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Characterization of the *bla*$_{CTX-M}$ gene in *E. coli* isolates from human and chicken feces from rural communities of Esmeraldas and an industrial chicken operation in Santo Domingo

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DEDICATORIA

A mi familia que siempre me ha apoyado y ha estado presente en todo momento sea este bueno o malo, me ha guiado y enseñado lo importante de vivir, y los valores necesarios para ser una persona de bien. Y en especial a mi hijo, la persona más importante en mi vida, que llego a iluminar mi mundo y llenar mi corazón; es gracias a ti, Martin Nicolás, que encuentro cada día las fuerzas y ganas de ser mejor.

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RESUMEN

La resistencia a los antibióticos es un problema de salud pública mundial en crecimiento que causa 700.000 muertes anuales y con una rápida difusión impulsada por presión selectiva debido al uso masivo de antibióticos. Este estudio tuvo como objetivo investigar la posibilidad de transferencia horizontal de genes bla_{CTX-M} entre aislados de *E. coli* de humanos y pollos en una comunidad remota. Para lograr nuestro objetivo, caracterizamos los genes bla_{CTX-M} y sus regiones flanqueantes mediante PCR y secuenciación de ADN. El más abundante fue $bla_{CTX-M-2}$ (28,97%) seguido de $bla_{CTX-M-15}$ (11,21%) y $bla_{CTX-M-55}$ (11,21%); los genes bla_{CTX-M} de aislados de *E. coli* de pollo fueron similares a los de humanos en la misma comunidad pero diferentes a los de la operación industrial avícola, ubicada a 200 km de distancia. Los genes bla_{CTX-M} comunes entre aislados humanos y de pollo en la comunidad fueron $bla_{CTX-M-2}$, $bla_{CTX-M-14}$, $bla_{CTX-M-15}$, $bla_{CTX-M-27}$, $bla_{CTX-M-55}$ y $bla_{CTX-M-65}$. El análisis de las secuencias flanqueantes a los genes bla_{CTX-M} también mostró similitudes entre *E. coli* aisladas de humanos y pollos en la comunidad y fueron diferentes de las de la operación industrial avícola. Este estudio mostró evidencia de transferencia horizontal de genes bla_{CTX-M} entre humanos y pollos en una comunidad remota.

Palabras clave: *E. coli*, resistencia a antibióticos, transferencia horizontal de genes, bla_{CTX-M}, secuencias flanqueantes.

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ABSTRACT

Antibiotic resistance is a growing global public health problem causing 700.000 deaths annually with rapid dissemination fueled by selection pressure due to massive antibiotic use. This study aimed to investigate the possibility of horizontal gene transfer of *bla*$_{CTX-M}$ between *E. coli* isolates from humans and chickens in a remote community. In order to achieve our goal, we characterized the *bla*$_{CTX-M}$ genes and their flanking regions using PCR and DNA sequencing. The most abundant was *bla*$_{CTX-M-2}$ (28.97%) followed by *bla*$_{CTX-M-15}$ (11.21%) and *bla*$_{CTX-M-55}$ (11.21%); *bla*$_{CTX-M}$s from chicken isolates were similar to those from humans in the same community but different from those of the industrial chicken operation located 200 km away. Common *bla*$_{CTX-M}$s from human and chicken isolates in the community were *bla*$_{CTX-M-2}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-27}$, *bla*$_{CTX-M-55}$, and *bla*$_{CTX-M-65}$. Analysis of *bla*$_{CTX-M}$s' flanking sequences also showed similarity among *E. coli* isolated from humans and chickens in the community and they were different from those of the industrial chicken operation. This study showed evidence of horizontal transfer of *bla*$_{CTX-M}$ genes between humans and chickens in a remote community.

Key words: *E. coli*, antibiotic resistance, horizontal gene transfer, *bla*$_{CTX-M}$, flanking sequences.

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PART I: GENERAL INTRODUCTION

Antibiotic resistance has become a global public health problem that had been traditionally focused on humans and health care facilities. The World Health Organization (WHO) acknowledges that antimicrobial resistance is a big problem that threats worldwide health and food safety; it is no longer a future issue and now it is a reality that affects anyone of any age (WHO, 2020). We are heading into a post-antibiotic era where no infection will be treatable (Reardon, 2014). To date, it is accepted that animals and most ecosystems may contribute to antibiotic resistance selection and dissemination (Martinez et al., 2009; Hernando-Amado et al., 2020). In fact, we can find antibiotic-resistance bacteria in wild animals, natural ecosystems, or even in isolated human populations without contact with antibiotics (Clemente et al., 2015; Alonso et al., 2016).

Antimicrobial resistance as a global threat

According to the CDC's Antibiotic Resistance Threats Report, more than 2.8 million infections in the U.S. are due to antibiotic-resistance bacteria each year; further, 35.000 people die as consequence of the infections. Prevention measures taken in the U.S. reduced only 18% of all deaths caused by antibiotic-resistant infections and hospital infections by 30%; however, the population remains at high risk (CDC, 2019). These data are similar to those of the European Union, where mortality is around 30,000 each year (Cassini, 2019; Dadgostar, 2019). The WHO estimates that 700,000 people die each year due to antibiotic-resistant infections around the globe (WHO, 2019), furthermore, world reports on this problem predict that 10 million people will die across the world by 2050 due to antibiotic-resistant infections (Dadgostar, 2019). Additionally, resistant bacteria will double the chance

of developing a serious health problem in people with comorbidities, and triple the chance of death (CDC, 2019).

According to World Bank, 24 million people will be economically affected by antibiotic resistance in 2050 and they could fall into extreme poverty. World Bank also estimates that antibiotic resistance could have more impact in the economy than climate change (Hernando-Amado et al., 2020). Therefore, antibiotic resistance not only has an impact on global health, but it also has a significant economic impact (Rudholm, 2002; Jonas et al., 2017).

The information about antibiotic resistance around the globe is constantly changing (Canton et al., 2012). The One Health approach to this matter is giving light on how antibiotic-resistance genes are spreading due to use and abuse of antimicrobial in humans and domestic animals (McEwen and Collignon, 2018; Hernando-Amado et al., 2020). The spread of antibiotic-resistant genes is influenced by selection pressure due to uncontrolled use of antibiotics (leading to selection of antimicrobial-resistant bacteria), poor management of wastewater systems, close contact with domestic animals, antibiotic resistant bacteria in the food chain (Alonso et al., 2017). New studies on this topic help to have a better comprehension to develop strategies to better manage antimicrobial resistance around the globe (McEwen and Collignon, 2018).

