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## Distribution of *bla*<sub>CTX-M</sub> gene variants in *E. coli* from different origins in Ecuador

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### Abstract

The increasing abundance of extended spectrum ( $\beta$ -lactamase (ESBL) genes in *E. coli*, and other commensal and pathogenic bacteria, endangers the utility of third or more recent generation cephalosporins, which are major tools for fighting deadly infections. The role of domestic animals in the transmission of ESBL carrying bacteria has been recognized, especially in low- and middle-income countries, however the horizontal gene transfer of these genes is difficult to assess. Here we investigate *bla*<sub>CTX-M</sub> gene diversity (and flanking nucleotide sequences) in *E. coli* from chicken and humans, in an Ecuadorian rural community and from chickens in another location in Ecuador. The *bla*<sub>CTX-M</sub> associated sequences in isolates from humans and chickens in the same remote community showed greater similarity than those found in *E. coli* in a chicken industrial

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#### Author contribution

Xavier Valenzuela: DNA experiments and analysis, selection of isolates, conjugation experiments, writing the manuscript. Hayden Hedman: collected samples and obtained metadata. Alma Villagomez: bacterial isolation and antibiotic susceptibility tests. Paul Cardenas: DNA analysis, data curation, and statistical analysis. Joseph Eisenberg: writing the manuscript, writing of the grant proposal. Karen Levy: writing the manuscript, writing of the grant proposal. Lixin Zhang: conceptualization, writing the manuscript, DNA sequencing, writing of the grant proposal, Gabriel Trueba: writing of the grant proposal, writing the manuscript, conceptualization, direction of the work in the laboratory.

#### Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.medmic.2023.100092>.

operation 200 km away. Our study may provide evidence of *bla*<sub>CTX-M</sub> transfer between chickens and humans in the community.

## Keywords

*E. coli*; Antimicrobial resistance; Third-generation cephalosporin resistance (TGCR); *bla*<sub>CTX-M</sub>; *ISEcp1*, *spacer sequence*; Ecuador; Chickens

## 1. Introduction

Antimicrobial resistance is recognized as one of the top 10 global public health threats facing humanity [1–3], with an estimated 700,000 annual deaths due to antibiotic-resistant infections, and predictions of 10 million deaths by 2050 [1,3]. Every year, reports on multi-resistant microorganisms increase in different countries, indicating that we are heading into a post-antimicrobial era in which common infections and minor wounds can cause death [4]. Different prevention and control measures have been proposed to mitigate this problem but with minimal success [1,2,5].

Most antimicrobials are administered to animals raised for food, and antimicrobial use in animals is projected to increase to over 100,000 tons by 2030, as global demand for animal protein increases [6]. In Ecuador and in other low- and middle-income countries (LMICs), increased small-scale farming has resulted in greater use of antibiotics as growth promoters (and prophylactics), selecting antimicrobial-resistant bacteria which colonize different hosts and contribute to the dissemination of antimicrobial genes [7,8,9,10].

One of the main antimicrobial resistance genes codes for extended spectrum  $\beta$ -lactamases (ESBLs) which are a rapidly evolving group of enzymes able to hydrolyze third generation cephalosporins, one of the most widely used antibiotics in hospitals and intensive care units [11]. These genes are carried on mobile genetic elements (MGE) which facilitate their spread between bacterial species belonging to different animal microbiomes [12].

*Escherichia coli* is considered one of the most serious threats in the antimicrobial resistance crisis [13] and it belongs to a type of intestinal bacteria that transfers the most amongst different animal hosts [14]. The *bla*<sub>CTX-M</sub> most widely distributed ESBL gene, even though it started to disseminate in *E. coli* (from the environmental bacteria *Kluyvera* spp.) since the 1990s [15–17]. The *bla*<sub>CTX-M</sub> genes have displaced other ESBLs genes in Enterobacteriaceae, maybe due to highly active MGEs (transposons and plasmids) and successful bacterial clones [15,18,19]. Currently, there are more than 234 *bla*<sub>CTX-M</sub> allelic variants in *E. coli* [20] and only a small number are successful.

