.*, 2010)

Quinolone resistance has been associated mainly with chromosomal mutations in the gyrase gene (*gyrA* and *gyrB*) or topoisomerase IV (*parC* and *parE*) gene (Allesiani *et al.*, 2009; Strahilevitz *et al.*, 2009; Hawkey *et al.*, 2009). More recently, plasmid mediated quinolore resistance is associated to *qnr* genes (*qnrA, qnrB, qnrS, qnrC, qnrD, Aac(6´)-Ib-cr* and *QepA*) (Robicsek *et al.*, 2006; Jacoby, *et al.*, 2008; Wang M, *et al.*, 2009; Cavaco *et al.*, 2009a; Cavaco *et al.*, 2009b) which are transferred trough conjugation (Kuo *et al.*, 2009; Ma *et al.*, 2009; Wu *et al.*, 2007; Huang *et al.*, 2009; Garacía-Fernández *et al.*, 2009; Zhou *et al.*, 2011). These genes

code for peptides that prevent quinolone interaction with DNA gyrase and topoisomerase IV (Robicsek *et al*., 2006; Strahilevitz *et al*., 2009; Xiong *et al*., 2011).

It is thought that the use of fluoroquinolones in food-producing animals has contributed to the problem of quinolone resistance in human pathogens such as *Escherichia coli* , *Enterobacter*spp. , *Klebsiella*spp., *Citrobactersp* ,*Salmonella sp, Campylobacter sp*(Nelson *et al*., 2007; Yue *et al*., 2008; Thorsteinsdottir *et al*., 2010; Xia, *et al*., 2010). Plasmid mediated quinolone resistance in human pathogens has been associated with food-producing animals (McDermott *et al*., 2001; Johnson *et al*., 2006; Mathew *et al*., 2007; Huang *et al*., 2009; Xia *et al*., 2010). Plasmid mediated quinolone resistance is also increasing in chicken *E. coli* in some parts of the world (Huang *et al*., 2009: Riccobono *et al*., 2011).

This report describes the presence of *qnrB* genes in human and chicken *E. coli* isolates from remote communities located in the northern region of the Ecuadorian coast. Additionally it suggested that a fluoroquinolone resistance gene was transferred from chicken to human micobiota or vice versa in this region.

Materials and Methods

Study area

In 1996, the Ecuadorian government began the construction of a highway along the north coast to facilitate the access to this region; this building was completed in 2001. The secondary roads that link communities with urban centers are being built today (Bates *et al.*, 2007).

At this region there is limited sanitation infrastructure (Levy *et al.*, 2008), which inevitably leads to contamination of large river system in the area, which correlates with high rates of diarrheal disease observed in the population (Eisenberg *et al*, 2006; Vieira *et al.*, 2007).

Borbón area has been a victim of deforestation and mining exploitation without technical measures, all of which has affected the local climate and hydrological patterns (Levy *et al*,2009).

Bacterial strains were obtained from 21 remote communities of Borbn, all located within the drainage system of three rivers: the Cayapas, the Santiago, and the Onzole, in northwestern Ecuador from 2009 to 2010. The study was part of a larger ongoing research project in this region (Viera *et al.*, 2007; Eisenberg *et al.*, 2006). All protocols were approved by the University of Michigan Institutional Review Board and Universidad San Francisco de Quito's bioethics committees.

Samples and bacterial isolates

A total of 2366 isolates were obtained from remote communities, 1167 from humans (fecal samples) and 1199 from chickens (rectal swab). Chicken samples came from two groups of birds: a) 224isolates from45 mixed-breed and raised free range chickens and b)975isolates from217 broiler chickens which were purchased by local farmers few days after hatching from an industrial operator and raised in confined spaces (houses). Additionally we obtained 50 fluoroquinolone resistant *E. coli* isolates from a chicken industrial operation (located in the Ecuadorian Coast at approximately 300 kilometers from the remote communities), 61uropathogenicfluoroquinolone resistant *E. coli* isolates from hospital Vozandes in Quito (generously donated by Janet Zurita), 42 from hospital Carlos Andrade Maríndonated by Isabel Narváez, and 11 from Microbiology Institute, USFQ. For each stool sample five lactose-fermenting colonies were isolated on a MacConkey agar plate. Colonies with typical *E. coli* morphology on MacConkey agar were confirmed with biochemical testing.

Antimicrobial susceptibility tests.

Isolates were subjected to a Kirby-Bauer antibiotic susceptibility test in accordance with the Clinical and Laboratory Standards Institute guidelines. Twelve antibiotics were tested: Amoxicillin-Clavulanic Acid, Ampicillin, Cefotaxime, Cephalothine,

Chloramphenicol, Ciprofloxacine, Enrofloxacine, Gentamicin, Streptomycin, Sulfisoxazole, Tetracycline and Trimethoprim-Sulfamethoxazole.