Another current concern about antibiotic resistance is the stability in absence of selection pressure (Hernando-Amado et al., 2020). It was proposed that antibiotic resistance increased bacterial fitness cost (Andersson and Hughes, 2010); hence, it was considered that reducing antibiotics use or antibiotic-cycling strategies would decrease antibiotic resistance (Beardmore et al., 2017). Unfortunately, several studies have shown otherwise; antibiotic

resistance can increase bacterial competitiveness and not always reduces bacterial fitness cost (Schaufler et al., 2016; Dimitriu et al., 2019).

There are several resistances to antimicrobial agents that have been described since the first identification of β-lactamase on 1940; the most important have been the aminoglycoside resistance, β-lactam resistance, vancomycin resistance, macrolide resistance, quinolone resistance, colistin resistance, among others (Davies and Davies, 2010; Canton et al., 2012; Aghapour et al., 2019). There has been a special interest in antibiotic resistance due to β-lactamases, enzymes that hydrolyze the β-lactam family of antibiotics. β-lactams are the most widely used antibiotics for infections in clinical practice (Bush and Jacoby, 2010), it is understandable that β-lactam resistance is one of the most studied (Canton et al., 2012).

β-lactamases

β-lactamases are enzymes that hydrolyze β-lactam antibiotics such as penicillin, cephalosporin, monobactam, and carbapenem family; by cleaving the β-lactam ring and destroying it, which causes loss of antimicrobial function (Dhillon and Clark, 2012).

Since 1980s there have been increasing concern about β-lactamases (Davies and Davies, 2010), especially the extended spectrum β-lactamases (ESBLs) of classes A and D (Bush and Jacoby, 2010). Among the class A β-lactamases, the TEM, SHV, CTX-M, VEB, and GES enzymes represent a public health concern (Coque et al., 2008), particularly the $bla_{\text{CTX-M}}$ gene family is one of the most interesting to study because it had a rapid spread around the globe and has evolved into 234 variants until 2021 (NCBI, 2021). Without a doubt, the $bla_{\text{CTX-}}$

$_M$ gene family has had the greatest expansion worldwide among ESBLs, representing one of the greatest current public health problems (Canton et al., 2012; WHO, 2020).

Extended Spectrum β-Lactamases (ESBLs)

Extended spectrum β-lactamases (ESBLs) are a rapidly evolving group of enzymes capable of hydrolyze third-generation cephalosporins, and monobactams, they are usually inhibited by beta-lactamase inhibitors such as clavulanic acid (Rawat and Nair, 2010). β-lactamases are coded by genes carried on bacterial chromosomes and mobile plasmids making them able to spread by horizontal gene transfer (Falagas and Karageorgopoulos, 2009).

ESBLs, unlike classical β-lactamases, are able to hydrolyze third-generation cephalosporins, which is why they were given the name of extended spectrum. β-lactamases are classified according to Ambler classification in A, B, C, and D in basis on their molecular structure (Ambler, 1980). The classes A, C, and D hydrolyze the β-lactam ring using an active site of serine, class B are metalloenzymes that needs divalent zinc ions from the active site to hydrolyze the β-lactam ring (Bush and Jacoby, 2010). Later, in 1995 Bush et al. proposed a functional classification that considers substrate and inhibitor profiles in order to group β-lactamases according to their phenotype in clinical isolates: Group 1 (class C) cephalosporinases which are not inhibited by clavulanic acid, group 2 (classes A and D) broad spectrum mostly inhibited by clavulanic acid, and group 3 metallo-β-lactamases (Bush et al,. 1995). An updated system is now being used to classify β-lactamases (Bush and Jacoby, 2010), as shown in Table 1.

ESBLs have spread during the last decades rapidly around the globe, to date, we have various genotypes but the most common is the *bla*$_{CTX-M}$ gene family (Rupp and Fey, 2003). The ESBLs main families are: TEM, SHV, CTX-M, GES and VEB (class A), and OXA (class D) (Paterson and Bonomo, 2005). During the 1980s and 1990s *bla*$_{TEM}$ and *bla*$_{SHV}$ were associated with hospital outbreaks mainly by *Klebsiella pneumoniae* and *Escherichia coli*, *bla*$_{CTX-M}$ were less prevalent during this period until the 2000s when this gene rapidly disseminated (Cantón, 2012).

CTX-M family

The CTX-M family is made of a complex group of non-homogeneous enzymes, they were discovered in 1989 and since then, new variants have been described (Canton et al., 2012); 234 variants have been identified to date (NCBI, 2021). The *bla*$_{CTX-M}$ genes had rapidly spread around the globe and have displaced other ESBLs genes in Enterobacteriaceae, due to dissemination in mobile genetic elements within successful clones which have other resistances causing co-selection (Coque et al., 2008; Alonso et al., 2017). The most common are *bla*$_{CTX-M-15}$ and *bla*$_{CTX-M-14}$, they have been invading bacteria in intestines of humans and other animals (Canton et al., 2012).

Amino acid sequence alignments allowed the CTX-M family to be classified into 5 main clusters (Bonnet, 2004; Rossolini et al., 2008) they are the CTX-M-1 cluster (*bla*$_{CTX-M-1}$, *bla*$_{CTX-M-3}$, *bla*$_{CTX-M-10}$, *bla*$_{CTX-M-12}$, *bla*$_{CTX-M-15}$, *bla*$_{CTXM-32}$), CTX-M-2 cluster (*bla*$_{CTX-M-2}$, *bla*$_{CTX-M-4}$, *bla*$_{CTX-M-5}$, *bla*$_{CTX-M-6}$, *bla*$_{CTX-M-7}$, *bla*$_{CTX-20}$), CTX-M-8 cluster (*bla*$_{CTX-M-8}$, *bla*$_{CTX-M-40}$, *bla*$_{CTX-M-63}$), CTX-M-9 cluster (*bla*$_{CTX-M-9}$, *bla*$_{CTX-M-13}$, *bla*$_{CTXM-14}$, *bla*$_{CTX-M-21}$, *bla*$_{CTX-M-24}$, *bla*$_{CTX-M-27}$), and CTX-

M-25 cluster (*bla*$_{CTX-M-25}$, *bla*$_{CTX-M-26}$, *bla*$_{CTX-M-91}$) (Galvis and Moreno, 2009; Canton et al., 2012).

Origin of the *bla*$_{CTX-M}$ gene family

Historically, *bla*$_{CTX-M}$ genes emerged from different and distant geographic areas in the 1990, whereupon a wide and rapid spread of *bla*$_{CTX-M}$ genes befell, this allowed the evolution of new variants including *bla*$_{CTX-M-3}$, *bla*$_{CTX-M-9}$, *bla*$_{CTX-M-14}$, and *bla*$_{CTX-M-15}$ from 1994 to 2000. Finally, since 2000 a global dispersion of the *bla*CTX-M genes took place (Canton et al., 2012).