In *E. coli*, the dissemination of *bla*<sub>CTX-M</sub> genes occurs clonally and through horizontal gene transfer (HGT). Evidence of antimicrobial-resistant bacteria transmission, from domestic animals to humans, is difficult to obtain; clonal transmission between domestic animals and humans requires the search for genetically identical isolates from a large diversity of *E. coli* lineages [9,10]. Evidence of HGT requires finding identical plasmids, with the complication that plasmids are diverse, plasmid DNA could be constantly rearranged [21], and resistance

genes are usually in highly active transposons (moving back and forth among plasmids, from plasmid to chromosome, or to other MGEs) [22–24,25].

Some reports showing different *bla*<sub>CTX-M</sub> allelic variants in *E. coli* isolates from humans and domestic animals, concluded that role of domestic animals in the current antimicrobial crisis may have been over-estimated [26,27]. However, other studies using isolates with closer spatial-temporal relationships, showed similar allelic variants in isolates from humans and domestic animals [9,10]. Here we analyzed the distribution of *bla*<sub>CTX-M</sub> allelic variants and flanking sequences (in conjugable plasmids) from human and chicken isolates in the same community from 2010 to 2017 and compared with similar *bla*<sub>CTX-M</sub> allelic variants in *E. coli* isolated from a chicken industrial operation 200 km away, during overlapping periods.

## 2. Materials and methods

### 2.1. Isolates

We analyzed 107 3<sup>rd</sup> generation cephalosporin resistant *E. coli* isolates (from a total of 4,518 *E. coli* isolates analyzed): 38 (35.5 %) from humans (in a rural community) and 69 (64.5 %) from chickens of which 26 (37.7 %) from the same rural community and 43 (62.3 %) were from an industrial operation (Table 2). The isolates were obtained from previous studies (from humans and chickens) in rural communities in northern Coastal Ecuador during 2017 [7,8], in 2009 [28], and 2010–2013 [29] and from chickens from an industrial operation (200 km apart from the remote community) during 2010. In these studies, *E. coli* was isolated from fecal samples by streaking on MacConkey Agar and incubating for 24 h at 37 °C. Lactose fermenting colonies were tested for β-glucuronidase production, as described previously [7,8]. Resistance profiles were assessed using the Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) recommendations. *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 were used as controls for antimicrobial susceptibility test. All *E. coli* were stored frozen at –80 °C until analyzed. Out of 4,518 *E. coli* isolates, 107 with phenotypic third-generation cephalosporin-resistance (TGCR) were identified. All the protocols were approved by Universidad San Francisco de Quito's Bioethics committee and University of Michigan's IRB.

### 2.2. Conjugation experiments

Conjugation experiments were conducted to identify the *bla*<sub>CTX-M</sub> genes that had the potential to be horizontally transferred by plasmids. Conjugation experiments were performed in Lysogeny Broth (LB) with *E. coli* J53Az<sup>r</sup> (resistant to sodium azide and susceptible to cefotaxime) as the recipient, and all of our *E. coli* TGCR isolates as donors [30]. Donor and recipient isolates were cultured in tubes with 5 ml of LB incubated for 12 h at 37 °C, to get cells in a logarithmic growth phase. A 0.5 ml-aliquot of each tube was transferred to 4 ml of fresh LB and incubated for 16 h at 37 °C without shaking. Transconjugants were selected using selective culture media made of Trypticase soy agar (TSA) supplied with sodium azide (200 µg/ml) and cefotaxime (1 mg/ml). All transconjugants were stored frozen at –80 °C.

### 2.3. Polymerase chain reaction (PCR) and DNA sequencing

Genetic materials from all 107 *E. coli* TGCR isolates were extracted using DNAzol<sup>®</sup> (Invitrogen<sup>™</sup>, USA) following manufacturer protocol and recommendations. A conventional PCR was performed to identify samples carrying *bla*<sub>CTX-M</sub> gene (PCR1); to identify the sequences upstream from the *bla*<sub>CTX-M</sub> gene (PCR2), and to identify sequences downstream from the *bla*<sub>CTX-M</sub> (PCR3).

PCR1 was carried out using degenerate primers [31]. PCR2 used the *bla*<sub>CTX-M</sub> gene and *ISEcp1* sequences [31]. Both sequences are often in close proximity. PCR3 was designed to obtain complete coding sequences of *bla*<sub>CTX-M</sub> genes and the downstream sequences. We used degenerate primers of *bla*<sub>CTX-M</sub> and *orf477* [32]. All amplicons obtained were sequenced. Primers used in this study are listed in Table 1 and the locations of the flanking regions are shown in Fig. 1. Accession numbers of the sequences are in supplemental material, Table S1. Amplicons were sequenced using Sanger's method at Research Technology Support Facility, Michigan State University.