Polymerase chain reaction amplification and DNA sequencing

Bacterial DNA from single fluoroquinolone resistant colonies was released from cells using a boiling technique (Vieira *et al.,* 2006). A PCR reaction for *qnrB* genes was carried out as previously described (Huang *et al*., 2009) using primers to amplify internal fragments of the target genes: qnrB F 5¨-GGMATHGAAATTCGCCACTG-3¨ and qnrB R 5´-TTTGCYGYYCGCCAGTCGAA-3´. PCR conditions were as follows: 95 °C for 5 min and 35 cycles, 94^0 C, for 30 sec, 56^0 C for 40 sec and 72^0 C for 1 min and a final incubation at 72^0 C for 10 min.

DNA Sequences from these isolates RemHUM2, RemHUM3, RemCHK10, RemCHK12, RemCHK13 and RemCHK17 were sequenced both strands, by company in Wisconsin (Functional Biosciences); however sequences from these RemHUM1,RemHUM4, RemHUM5, RemCHK6, RemCHK7, RemCHK8, RemCHK9, RemCHK11, RemCHK14, RemCHK15, RemCHK16, RemCHK18, RemCHK19, RemCHK20, RemCHK21, RemCHK22, RemCHK23, IndCHK24, IndCHK25, IndCHK26, *E.coli*Quito27 isolates were sequenced only one strand by Michigan of University DNA Sequencing Core. Accession numbers for the *qnrB* genes described in this report are strain RemHUM, RemCHK, IndCHK, E. coli, accession number: JN714812 to JN714838 or (RemHUM1 JN714812, RemHUM2 JN714813, RemHUM3

JN714814, RemHUM4 JN714815, RemHUM5 JN714816, RemCHK6 JN714817, RemCHK7 JN714818, RemCHK8 JN714819, RemCHK9 JN714820, RemCHK10 JN714821, RemCHK11 JN714822, RemCHK12 JN714823, RemCHK13 JN714824, RemCHK14 JN714825, RemCHK15 JN714826, RemCHK16 JN714827, RemCHK17 JN714828, RemCHK18 JN714829, RemCHK19 JN714830, RemCHK20 JN714831, RemCHK21 JN714832, RemCHK22 JN714833, RemCHK23 JN714834, IndCHK24 JN714835, IndCHK25 JN714836, IndCHK26 JN714837, *E.coli* JN714838).

Sequence analysis

The analysis of Single nucleotide polymorphisms (SNP) was performed with each one of the obtained sequences.

The statistical analysis is purely descriptive, based on frequencies and percents.

Results

Antibiotic resistance:

No major differences in antibiotic susceptibility patterns of human and chicken isolates from remote communities were observed, except for an increased resistance to ciprofloxacin (19%) and gentamycin (14%) in chicken isolates compared to 3% ciprofloxacin and 1,3% gentamycin resistances in human isolates (Table1A)

Presence of gene *qnrB* in *E. coli* isolates from humans and chickens:

There was a notorious difference in the percentage of fluoroquinolone resistant isolates (FRI) carrying qnrBgenes in isolates from chickens and humans. In remote communities 31.25% of human isolates, 31.25% of mixed breed chickens and 3.6% of the broiler chicken isolates carried qnrB(table 1B). On the other hand FRI from the poultry industrial operation showed 6% of *qnrB* gene carriage and fluoroquinolone resistant isolates obtained from hospitals in Quito showed 1.6 % of *qnrB*gene carriage.

Phylogenetic analysis of *qnrB* genes:

Analysis of nucleotide sequences showed that *qnrB* genes amplified from *E. coli* isolates (from humans and chickens) from remote communities, *qnrB* genes amplified from *E. coli* isolated from industrial operation and *qnrB* from clinical *E.coli* isolated from hospital in Quito clustered together using Maximun Parsimony analysis (Fig 1).

Discussion

All Ecuadorian *qnrB* sequences seemed to be the same including the sequences from the industrial operation which clustered together to the rest of Ecuadorian sequences but showed 5 nucleotide diference which cuould be sequence error because these amplicons were sequenced just one strand. The fact that all Ecuadorian qnrB sequences analyzed in Borbon and urban hospital in Quito seemed to be the same allele and clustered apart form other qnrB suggests that they come from a common ancestor and that the gene was transfered form human *E. coli* to chicken *E. coli* or viceversa. A recent study in Bolivia found evidence of cross-transmission of *E. coli* between home-raised chickens and humans (Riccobono, *et al*., 2011).

These genes have been found human commensal *E. coli* and *Klebsiella*sp. in urban settings in Peru and Bolivia (Pallecchi *et al*., 2009) and clinical *E. coli* and *Klebsiella* sp.isolates in China (Wang A *et al*., 2008). However other studies found no genetic relationship between Fluoroquinolone Resistance Isolated from humans and chickens (Xia *et al.*, 2010).