Phylogenetic studies suggest that *bla*$_{CTX-M}$ were originated by the incorporation of *bla* genes into mobile genetic elements from the environmental bacteria *Kluyvera* spp. chromosome (Table 2). These original mobilized *bla*$_{CTX-M}$ genes conferred resistance to cefotaxime, making them successful ESBLs (Canton et al., 2012). Later *bla*$_{CTX-M}$ diverged by single nucleotide mutations and variants with enhanced hydrolytic activity were selected by antibiotic pressure (Bonnet, 2004).

The CTX-M clusters have different origins in different species of a soil bacterium *Kluyvera*. The ancestor of the CTX-M-1 cluster is the chromosomal gene *kluC* in *Kluyvera cryocrescens* (Decousser et al., 2001), the CTX-M-2 cluster was originated from *kluA* gene in *Kluyvera ascorbata* (Humeniuk et al., 2002), the chromosomal genes of *Kluyvera georgiana* (*kluG*, *kluY*, and *bla*$_{CTX-M-78}$) gave rise to the CTX-M-8 cluster (Poirel et al., 2002), the CTX-M-9 cluster (Olson et al., 2005), and the CTX-M-25 cluster (Rodríguez et al., 2010) respectively (Table 2). A new cluster was described by Minarini et al. 2009, with the characterization of *bla*$_{CTX-M-74}$ and *bla*$_{CTX-M-75}$ with one amino acid change respect to *bla*$_{CTX-M-2}$ (Minarini et al.,

2009), several authors have observed nucleotide divergence in new variants; suggesting a convergent evolution of *bla*$_{CTX-M}$ genes (Stepanova et al., 2008). It has been speculated that *bla*$_{CTX-M}$ genes have been circulating among different *Kluyvera* spp. and that they could have gained the genes after species diverged, however it is not possible to completely understand this topic because there are not studies about *bla*$_{klu}$ genes and their genetic environment (upstream and downstream sequences) in susceptible *Kluyvera* spp. isolates (Canton et al., 2012).

Genetic environment of *bla*$_{CTX-M}$ genes

The original *bla*$_{klu}$ genes of *Kluyvera* spp. do not have a strong promoter upstream resulting in weak gene expression and low MIC values, however the insertion sequence (IS) provided the missing strong promoter (Canton et al., 2012), resulting in increased expression. This suggests that the insertion sequence has a role in the spread and selection of *bla*$_{CTX-M}$ genes by causing the bacteria to express antibiotic resistance. In this context, the study of the genetic environment of the *bla*$_{CTX-M}$ genes has shed light on understanding the mobilization events that have occurred.

The capture of *bla* genes by IS*Ecp*1 is highly frequent and it was demonstrated in vitro experiments by Lartigue et al. (2006) proving the mobilization of *bla* genes (Lartigue et al., 2006). Several studies have reported that the most prevalent insertion sequence associated with *bla*$_{CTX-M}$ genes is the IS*Ecp*1. Unfortunately, information about downstream nucleotide sequences is missing (Canton et al., 2012), but *orf477* was reported as one of the most prevalent (Hu et al., 2018). Besides IS*Ecp*1, other insertion sequences have been described upstream *bla*$_{CTX-M}$ genes, including IS*CR*1, IS*10*, and IS*26.* IS*Ecp*1 has been

associated with almost all CTX-M clusters, except for the CTX-M-8 cluster (Canton et al., 2012). Finally, it has been reported that other elements have been found interrupting IS*Ecp*1 or the corresponding insertion sequence located upstream *bla*$_{CTX-M}$ genes (Eckert et al., 2006).

There are spacers sequences located between IS*Ecp*1 and *bla*$_{CTX-M}$ genes that have been well studied. The distance between the IS and *bla*$_{CTX-M}$ genes is related to resistance phenotypic, expressed in high MIC values, the lengths of these spacers are closely linked to the gene expression of *bla*$_{CTX-M}$ genes. It has been observed that plasmids carrying *bla*$_{CTX-M}$ genes with IS*Ecp1* located upstream and with shorter length of spacer sequence have higher gene expression, possibly due to the strong promoter provided by IS*Ecp1* (Ma et al., 2011). Sequence analyses of these elements, belonging to CTX-M-1 cluster, have shown homology among spacer sequences; suggesting that these *bla*$_{CTX-M}$ genes could derive from a single transposition event. The spacer sequences of CTX-M-8 cluster, CTX-M-9 cluster, and CTX-M-25 cluster were analyzed and they showed no homology among their spacers; suggesting different sources, even though they all come from the same ancestor, *Kluyvera georgiana.* These results also suggest that it is possible to have another organism as origin of clusters attributed to *K. georgiana,* they could even be other non-described *Kluyera* spp. (Canton et al., 2012).

Several studies have reported the rapid and wide distribution of *bla*$_{CTX-M}$ genes around the globe, even international organizations recognize this problem as a potential threat to global health. Molecular epidemiological surveillance of these genes is required to better understand how this global dissemination occurs in order to make correct decisions in public health. With the advent of whole genome sequencing it has been possible to know

the origin of these genes but it is expensive for developing countries as Ecuador, whereby it is important to find cheaper and faster alternatives to trace the origin of these genes. The study of flanking sequences of *bla*$_{CTX-M}$ genes can partially provide data to this lack of information without having to depend on whole genome sequencing.

The aim of this study was to investigate the possibility of horizontal gene transfer of *bla*$_{CTX-M}$ between *E. coli* isolates from humans and chickens feces, in a community setting. In order to achieve our goal, we characterized the *bla*$_{CTX-M}$ genes in these *E. coli* isolates and their respective flanking regions.

Table 1. β-lactamases classification scheme from Bush et al., 2010.

Bush-Jacoby	Ambler	Substrate	CA or TZB Inhibition	EDTA Inhibition	Characteristics	Enzymes
1	C	Cephalosporins	No	No	Greater hydrolysis of cephalosporins than benzylpenicillin; hydrolyzes cephamycins	*E. coli* AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1
1e	C	Cephalosporins	No	No	Increased hydrolysis of ceftazidime and often other oxyimino-β-lactams	GC1, CMY-37
2a	A	Penicillins	Yes	No	Greater hydrolysis of benzylpenicillin than cephalosporins	PC1
2b	A	Penicillins, I generation cephalosporins	Yes	No	Similar hydrolysis of benzylpenicillin and cephalosporins	TEM-1, TEM-2, SHV-1
2be	A	Extended-spectrum cephalosporins, monobactams	Yes	No	Increased hydrolysis of oxyimino-β-lactams (cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam)	TEM-3, SHV-2, CTX-M-15, PER-1, VEB-1
2br	A	Penicillins	No	No	Resistance to clavulanic acid, sulbactam, and tazobactam	TEM-30, SHV-10
2ber	A	Extended-spectrum cephalosporins, monobactams	No	No	Increased hydrolysis of oxyimino-β-lactams combined with resistance to clavulanic acid, sulbactam, and tazobactam	TEM-50
2c	A	Carbenicillin	Yes	No	Increased hydrolysis of	PSE-1, CARB-3