### 2.4. *bla*<sub>CTX-M</sub> variant distribution analysis

We assumed that an evidence of gene transfer in the local community were the presence of isolates with: 1) same *bla*<sub>CTX-M</sub> allele variant; 2) same number of nucleotides and same nucleotide sequences between the *bla*<sub>CTX-M</sub> gene and the transposable element; 3) had the same transposon (or Insertion Sequence); and 4) had the same downstream gene (Fig. 1).

### 2.5. Statistical analysis

We used R statistical software to run principal component analysis (PCA) and ANOVA to evaluate the relationship between *bla*<sub>CTX-M</sub> allele variants (from humans' and chickens' *E. coli*) in the community and those from chickens in the industrial operation. We used GraphPrim 9.5 for the graphic representation.

## 3. Results

### 3.1. Conjugation experiments

We obtained 102 transconjugants (from isolates carrying *bla*<sub>CTX-M</sub> genes); 37 (36.3 %) from humans and 65 (63.7 %) from chickens. The origin of chicken's isolates was: 40 (61.5 %) were from industrial operation and 25 (38.5 %) were from the rural community (Table 2).

### 3.2. Nucleotide sequence analysis of *bla*<sub>CTX-M</sub> genes

We obtained PCR1 amplicons from all transconjugants, however only 94 produced clean DNA sequences. All *bla*<sub>CTX-M</sub> genes found in isolates from chickens in the rural community were also present in isolates from humans of the same community (*bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-27</sub>, *bla*<sub>CTX-M-55</sub>, *bla*<sub>CTX-M-64</sub>, *bla*<sub>CTX-M-65</sub>); none of these variants were present in isolates from the chicken industrial operation. Isolates from humans in the community shared only 2 variants with chickens in the industrial operation (*bla*<sub>CTX-M-2</sub> and *bla*<sub>CTX-M-14</sub>) (Table 2, Fig. 3). The prevalence of some shared variants was different for humans and chickens in the community: *bla*<sub>CTX-M-15</sub> was present in 9 human and 2 chicken isolates, *bla*<sub>CTX-M-55</sub> was present in 1 human and 11 chicken isolates; *bla*<sub>CTX-M-64</sub> was

present in 2 human and 7 chicken isolates. The prevalence of the shared *bla*<sub>CTX-M-2</sub> gene was different for isolates from humans and chickens in the industrial operation (3 and 28 respectively). Three variants (*bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-9</sub>, *bla*<sub>CTX-M-137/15</sub>) were present only in human isolates and 2 variants (*bla*<sub>CTX-M-8</sub>, *bla*<sub>CTX-M-12</sub>) were present only in isolates from chickens in the industrial operation (Table 2).

### 3.3. Nucleotide sequence analysis of *bla*<sub>CTX-M</sub> flanking regions

The transposable element (*ISEcpI*), and the spacer sequences (between the *bla*<sub>CTX-M</sub> and *ISEcpI*) (Table 2) were detected in 51 *bla*<sub>CTX-M</sub> positive isolates. The isolates that have the same *bla*<sub>CTX-M</sub> seemed to share the same spacer size except for *bla*<sub>CTX-M-15</sub> (2 isolates had a 127bp spacer, while 6 isolates had a 48bp spacer) and *bla*<sub>CTX-M-65</sub> (1 isolate had 48bp while 8 isolates had 45 bp) (Table 2). In 18 isolates we identified the *orf477* downstream *bla*<sub>CTX-M</sub> gene, however we did not see an association with any source of samples. We were able to identify a possible recombinant of *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-137</sub> in one isolate (Fig. 2). All accession numbers for these allelic variants are listed in Table S1.