Alternatively, these *qnrB* genes may have come from chickens and passed to humans.This idea is in agreement with a previous reports suggesting that some quinolone resistence in human pathogens originated in poultry (McDermott *et al*.,

2001; Johnson *et al.*, 2006; Mathew *et al.*, 2007; Huang *et al.*, 2009, Thorsteinsdottir *et al.*, 2010).

There was a large difference in the percentage of FRI carrying *qnrB* genes in isolates from mixed breed chickens (31.25%) and broiler chickens (3.6%) from remote communities, poultry industrial operation (6%) and FRI obtained from hospitals in Quito (1.6 %). This finding may indicate that clinical isolates carried fluoroquinolone resistance genes different from *qnrB.*Also these genes were found in larger percentage in isolates from mixed breed chickens and humans than in Broiler chickens which may indicate that the gene was circulating in the remote communities prior the arrival of the Broiler chickens to the region. When construction of the road had not started, the road then allowed a better access to remote communities.

The preset study found that commensal human isolates from remote communities had 3% fluoroquinolone resistance which is lower than corresponding percentage found in chickens isolates (19%) in the same location. On the other hand prevalence of fluoroquinolone resistance in clinical isolates of *E. coli* in Ecuador has been estimated in 43.7% (REDNARBEC, 2008), however there are no data from human commensal *E. coli*.

Previous studies have raised concerns about the use of quinolones in the animal industry and the potential risk of transference to human pathogens (McDermott *et al.*, 2001; Mathew *et al.*, 2007). Our study provides additional evidence regarding the importance of agricultural use of antibiotics in human health.

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Figure Legend

Figure1. **Maximum Parsimony analysis of qnrB genes:** Bacterial sequences obtained from humans in Ecuadorian remote communities are named RemHum; sequences from chickens in remote communities are RemChk; *qnrB* from hospital isolate in Quito : *E. coli* Quito27 (hospital) and sequences from isolates from a poultry industrial operation are IndCHK. The rest of the sequences were obtained from GenBank. Numbers are bootstrap values obtained after 500 pseudoreplicates.

Table 1A. **Fluoroquinolone and Gentamycin resistant *E. coli* from remote communities**

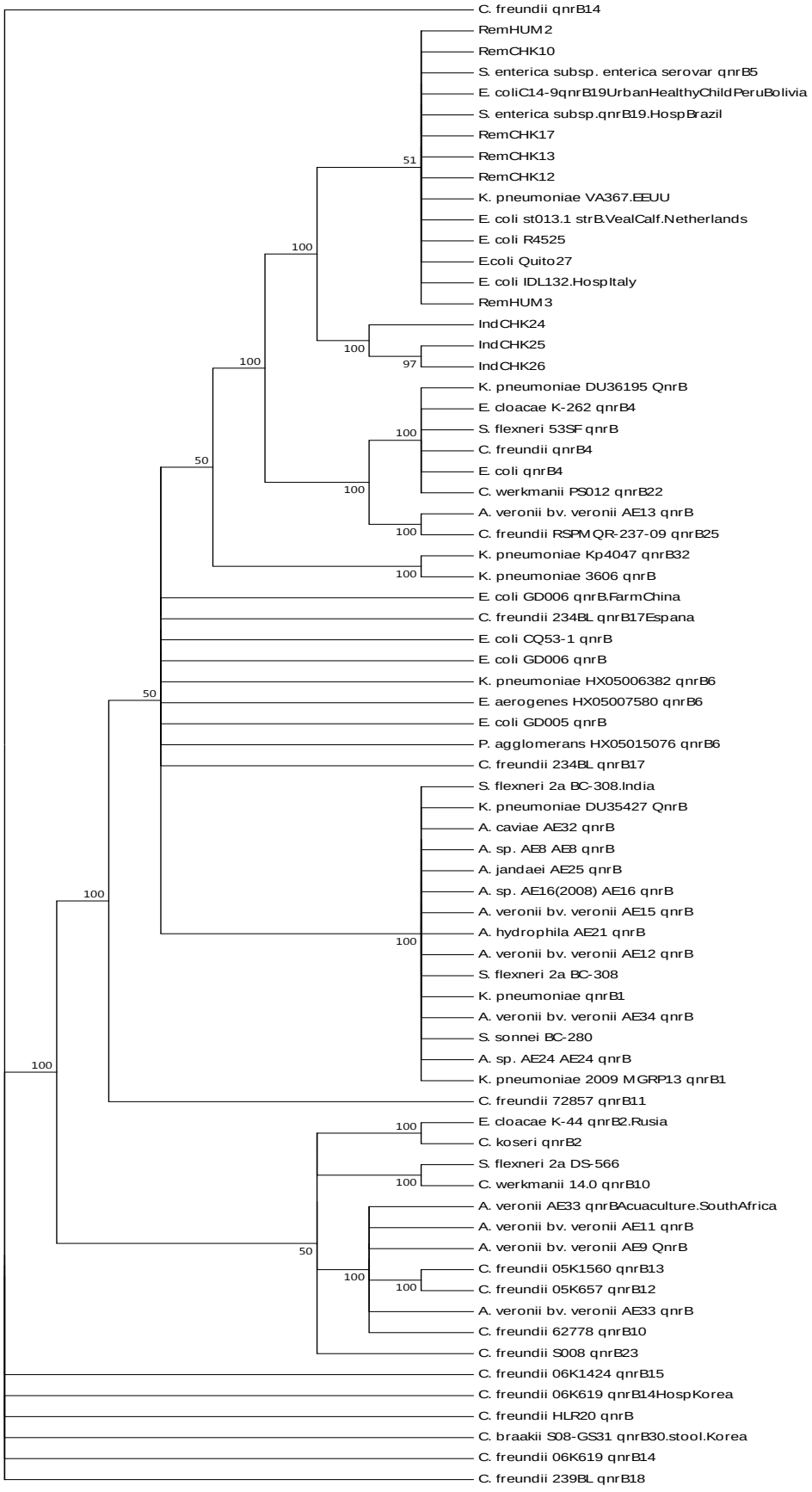
	Total strains analyzed	**% Quinolone Resistant isolates**	**% Gentamycin Resistant**
Isolates from humans	1167	3	1.3
Isolates from chickens	1199	19	14