					carbenicillin	
2ce	A	Carbenicillin, cefepime	Yes	No	Increased hydrolysis of carbenicillin, cefepime, and cefpirome	RTG-4
2d	D	Cloxacillin	Variable	No	Increased hydrolysis of cloxacillin or oxacillin	OXA-1, OXA-10
2de	D	Extended-spectrum cephalosporins	Variable	No	Hydrolyzes cloxacillin or oxacillin and oxyimino-β-lactams	OXA-11, OXA-15
2df	D	Carbapenems	Variable	No	Hydrolyzes cloxacillin or oxacillin and carbapenems	OXA-23, OXA-48
2e	A	Extended-spectrum cephalosporins	Yes	No	Hydrolyzes cephalosporins. Inhibited by clavulanic acid but not aztreonam	CepA
2f	A	Carbapenems	Variable	No	Increased hydrolysis of carbapenems, oxyimino-β-lactams, cephamycins	KPC-2, IMI-1, SME-1
3a	B (B1)	Carbapenems	No	Yes	Broad-spectrum hydrolysis including carbapenems but not monobactams	IMP-1, VIM-1, CcrA, IND-1
	B (B3)					L1, CAU-1, GOB-1, FEZ-1
3b	B (B2)	Carbapenems	No	Yes	Preferential hydrolysis of carbapenems	CphA, Sfh-1

CA: clavulanic acid
TZB: tazobactam

Table 2. CTX-M clusters and origin in *Kluivera* spp.

CTX-M Cluster	***Kluivera* spp.**	**Chromosomal gen**
CTX-M-1	*K. cryocrencens*	*KluC*
CTX-M-2	*K. ascorbata*	*KluA*
CTX-M-8	*K. gregoriana*	*KluG*
CTX-M-9	*K. gregoriana*	*KluY*
CTX-M-25	*K. gregoriana*	$bla_{\text{CTX-M-78}}$

PART II: SCIENTIFIC ARTICLE

Characterization of the *bla*$_{CTX-M}$ gene in *E. coli* isolates from human and chicken feces from rural communities of Esmeraldas and an industrial chicken operation in Santo Domingo.

Introduction

Antibiotic resistance is currently recognized, by multiple international institutions, as a global public health problem (CDC, 2019; WHO, 2020). The World Health Organization (WHO) estimates that 700.000 people die annually, due to antibiotic-resistant infections, and will be 10 million by 2050 (WHO, 2019; Dadgostar, 2019). Every year reports on multi-resistant microorganisms increase in different countries, indicating that we are heading into a post-antibiotic era where no antibiotic will work to combat infectious diseases (Reardon, 2014). Different prevention and control measures have been proposed to mitigate this problem, but they seem to have a low impact (CDC, 2019; Cassini, 2019; Dadgostar, 2019).

Antibiotic-resistance genes (ARGs) are spreading due to selection pressure induced by uncontrolled use of antibiotics in humans and domestic animals (Alonso et al., 2017; McEwen and Collignon, 2018; Hernando-Amado et al., 2020). It is now known that animals and most ecosystems may contribute to antibiotic resistance selection and dissemination (Martinez et al., 2009; Hernando-Amado et al., 2020). Increased small-scale farming in low-resource settings has allowed for greater use of antibiotics as growth promoters globally,

therefore creating reservoirs of antibiotic-resistant bacteria in animals and playing an important role in their spread (Hedman et al., 2019a).

While some ARGs are widely disseminated in the *E. coli* because they emerged more than 60 years ago (Tadesse et al., 2012), other genes (such as bla_{CTX-M}) entered *E. coli* in 1990s (Canton et al., 2012) and different allelic variants are associated to *E. coli* from different locations and sources (Day et al., 2019; Ludden et al., 2019).

The aim of this study was to investigate the possibility of horizontal gene transfer of bla_{CTX-M} genes between *E. coli* from humans and chickens, in a community setting and to compare with allelic variants of bla_{CTX-M} associated with an industrial chicken operation located 200 km from the community.

Materials and methods

Samples

Samples were previously collected from human and chicken feces in rural communities of Esmeraldas during 2010-2013 (Braykov et al., 2016) and during 2017 (Hedman et al., 2019a), and in an industrial chicken operation in Santo Domingo during 2010. Fecal samples were collected from chickens by cloacal swabs, human fecal samples were provided by parents in the case of minors or by themselves in the case of adults, with prior consent. The humans sampled from the community lived in close contact with the sampled chickens. (Braykov et al., 2016; Hedman et al., 2019a).

Samples were direct cultured in MacConkey Agar for 24 hours at 37˚C, every Gram-negative lactose fermenting colony was tested using biochemical reactions in different

culture media to identify genera and specie; *E. coli* isolates were obtained and tested to antibiotics to determinate their resistance profile. All *E. coli* isolates were stored frozen at -80°C. We revived 166 isolates of *E. coli* third-generation cephalosporin resistant (TGCR) previously tested. All *E. coli* TGCR isolates were cultured in MacConkey Agar and incubated for 24 hours at 37°C, we obtained lactose fermenting colonies that were cultured in Chromocult® Agar to confirm genus and species. Isolates were finally tested with cefotaxime, using Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) recommendations, to confirm TGCR bacteria. We obtained 107 *E. coli* TGCR.

Conjugation experiments

We conducted conjugation experiments to find out if resistance to cefotaxime could be transferred due to mobile genetic elements. We used *E. coli* J53 strain (resistant to sodium azide and susceptible to cefotaxime) as recipient bacteria, and all our previously isolated *E. coli* TGCR as donors. Conjugation experiments were performed in Lysogeny Broth (LB) with *E. coli* J53Az^{r} as the recipient. We culture donor and recipient in tubes with 5 ml of LB incubated for 12 hours at 37°C, to get cells in logarithmic phase (Zhang et al., 2017), after then we added 0.5 ml of each tube to 4 ml of fresh LB and incubated for 16 hours at 37°C without shaking (Wang et al., 2003). Transconjugants were selected using selective culture media made of Trypticase soy agar (TSA) supplied with sodium azide (200 ug/ml) and cefotaxime (1mg/ml). All transconjugants were stored frozen at -80°C.

Conventional PCR

Genetic materials from all *E. coli* TGCR isolates were extracted using DNAzol® (Invitrogen™, USA) following manufacturer protocol and recommendations. We performed a conventional PCR to find samples carrying bla_{CTX-M} gene (PCR1); to find the sequences upstream bla_{CTX-M} gene (PCR2), and to find sequences downstream bla_{CTX-M} (PCR3).