We found difference in *bla*<sub>CTX-M</sub> genes (and adjacent sequences) associated with different animal species; some *bla*<sub>CTX-M</sub> variants (*bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-9</sub>) were found only in human isolates and not in chickens in the community. Additionally, spacers flanking *bla*<sub>CTX-M-15</sub> in human and chicken isolates (in the same community) were different (Table 2); we also found different proportion of strains carrying same variants: human isolates had more *bla*<sub>CTX-M-15</sub> than chickens in the same community (45 %; n = 9 vs. 8 %; n = 2); chicken isolates had more *bla*<sub>CTX-M-55</sub> than human isolates in same community (45 %; n = 11 vs. 5 %, n = 1).

### 3.4. Statistical analysis

The ANOVA and Pearson correlation indicate that human isolates (from the community) have larger differences with chickens in the industrial operation than with isolates from the same community ( $p < 0.0001$ ) (Fig. S1). Similarly, Principal Component Analysis indicate that location (community or industrial operation) has more influence in variability than animal species ( $p < 0.0001$ ) (Fig. S1).

## 4. Discussion

The results of our study show a difference in the distribution of *bla*<sub>CTX-M</sub> variants in *E. coli* isolates from 2 different locations in Ecuador, from 2010 to 2017. The same *bla*<sub>CTX-M</sub> variants and the same flanking sequences (spacer and transposons) found in chicken isolates were also found in human isolates in the community. Contrastingly, no *bla*<sub>CTX-M</sub> variants were shared between chicken's isolates in the community and those from the industrial operation 200 km apart (even in isolates from the same year) (Table 2 and Fig. 3); the ANOVA and PCA were consistent with this observation. We suggest that the distribution of *bla*<sub>CTX-M</sub> variants is a supportive evidence for transference of *bla*<sub>CTX-M</sub> genes among *E. coli* from chickens and humans in the same community. Transposable elements (insertion sequences, like those found in our study) are actively moving important antimicrobial resistance genes among plasmids. The HGT of *bla*<sub>CTX-M</sub> (and other antimicrobial genes) is complex; these *bla*<sub>CTX-M</sub> genes (and flanking sequences) are associated with transposable

elements (such as *ISEpc1* or *IS26*) and promote high frequency transposition among different plasmids and chromosomes [33,34,35–38] and the transposition of genes among plasmids increases the possibilities of HGT [34,38]. Transposition also makes it difficult to detect transmission, even with the use of whole genome sequencing [39,40]. Despite our arguments in favor of HGT, it is also possible that the statistically significant association of *bla*<sub>CTX-M</sub> variants with locations may be due to the dissemination of one clone or few clonally related strains in the community, however, clonally transmission of *bla*<sub>CTX-M</sub> variants is rare in rural communities [39].

We did find 2 variants in isolates from chickens at the industrial operation and humans in the community (*bla*<sub>CTX-M-2</sub>, *bla*<sub>CTX-M-14</sub>), however, the proportion of isolates carrying the *bla*<sub>CTX-M-2</sub> in the two species was different (15 % in human's compared to 78 % in chicken's).

There were differences in the frequencies *E. coli* (carrying the same *bla*<sub>CTX-M</sub> variant and flanking sequences) obtained from chicken and humans (Table 2). These results suggest that transmission of these genes may occur more frequently among strains from the same animal species and less frequently among strains from different animal species; greater interaction with members of the same animal species may contribute to the transference of bacteria and bacterial genes among these bacteria.

Diference in variant distribution overtime was also evident as 31 % of 2017 isolates had *bla*<sub>CTX-M-55</sub> whereas no isolate from 2010 to 2013 had this variant; 21 % of 2017 isolates (from humans and chickens) had *bla*<sub>CTX-M-15</sub> compared with 5 % in isolates obtained in 2010–2013; 21 % of 2017 isolates had *bla*<sub>CTX-M-64</sub> whereas 1.8 % of isolates from 2010 to 2013 had this variant (see Table 2). These results are also in agreement with previous research showing that *bla*<sub>CTX-M</sub> variants change overtime in a given location [22].

We observed more *bla*<sub>CTX-M</sub> variant diversity in human isolates than in chicken isolates in the community. Additionally, we found *bla*<sub>CTX-M-64</sub> and *bla*<sub>CTX-M-65</sub> first in humans in the community in 2010–2013 and later in chickens in the community in 2017 (Table 2). It is tempting to speculate that these results indicate that humans may be the source of some of these gene variants found in *E. coli* from chickens. This transmission may be associated with low sanitary infrastructure in this community [41], allowing the chickens to become colonized with *E. coli* from humans. Other researchers have presented evidence of ARG transmission between *E. coli* from humans and domestic animals due to a shared environment, direct contact between humans and domestic animals, or ingestion of food contaminated with *E. coli* [7,8,9,10].