Table 1B . **Fluoroquinolone resistant *E. coli* from remote communities**

	Total strains analyzed	**% Quinolone Resistant isolates**	**% Quinolone Resistant strains carrying *qnrB***
Isolates from humans	1167	3	31.25
Isolatesfromchickens (Broilers)	975	20	3.6
Isolates from chickens (mixed breed)	224	14.3	31.25

Figures

Figure 1.


C. freundii qnrB14
RemHUM2
RemCHK10
S. enterica subsp. enterica serovar qnrB5
E. coliC14-9qnrB19UrbanHealthyChildPeruBolivia
S. enterica subsp.qnrB19.HospBrazil
RemCHK17
RemCHK13
RemCHK12
K. pneumoniae VA367.EEUU
E. coli st013.1 strB.VealCalf.Netherlands
E. coli R4525
Ecoli Quito27
E. coli IDL132.HospItaly
RemHUM3
IndCHK24
IndCHK25
IndCHK26
K. pneumoniae DU36195 QnrB
E. cloacae K-262 qnrB4
S. flexneri 53SF qnrB
C. freundii qnrB4
E. coli qnrB4
C. werkmanii PS012 qnrB22
A. veronii bv. veronii AE13 qnrB
C. freundii RSPMQR-237-09 qnrB25
K. pneumoniae Kp4047 qnrB32
K. pneumoniae 3606 qnrB
E. coli GD006 qnrB.FarmChina
C. freundii 234BL qnrB17Espana
E. coli CQ53-1 qnrB
E. coli GD006 qnrB
K. pneumoniae HX05006382 qnrB6
E. aerogenes HX05007580 qnrB6
E. coli GD005 qnrB
P. agglomerans HX05015076 qnrB6
C. freundii 234BL qnrB17
S. flexneri 2a BC-308.India
K. pneumoniae DU35427 QnrB
A. caviae AE32 qnrB
A. sp. AE8 AE8 qnrB
A. jandaei AE25 qnrB
A. sp. AE16(2008) AE16 qnrB
A. veronii bv. veronii AE15 qnrB
A. hydrophila AE21 qnrB
A. veronii bv. veronii AE12 qnrB
S. flexneri 2a BC-308
K. pneumoniae qnrB1
A. veronii bv. veronii AE34 qnrB
S. sonnei BC-280
A. sp. AE24 AE24 qnrB
K. pneumoniae 2009 MGRP13 qnrB1
C. freundii 72857 qnrB11
E. cloacae K-44 qnrB2.Rusia
C. koseri qnrB2
S. flexneri 2a DS-566
C. werkmanii 14.0 qnrB10
A. veronii AE33 qnrBAcuaculture.SouthAfrica
A. veronii bv. veronii AE11 qnrB
A. veronii bv. veronii AE9 QnrB
C. freundii 05K1560 qnrB13
C. freundii 05K657 qnrB12
A. veronii bv. veronii AE33 qnrB
C. freundii 62778 qnrB10
C. freundii S008 qnrB23
C. freundii 06K1424 qnrB15
C. freundii 06K619 qnrB14HospKorea
C. freundii HLR20 qnrB
C. braakii S08-GS31 qnrB30.stool.Korea
C. freundii 06K619 qnrB14
C. freundii 239BL qnrB18