PCR1 was carried out using degenerated primers (Fang et al., 2008). PCR2 used bla_{CTX-M} gene and IS*Ecp1* sequences (Poirel et al., 2003); both sequences are often in close proximity. PCR3 was designed to obtain complete coding sequences of bla_{CTX-M} genes and the downstream sequences; we used degenerated primers of bla_{CTX-M} and *orf477* (Hu et al., 2018).

PCR amplification reactions were performed in 30 ul containing 6ul of 5X PCR buffer, 3.6 ul of 25 mM $MgCl_2$, 3 ul of 2mM dNTPs, 0.6 ul of 10 uM primer forward, 0.6 ul of 10 uM primer reverse, 0.2 ul of 5 u/ul Invitrogen Taq DNA Polymerases (Invitrogen™, USA), 14 ul of UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen™, USA), and 2 ul of DNA template. The cycling parameters were as follows: initial denaturation at 95°C for 15 min; followed by 30 cycles of 94°C for 30 s, 62°C for 90 s, and 72°C for 60 s; final extension at 72°C for 10 min (Fang et al., 2008). The obtained amplicons were subjected to electrophoresis at a 1.5% agarose gel in 1X TBE buffer. Finally, all amplicons obtained were sequenced in Michigan State University with Sanger sequencing. Primers used in this study are listed in Table 1 and Figure 1.

Results

Samples

A total of 107 *E. coli* TGCR were isolated from human and chicken feces; 38 (35.5%) were obtained from human feces and 69 (64.5%) were from chicken feces. The chicken isolates from industrial operation and community were 43 (62.3%) and 26 (37.7%), respectively (Table 2).

Conjugation

We obtained 105 transconjugants from individual isolates carrying $bla_{\text{CTX-M}}$ genes; 38 (36.2%) from human feces and 67 (63.8%) from chicken feces. Of the *E. coli* isolates obtained from chicken feces, 43 (64.2%) were from industrial operation and 26 (38.8%) were from the remote community. We performed conjugation experiments again on the 2 unconjugated isolates and obtained the same results. We suspect that the gene in these *E. coli* TGCR isolates was contained in a non-conjugative plasmid or was a bacterial chromosomal gene. The unconjugated isolates where from the industrial chicken operation.

PCR and sequence analysis of $bla_{CTX\text{-}M}$ genes

We obtained 102 amplicons, of the 105 transconjugants, in PCR1 assay. All amplicons were sequenced and we obtained DNA sequences from 94 of the 102 amplicons; nucleotide sequence indicated that the most abundant was $bla_{\text{CTX-M-2}}$ followed by $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-55}}$ (Table 3). $bla_{\text{CTX-M- 1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-64}}$, and $bla_{\text{CTX-M-65}}$ were present in isolates from human feces, and $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-}}$

$_{M\text{-}12}$, *bla*$_{CTX\text{-}M\text{-}14}$, *bla*$_{CTX\text{-}M\text{-}15}$, *bla*$_{CTX\text{-}M\text{-}27}$, *bla*$_{CTX\text{-}M\text{-}55}$, *bla*$_{CTX\text{-}M\text{-}64}$, and *bla*$_{CTX\text{-}M\text{-}65}$ were present in isolates from chicken feces; *bla*$_{CTX\text{-}M}$s from chickens feces isolates were different between chicken in industrial operation (*bla*$_{CTX\text{-}M\text{-}2}$, *bla*$_{CTX\text{-}M\text{-}8}$, *bla*$_{CTX\text{-}M\text{-}12}$, and *bla*$_{CTX\text{-}M\text{-}14}$) and chicken in the community (*bla*$_{CTX\text{-}M\text{-}15}$, *bla*$_{CTX\text{-}M\text{-}27}$, *bla*$_{CTX\text{-}M\text{-}55}$, *bla*$_{CTX\text{-}M\text{-}64}$, and *bla*$_{CTX\text{-}M\text{-}65}$). Common *bla*$_{CTX\text{-}M}$s from human and chicken isolates were *bla*$_{CTX\text{-}M\text{-}2}$, *bla*$_{CTX\text{-}M\text{-}14}$, *bla*$_{CTX\text{-}M\text{-}15}$, *bla*$_{CTX\text{-}M\text{-}27}$, *bla*$_{CTX\text{-}M\text{-}55}$, and *bla*$_{CTX\text{-}M\text{-}65}$. We found that *bla*$_{CTX\text{-}M\text{-}1}$ and *bla*$_{CTX\text{-}M\text{-}9}$ were present only in isolates from human feces, while *bla*$_{CTX\text{-}M\text{-}8}$ and *bla*$_{CTX\text{-}M\text{-}12}$ were present only in isolates from chicken feces.

We found that 63 isolates (61.8%) had the insertion sequence IS*Ecp1* upstream *bla*$_{CTX\text{-}M}$ gene, 32 (50.8%) corresponded to human isolates and 31 (49.2%) to chicken isolates; 6 (19.4%) from chicken in industrial operation and 25 (80.6%) from chicken in the community. Further, from the 63 isolates, 18 isolates (28.6%) had *orf477* downstream *bla*$_{CTX\text{-}M}$ gene, 3 (16.7%) corresponded to human isolates and 15 (83.3%) to chicken isolates; 1 (6.7%) from chicken in industrial operation and 14 (93.3%) from chicken in the community (Table 2).

We found 5 different spacer sequences located between IS*Ecp1* and *bla*$_{CTX\text{-}M}$ gene; 21 (33.33%) showed 42 bp spacer, 14 (22.22%) had a 127 bp spacer, 9 (14.29%) had a 48 bp spacer, 8 (12.70%) had a 45 bp spacer, and 1 (1.59%) had an 80 bp spacer (Table 4). The spacer sequences in human isolates were mainly 42 bp and 48 bp lengths, while chicken isolates were 127 bp, 42 bp and 45 bp. The most abundant spacer was 42 bp in human and chicken isolates. Further, chicken isolates from industrial operation and community were different sizes, 42 bp and 127 bp, respectively. We found that all same-size spacer contained identical sequences and were found in isolates from chickens and humans, one 80 bp spacer was present only in one human isolate.

We found 6 *bla*$_{CTX-M}$ allelic variants in different isolates, which have not been described before (Table 5). We identified several single nucleotide polymorphisms (SNPs) in these sequences; most of them were synonymous mutations. We were able to identify a possible recombinant of *bla*$_{CTX-M-15}$ and *bla*$_{CTX-M-137}$ in one isolate (Figure 2). All accession numbers for these allelic variants are listed in table 5.