In Ecuador, *bla*<sub>CTX-M-14</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-55</sub>, and *bla*<sub>CTX-M-65</sub> variants have been reported in clinical isolates [42–44]. Studies in other Ecuadorian rural communities have shown that humans and domestic animals in the same communities share *E. coli* with *bla*<sub>CTX-M-2</sub>, *bla*<sub>CTX-M-14</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-55</sub>, and *bla*<sub>CTX-M-65</sub>, suggesting a mechanism of spread (clonal and HGT of *bla*<sub>CTX-M</sub> genes) between humans and domestic animals [10].

Previous research has described the close relationship between the *ISEcp1* (and other insertion sequences) with *bla*<sub>CTX-M</sub> genes [15,45,46]. These insertion sequences are thought to have been involved in the original mobilization *bla*<sub>CTX-M</sub> genes from the bacteria *Kluyvera* spp [15]. Most of the members of the *bla*<sub>CTX-M</sub> gene family have been associated with *ISEcp1* and five different DNA spacer sequences between the insertion sequence and the *bla*<sub>CTX-M</sub> gene [15]. The length of the spacer sequence is often associated with specific a *bla*<sub>CTX-M</sub> gene [47] and differences in the location of the *ISEcp1* (Table 2) indicate frequent transposable activity, something that has been observed previously [23].

We also detected a possible recombinant of *bla*<sub>CTX-M-137</sub> and *bla*<sub>CTX-M-15</sub> in one isolate, being the first 218 nucleotides from *bla*<sub>CTX-M-137</sub> and the rest from *bla*<sub>CTX-M-15</sub> (Fig. 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).

The small number of TGCR isolates analyzed is a major limitation of our study. We obtained 3 to 5 *E. coli* colonies from each culture plate (without cephalosporin selection); this approach allows to obtain strains that are numerically dominant (regardless of the resistance status) in the intestines and feces [48]. Numerically dominant strains may correspond to the strains that disseminate the most in the environment. We present evidence that the combination of epidemiological data (location, time frame, animal species) and DNA sequence data from *bla*<sub>CTX-M</sub> genes and flanking sequences could provide evidence of *bla*<sub>CTX-M</sub> genes transfer between bacteria in humans and domestic animals inhabiting the same community. We showed that nucleotide sequences of *bla*<sub>CTX-M</sub> gene and flanking regions is a very useful tool to study the transmission of these genes. This information is critical to detect sources and trends in the transmission of this ESBL genes in communities.