Discussion

This study aimed to investigate the possibility of horizontal gene transfer of *bla*$_{CTX-M}$ genes between *E. coli* strains from humans and chickens in a remote community. Based on PCR experiments and sequencing analysis, of human and chicken *E. coli* isolates carrying *bla*$_{CTX-M}$ genes, we reconstructed the genes and their flanking upstream sequences allowing us to determine the relationship between the spacer and *bla*$_{CTX-M}$ alleles. We found the same spacer, and *bla*$_{CTX-M}$ alleles in different isolates from human and chickens in the same community; the *bla*$_{CTX-M}$ alleles in the community were different from those found in the industrial chicken operation. Taking together these data suggest horizontal transfer of *bla*$_{CTX-M}$ genes between *E. coli* from humans and chickens in the community. Other researchers have presented evidence of ARGs transmission between *E. coli* from humans and domestic animals, due to direct contact with domestic animals or by ingestion of food contaminated with *E. coli* (Sheppard et al., 2016; Alonso et al., 2017; Hedman et al., 2019a; Hedman et al., 2019b; Salinas et al., 2021). Also, other studies have shown that different *bla*$_{CTX-M}$ genes are found in isolates from different locations, sources, and periods (Day et al., 2019; Ludden et al., 2019)

In Ecuador, *bla*$_{CTX-M}$s have been frequently reported and the more prevalent variants are: *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-55}$, and *bla*$_{CTX-M-65}$ in clinical isolates (Delgado et al., 2016; Ortega-Paredes et al., 2019; Segarra et al., 2018). Studies in Ecuadorian rural communities have shown that humans in close contact with domestic animals carry *E. coli* with *bla*$_{CTX-M-2}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-55}$, and *bla*$_{CTX-M-65}$ in bacterial isolates of the community, suggesting that horizontal gene transfer is happening in *E. coli* from different sources in these rural communities (Parra, 2018; Salinas et al., 2021). Our findings support these previous reports; we identified *bla*$_{CTX-M-2}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-27}$, *bla*$_{CTX-M-55}$, and *bla*$_{CTX-M-65}$ in isolates from human and chickens, especially *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-27}$, *bla*$_{CTX-M-55}$, and *bla*$_{CTX-M-65}$ were found in isolates of human and chickens in the same community and in close contact. Further, we found that *bla*$_{CTX-M-1}$, and *bla*$_{CTX-M-9}$ were present only in isolates from human, while *bla*$_{CTX-M-8}$, and *bla*$_{CTX-M-12}$ were present only in isolates from chicken feces; these chicken isolates were from industrial operation and were not found in the community. Human isolates collected in Esmeraldas during 2010-2013 were mainly carrying *bla*$_{CTX-M-9}$, *bla*$_{CTX-M-14}$, and *bla*$_{CTX-M-15}$ while those collected during 2017 were mainly carrying *bla*$_{CTX-M-15}$; according with other authors *bla*$_{CTX-M-15}$ prevalence in clinical isolates has been increasing in most countries, and is dominant in most regions (Bevan, et al. 2017)

Previous research has described the close relationship between the IS*Ecp1* (and other insertion sequences) with *bla*$_{CTX-M}$ genes (Zong et al., 2010; Fei Tian et al., 2011). These insertion sequences are thought to have been involved in the mobilization and dissemination of these genes from the original bacteria *Kluyvera* (Canton et al, 2012). Most of the members of the *bla*$_{CTX-M}$ gene family have been associated with IS*Ecp1* and 5 different DNA spacer sequences between the insertion sequence and the *bla*$_{CTX-M}$ gene (Canton et al,

2012). The length of the spacer sequence is associated with specific *bla*$_{CTX-M}$ gene (Ma et al. 2011). Plasmids carrying *bla*$_{CTX-M}$ genes and IS*Ecp1* with shorter spacer (42 bp) may have higher gene expression (Ma et al. 2011). We found that almost all same *bla*$_{CTX-M}$ alleles had the same spacer sequence upstream, suggesting that these *bla*$_{CTX-M}$ genes could derive from a single transposition event from *Kluyvera*, as previously reported (Canton et al, 2012).

It is interesting that the *bla*$_{CTX-M}$ gene family have had a rapid and world wide spread with a high diversification from 2000 to the present, even displacing other ESBLs families from their niches (Livermore et al., 2007) our findings are consistent with this notion; we identified different *bla*$_{CTX-M}$ in *E. coli* from human and domestic animals in the same community, close by communities, and in an industrial operation separated by approximately 200 kms.

We detected a possible recombinant of *bla*$_{CTX-M-137}$ and *bla*$_{CTX-M-15}$ in only one isolate, being the first 218 nucleotides form *bla*$_{CTX-M-137}$ and the rest from *bla*$_{CTX-M-15}$ (Figure 2). It is the first report of this allelic variant; we could not find other sequences close to it during BLAST search (accession number MZ314224).

Additionally, we identified nonsynonymous mutation present in *bla*$_{CTX-M}$ alleles from different isolates (Table 5); one of these SNPs was located in the nucleotide 781 (c.781G>A) and coded for Isoleucine instead of Valine (p.V261I). This mutation has not been reported before and was present in different isolates that were carrying different *bla*$_{CTX-M}$ from humans and domestic animals; supporting the possibility of transmission of these genes in a community setting. We also identified 19 synonymous and 6 nonsynonymous mutations in a *bla*$_{CTX-M-64-like}$ gene from human feces isolate; this mutation has not been reported before. Unfortunately, we could not get complete coding sequences of all these allelic variants of

bla$_{CTX-M}$ genes. These findings may be sequencing artifacts and warrant additional investigation.

Studying flanking regions of *bla*$_{CTX-M}$ genes could help us determine if genes in different bacteria have recent common ancestor and close relationship. In this work we found isolates of *E. coli* carrying the same *bla*$_{CTX-M}$ gene, same insertion sequences and same spacer, but from different source; suggesting cross-colonization of animal species or horizontal gene transfer between *E. coli* of human and domestic animals. The more elements are the same, the greater the probability that it is the same plasmid that has spread.

We present evidence that transmission of antibiotic-resistance genes is frequently happening between bacteria in humans and domestic animals in a community setting. The importance of our work is that we could identify the presence of the same *bla*$_{CTX-M}$ gene with identical upstream regions in different isolates of the same community, suggesting the spread of plasmids between species.

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TABLES AND FIGURES

Table 1. Sequences of primers used in PCR experiments in this study

PCR	Primer forward	Primer reverse
PCR1	5'-ATGTGCAGYACCAGTAARGTKATGGC-3'	5'-TGGGTRAARTARGTSACCAGAAYCAGCGG-3'
PCR2	5'-TGCTCTGTGGATAACTTGC-3'	5'-GCCATMACYTTACTGGTRCTGCACAT-3'
PCR3A	5'-GAATACTGATGTAACACGGATTG-3'	5'-TCGTTTCGTGGTGCTGAATTT-3'
PCR3B	5'-CGTMTCTTYCAGAATAAGGAATCCC-3'	5'-TCGTTTCGTGGTGCTGAATTT-3'

Table 2. Overall data of *E.coli* isolates from human and chicken feces.

Isolate	Source	Collection place	Year	bla_{CTX-M}	Upstream Spacer	IS Upstream	Downstream
117	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
118	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
119	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
121	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
127	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
129	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
133	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
134	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
135	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
138	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
140	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
141	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
142	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
143	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
144	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
149	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
150	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
152	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
153	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
154	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
155	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
156	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
157	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
158	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
163	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
164	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
165	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
166	Chicken	Industrial operation	2010	2	N/A	N/A	N/A
136	Chicken	Industrial operation	2010	8	N/A	N/A	N/A
145	Chicken	Industrial operation	2010	8	N/A	N/A	N/A

146	Chicken	Industrial operation	2010	8	N/A	N/A	N/A
148	Chicken	Industrial operation	2010	8	N/A	N/A	N/A
151	Chicken	Industrial operation	2010	8	N/A	N/A	N/A
124	Chicken	Industrial operation	2010	12	48 bp	ISEcp1	N/A
126	Chicken	Industrial operation	2010	12	48 bp	ISEcp1	ORF477
130	Chicken	Industrial operation	2010	14	42 bp	ISEcp1	N/A
131	Chicken	Industrial operation	2010	14	42 bp	ISEcp1	N/A
139	Chicken	Industrial operation	2010	14	42 bp	ISEcp1	N/A
160	Chicken	Industrial operation	2010	14	42 bp	ISEcp1	N/A
40	Human	Community	2010-2013	2	N/A	N/A	N/A
41	Human	Community	2010-2013	2	N/A	N/A	N/A
42	Human	Community	2010-2013	2	N/A	N/A	N/A
51	Human	Community	2010-2013	9	42 bp	ISEcp1	N/A
53	Human	Community	2010-2013	9	42 bp	ISEcp1	N/A
54	Human	Community	2010-2013	9	42 bp	ISEcp1	N/A
56	Human	Community	2010-2013	9	42 bp	ISEcp1	N/A
36	Human	Community	2010-2013	14	42 bp	ISEcp1	N/A
38	Human	Community	2010-2013	14	42 bp	ISEcp1	N/A
39	Human	Community	2010-2013	14	N/A	ISEcp1	N/A
29	Human	Community	2010-2013	15	48 bp	ISEcp1	N/A
30	Human	Community	2010-2013	15	48 bp	ISEcp1	N/A
31	Human	Community	2010-2013	15	N/A	ISEcp1	N/A
34	Human	Community	2010-2013	64	48 bp	ISEcp1	N/A
55	Human	Community	2010-2013	65	42 bp	ISEcp1	N/A
35	Human	Community	2010-2013	137/15	42 bp	ISEcp1	N/A
63	Human	Community	2017	1	N/A	N/A	N/A
100	Human	Community	2017	1	80 bp	ISEcp1	N/A
90	Human	Community	2017	9	42 bp	ISEcp1	N/A
60	Human	Community	2017	15	N/A	N/A	N/A
68	Chicken	Community	2017	15	127 bp	ISEcp1	N/A
73	Human	Community	2017	15	N/A	ISEcp1	N/A
74	Human	Community	2017	15	48 bp	ISEcp1	N/A
87	Human	Community	2017	15	48 bp	ISEcp1	ORF477
94	Chicken	Community	2017	15	127 bp	ISEcp1	ORF477
96	Human	Community	2017	15	48 bp	ISEcp1	N/A
99	Human	Community	2017	15	48 bp	ISEcp1	N/A
65	Human	Community	2017	27	42 bp	ISEcp1	N/A
69	Human	Community	2017	27	42 bp	ISEcp1	N/A
89	Human	Community	2017	27	42 bp	ISEcp1	N/A
101	Chicken	Community	2017	27	42 bp	ISEcp1	N/A
66	Chicken	Community	2017	55	127 bp	ISEcp1	ORF477
67	Chicken	Community	2017	55	127 bp	ISEcp1	ORF477
76	Human	Community	2017	55	127 bp	ISEcp1	ORF477
78	Chicken	Community	2017	55	127 bp	ISEcp1	ORF477
85	Chicken	Community	2017	55	127 bp	ISEcp1	ORF477
93	Chicken	Community	2017	55	127 bp	ISEcp1	ORF477

95	Chicken	Community	2017	55	127 bp	ISEcp1	ORF477
102	Chicken	Community	2017	55	127 bp	ISEcp1	N/A
103	Chicken	Community	2017	55	127 bp	ISEcp1	ORF477
104	Chicken	Community	2017	55	127 bp	ISEcp1	ORF477
105	Chicken	Community	2017	55	127 bp	ISEcp1	ORF477
106	Chicken	Community	2017	55	127 bp	ISEcp1	ORF477
70	Chicken	Community	2017	64	45 bp	ISEcp1	N/A
71	Chicken	Community	2017	64	45 bp	ISEcp1	ORF477
72	Chicken	Community	2017	64	45 bp	ISEcp1	N/A
75	Human	Community	2017	64	45 bp	ISEcp1	ORF477
80	Chicken	Community	2017	64	45 bp	ISEcp1	ORF477
81	Chicken	Community	2017	64	45 bp	ISEcp1	N/A
82	Chicken	Community	2017	64	45 bp	ISEcp1	N/A
83	Chicken	Community	2017	64	45 bp	ISEcp1	ORF477
62	Chicken	Community	2017	65	42 bp	ISEcp1	N/A
77	Chicken	Community	2017	65	42 bp	ISEcp1	N/A
92	Chicken	Community	2017	65	42 bp	ISEcp1	N/A
98	Chicken	Community	2017	65	42 bp	ISEcp1	N/A

Table 3. Different types of *bla*$_{\text{CTX-M}}$ genes found in *E.coli* isolates from human and chicken feces.

***bla*$_{\text{CTX-M}}$**	**Human**	**Chicken**	**Total**	**Percent**	**Chicken (industrial operation)**	**Chicken (community)**
1	2	0	2	1.87	0	0
2	3	28	31	28.97	28	0
8	0	5	5	4.67	5	0
9	5	0	5	4.67	0	0
12	0	2	2	1.87	2	0
14	3	4	7	6.54	4	0
15	10	2	12	11.21	0	2
27	3	1	4	3.74	0	1
55	1	11	12	11.21	0	11
64	2	7	9	8.41	0	7
65	1	4	5	4.67	0	4
No Data	8	5	13	12.15	4	1

Table 4. Spacers upstream *bla*$_{CTX-M}$ genes in *E.coli* isolates from humans and chickens.