## 5. Conclusions

The results of our research suggest that the *bla*<sub>CTX-M</sub>- variants are transmitted between chickens and humans in a rural community in Ecuador. We also present indirect evidence of transmission of antimicrobial resistance genes from humans to chickens in a rural community with low sanitary infrastructure. This study supports the notion that domestic animals play an important role in the circulation of ESBL genes.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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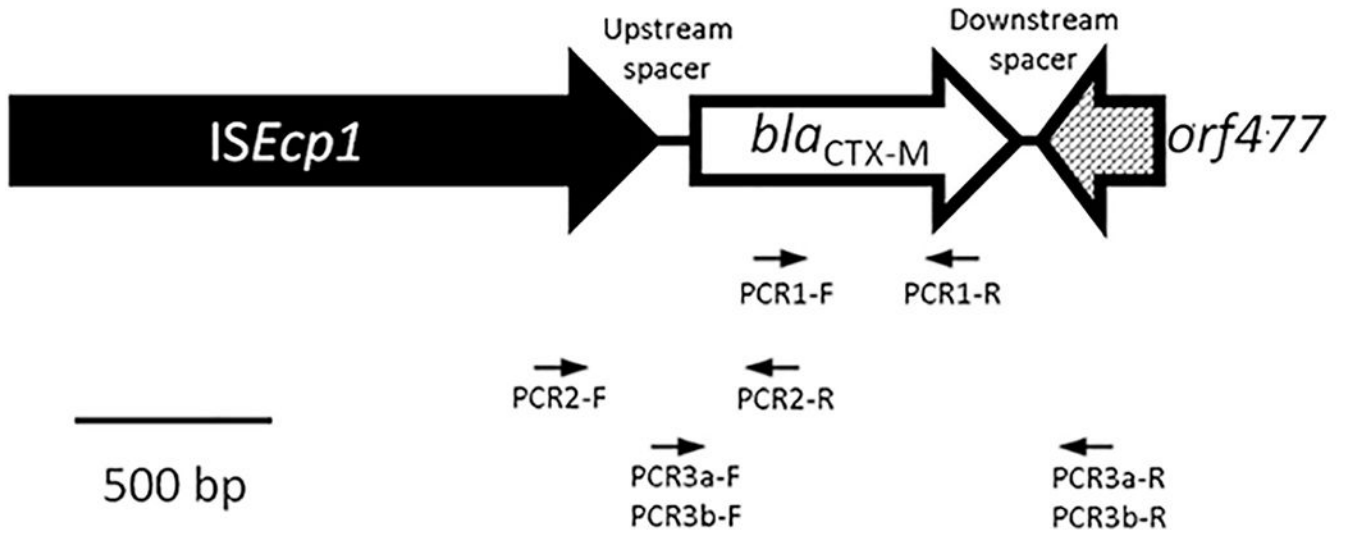
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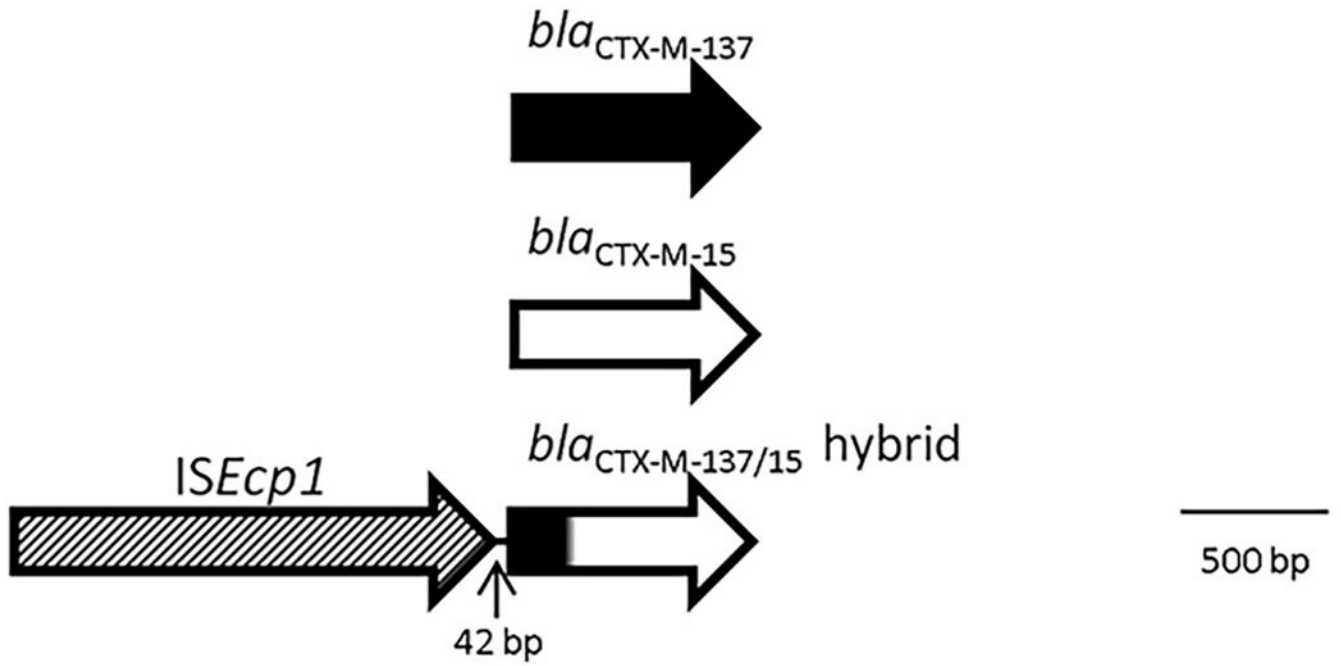