Spacer	Human	Chicken	Total	Percent	Chicken (industrial operation)	Chicken (community)
42 bp	14	9	21	33.3	4	5
45 bp	1	7	8	12.7	0	7
48 bp	8	2	9	14.3	2	0
80 bp	1	0	1	1.6	0	0
127 bp	1	13	14	22.2	0	13
No Data	10	0	10	15.9	0	0

Table 5. Allelic variants of *bla*$_{CTX-M}$ genes found in *E.coli* isolates from human and chicken feces.

Isolate	Source	Collection place	*bla*$_{CTX-M}$	SNPs	Mutation	DNA sequence change	Amino acid change	Accession number
51	Human	Community	9	3	1 synonymous	c.219T>C	Not change	MZ314220
53	Human	Community			2 nonsynonymous	c.701C>T	p.A234V	
54	Human	Community				c.781G>A	p.V261I	
56	Human	Community						
90	Human	Community						
36	Human	Community	14	2	1 synonymous	c.219T>C	Not change	MZ314221
38	Human	Community			1 nonsynonymous	c.781G>A	p.V261I	
130	Chicken	Industrial						
131	Chicken	operation						
139	Chicken	Industrial						
160	Chicken	operation						
65	Human	Community	27	1	nonsynonymous	c.781G>A	p.V261I	MZ314222
69	Human	Community						
89	Human	Community						
101	Chicken	Community						
55	Human	Community	65	3	2 synonymous	c.219T>C	Not change	MZ314223
62	Chicken	Community			1 nonsynonymous	c.228A>G	Not change	
77	Chicken	Community				c.781G>A	p.V261I	
92	Chicken	Community						
98	Chicken	Community						
34	Human	Community	64	25	19 synonymous	c.208C>G	p.P70A	MZ314225
					6 nonsynonymous	c.210A>G	p.P70A	
						c.219T>C	Not change	
						c.678T>C	Not change	
						c.681A>C	Not change	
						c.682C>T	Not change	

					c.684G>A	Not change	
					c.687T>G	Not change	
					c.688G>A	p.A230T	
					c.690T>G	p.A230T	
					c.693C>G	Not change	
					c.697G>A	p.V233T	
					c.698T>C	p.V233T	
					c.705G>T	Not change	
					c.711A>G	Not change	
					c.723T>C	Not change	
					c.725G>A	p.G242D	
					c.729T>C	Not change	
					c.741C>T	Not change	
					c.747C>T	Not change	
					c.762A>G	Not change	
					c.763A>C	p.K255Q	
					c.765A>G	p.K255Q	
					c.767C>G	p.D256G	
					c.789A>G	Not change	
35	Human	Community	137 and 15 hybrid	Recombination			MZ314224

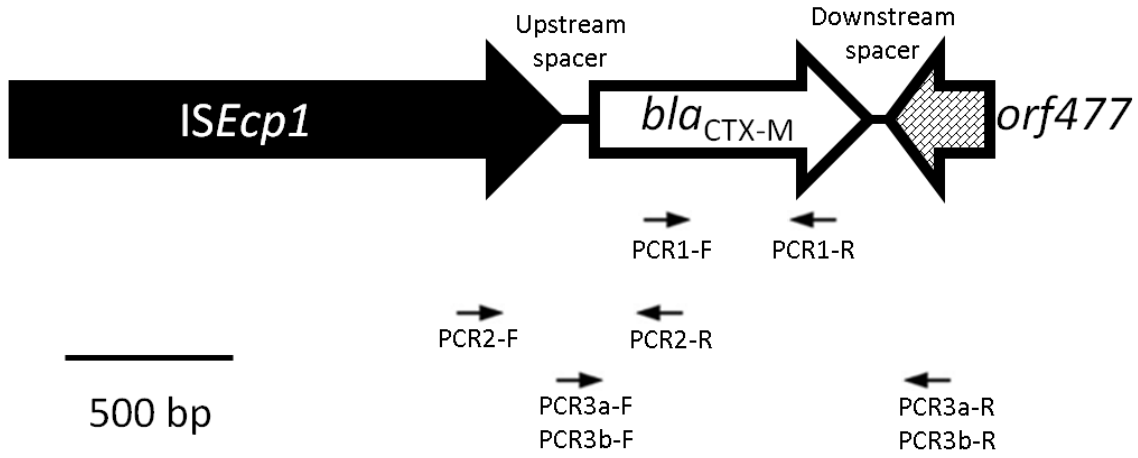

Figure 1. Schematic representation of usual orientation of the *bla*$_{CTX-M}$ genes, upstream and downstream regions. Arrows indicates the primers used in this study.

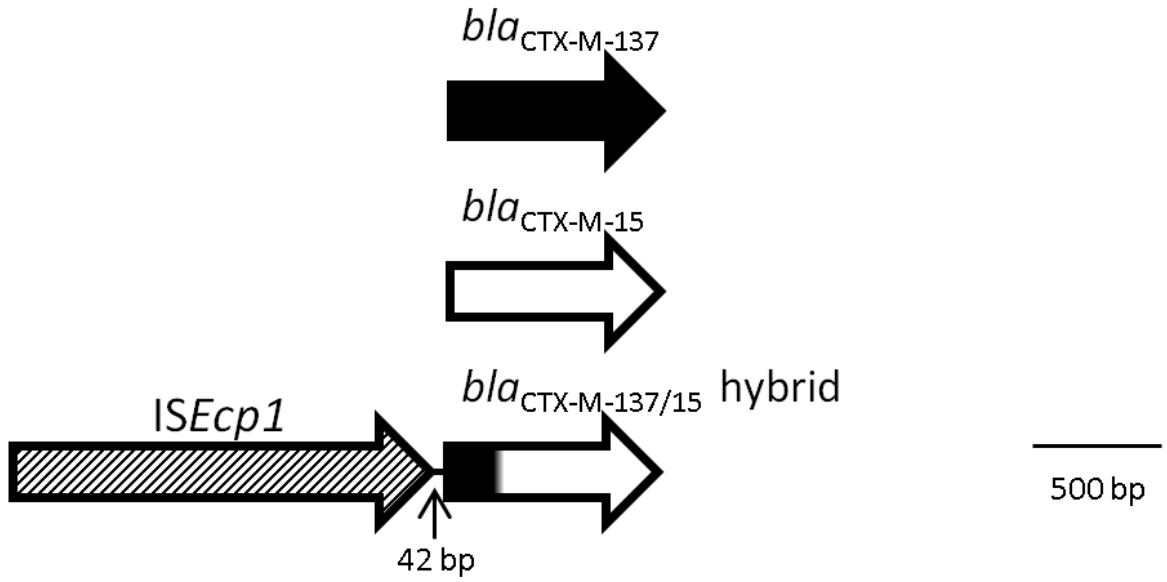

Figure 2. Recombination scheme between *bla*$_{CTX-M-137}$ and *bla*$_{CTX-M-15}$, sample isolated from human feces in a remote community, GenBank accession number MZ314224.