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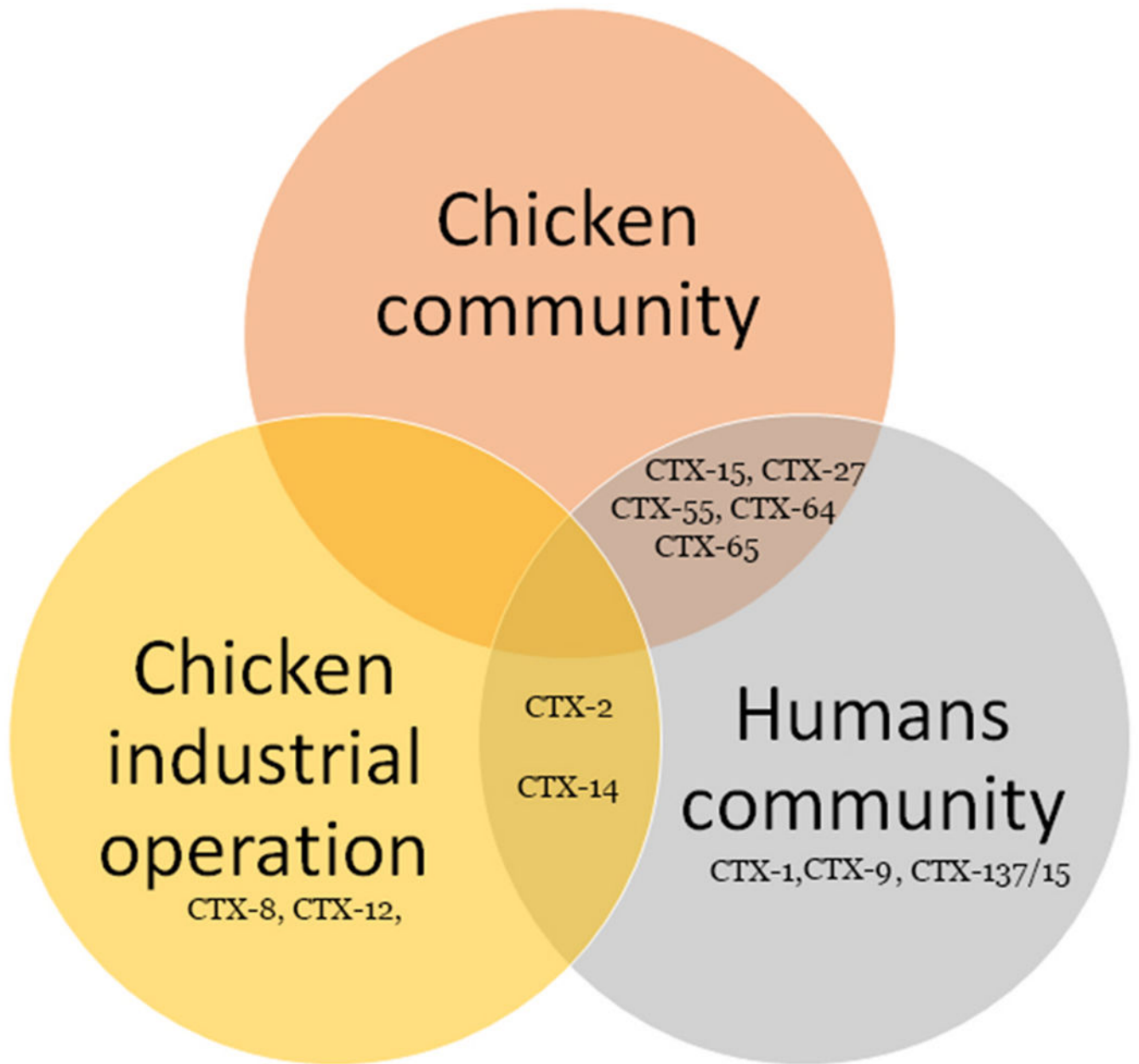
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**Fig. 1.** Schematic representation of usual orientation of the *bla*<sub>CTX-M</sub> genes. Here we see the transposon *ISEcp1* [Insertion Sequence] upstream from the *bla*<sub>CTX-M</sub> gene, and the *orf477* gene in the downstream region. Arrows indicates the primers used in this study.



**Fig. 2.** Recombination scheme between *bla*<sub>CTX-M-137</sub> and *bla*<sub>CTX-M-15</sub>, sample isolated from human feces, GenBank accession number MZ314224.



**Fig. 3.** Venn Diagram showing the distribution of *bla*<sub>CTX-M</sub> gene variants found in humans and chickens in different locations.

**Table 1**

Sequences of primers used in PCR experiments in this study. PCR experiment described in the text.

PCR	Primer forward	Primer reverse	Reference
PCR1	5'-ATGTGCAGYACCAGTAAARGTKATGGC-3'	5'-TGGGTRAAARTARGTSACCAGAAYCAGCGG-3'	Fang et al., 2008
PCR2	5'-TGCCTCTGTGGATAAAGTTGC-3'	5'-GCCATMACYTTACTGGTRCTGCACAT-3'	Poirel et al., 2003
PCR3A	5'-GAATAFACTGATGTAAACACGGGATTTG-3'	5'-TCGTTTTCGTGGTGTGCTGAAATTT-3'	Hu et al., 2018
PCR3B	5'-CGTMTCTTTCAGATAAAGGAATCCC-3'	5'-TCGTTTTCGTGGTGTGCTGAAATTT-3'	Hu et al., 2018

Characteristics of *E. coli* isolates from 94 human and chicken fecal isolates, including year sample was collected, the variant number for the *bla*<sub>CTX-M</sub>- gene, spacer [number of base pairs between the *bla*<sub>CTX-M</sub>- gene and the downstream transposon], Insertion Sequence [IS], and the gene that is downstream from the *bla*<sub>CTX-M</sub>- gene. The sequences are ordered according to the type of *bla*<sub>CTX-M</sub>- variant.

**Table 2**

Source	Number isolates	Year	<i>bla</i> <sub>CTX-M</sub> - variant	Upstream Spacer	IS [transposon]	Down-stream Sequences
Chicken Community	1	2017	15	127 bp	ISEcp1	N/A
Chicken Community	1	2017	15	127 bp	ISEcp1	ORF477
Chicken Community	11	2017	55	127 bp	ISEcp1	ORF477
Chicken Community	4	2017	64	45 bp	ISEcp1	N/A
Chicken Community	3	2017	64	45 bp	ISEcp1	ORF477
Chicken Community	4	2017	65	42 bp	ISEcp1	N/A
Chicken industrial	28	2010	2	N/A	N/A	N/A
Chicken industrial	5	2010	8	N/A	N/A	N/A
Chicken industrial	1	2010	12	48 bp	ISEcp1	N/A
Chicken industrial	1	2010	12	48 bp	ISEcp1	ORF477
Chicken industrial	4	2010	14	42 bp	ISEcp1	N/A
Human Community	2	2010–2013	15	48 bp	ISEcp1	N/A
Human Community	3	2010–2013	2	N/A	N/A	N/A
Human Community	4	2010–2013	9	42 bp	ISEcp1	N/A
Human Community	1	2010–2013	137/15	42 bp	ISEcp1	N/A
Human Community	2	2010–2013	14	42 bp	ISEcp1	N/A
Human Community	1	2010–2013	14	N/A	ISEcp1	N/A
Human Community	1	2010–2013	15	N/A	ISEcp1	N/A
Human Community	1	2010–2013	64	48 bp	ISEcp1	N/A
Human Community	1	2010–2013	65	42 bp	ISEcp1	N/A
Human Community	1	2017	1	80 bp	ISEcp1	N/A
Human Community	1	2017	1	N/A	N/A	N/A
Human Community	1	2017	9	42 bp	ISEcp1	N/A
Human Community	3	2017	15	48 bp	ISEcp1	N/A

Source	Number isolates	Year	<i>bla</i> <sub>CTX-M</sub> variant	Upstream Spacer	<i>IS</i> [transposon]	Down-stream Sequences
Human Community	1	2017	15	48 bp	<i>ISEcpI</i>	<i>ORF477</i>
Human Community	1	2017	15	N/A	<i>ISEcpI</i>	N/A
Human Community	1	2017	15	N/A	N/A	N/A
Human Community	4	2017	27	42 bp	<i>ISEcpI</i>	N/A
Human Community	1	2017	55	127 bp	<i>ISEcpI</i>	N/A
Human Community	1	2017	64	45 bp	<i>ISEcpI</i>	<i>ORF477</i>

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