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Article

Isolated *Escherichia coli* resistance genes in broiler chicken

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Abstract:

Poultry production due to consumer demand has increased annually, which leads to the use of additives such as antibiotics to favor rearing conditions, this increases the deficiency in the composition of production animals' intestinal microbiota and can generate microbiological and genetic changes; this microbiota can reach humans through food chain, generating a possible horizontal transfer of genes that encode resistance to antibiotics. The objective was to identify resistance profiles and the genes that encode it. Materials and

methods: From 200 chickens, 35 strains of *Escherichia coli* with extended spectrum beta-lactamase resistance phenotype were isolated from healthy broilers, from production farms in Santander (Colombia). 83 % of the *AmpC* gene, 86 % of the *blaCTXM* gene, 54 % of the *blaSHV* gene and 57 % of the *blaTEM* gene were identified. Regarding the genes that code for resistance to quinolones, 94 % of the *qnrB* gene, 9 % of the *qnrC* gene and 0 % of the *qnrA* gene were identified**.** The coexistence of the genes that encode for resistance to antibiotics is a serious problem that requires vigilance, in view of this; control strategies must be generated to avoid the spread through the food chain, as well as strategies for the control of the use of antibiotics in the production.

Key words: Poultry, Resistance, Gene, Antibiotics.

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Introduction

In Latin-American countries, chicken is one of most consumed foods because of its easiness for getting it, its low price, its high protein content, and low lipid content, it is the second favorite meat[(1)]. Poultry production increases every year, that is why additives as antibiotics have been employed to encourage upbringing conditions, these additives increase deficiencies in gut microbiota composition. When chickens are born their small intestine is immature and requires morphological, biochemical, and molecular changes that occur during the first two weeks of life, as the animal grows, it is established a microbial community which is more complex through the time. Antibiotics consummation causes digestive disorders be more frequent and produces a low natural resistance to colonization by pathogen microorganisms[(2,3)]. Antibiotics residue can reach the consumer through the food chain causing allergic reactions, bacterial resistance and microflora alteration. In different countries, there are difficulties in commercialization due to a breach in the established rules related to substances concentrations presented in the food. Likewise, several studies have been developed in which bacterial pathogens are referred, including resistant isolations, can be transmitted from chicken to humans[(4)].

In general, antibiotics haven't been used as growing promoters in animals' diets around the world during decades. This fact has caused a great concern since human health can be affected when generating bacterial resistance; because antibiotics used for infections

treatments in humans are employed. Beta lactam antibiotics and fluoroquinolones are broad-spectrum agents commonly used for treating infections, the resistance to this type of antimicrobials has easily arisen. The last reports have demonstrated that resistance to this kind of antibiotics can lead several impacts, which depend on the bacterial strains[5]. Some countries present a restricted use of antibiotics as growing promoters, for instance Sweden since 1986, Finland since 1995[6], the European Union since January first 2006[7]; among others. In Colombia the use of antibiotics is regulated by different resolutions and decrees, however, there are not restrictions in antibiotics commercialization for veterinary use; for what in some cases, provision is empirical and with no specialized prescription[8]. They can be caused by mutations in chromosomal genes and the presence of conjugative and non-conjugative plasmids in the genes[9]. The objective of this article was to establish resistance profiles and the genes which codify it.

Material and methods

From 200 production chickens, samples were taken with a sterile swab from different organs (trachea, intestines, deep and superficial air-abdominal sacs, pericardium, manufacturing bag, intestine, intestinal contents and pancreas), they were sown in BHI broth and incubated at 37 °C for 24 h, later it was seeded on Mac Conkey agar and incubated at 37 °C for 24 h, they were isolated 35 *Escherichia coli* strains with extended-spectrum beta-lactamase (ESBL) resistance phenotype of healthy broiler chickens from farms in Santander (Colombia); it was made the microbiological confirmation of gender and species by using BBL Crystal® system and sensitivity tests by means of Kirby Bauer method following CLSI guidelines (2017), using *Klebsiella pneumoniae* ATCC 700603 strain as positive control and *Escherichia coli* ATCC 25922 strain as negative control. The susceptibility disks employed were ceftriaxone (CRO 30 µg)(Oxoid ®), cefotaxime (CTX 30 µg) (Oxoid ®), cefepime (FEP 30 µg) (Oxoid ®), nalidixic acid (FEP 30 µg) (Oxoid ®), ciprofloxacin (CIP 1 µg) (Oxoid ®), norfloxacin (NOR 2 µg) (Oxoid ®), piperacillin (PRL 30 µg) (Oxoid ®), aztreonam (ATM 30 µg) (Oxoid ®) and amoxicillin/clavulanic acid (AMC 30 µg) (Oxoid ®). The strains were cultivated in Brain Heart Infusion (BHI) to 37 °C all the night in stirring to make the DNA extraction according to the manufacturer's instructions (Wizard® Genomic DNA Purification Kit), they were considered ideal strains in concentration ≥100µg/µL and DNA-proteins relation A260/280 to determine optimal purity with an OD rate between 1.8 to 2.0 (MaestroNano Micro-Volume Spectrophotometer). They were identified by endpoint PCR *blaTEM* genes (700 pb), *blaSHV* genes (700 pb), *blaCTX* genes (500 pb) and *Amp-C* genes (550 pb) with the protocole modified by López *et al*[10]; and *qnrA* genes (580 pb), *qnrB* genes (264 pb), *qnrC* genes (428 pb) with Aguilar *et al* protocole [11]. The amplified products were visualized by

electrophoresis in agarose gel to 1% with TAE to 1% and Safeview classic as a colorant. Gels were visualized by using Ultra Slim Led Illuminator.

Results

Susceptibility profiles were 63 % (n=22/35) for ceftriaxone (Oxoid ®), 69 % (n= 23/35) cefepime (Oxoid ®), 77 % (n =27/35) cefotaxime (Oxoid ®), 86 % (n= 30/35) norfloxacin (Oxoid ®), 89 % (n =31/35) ciprofloxacin (Oxoid ®), 91 % (n =32/35) piperacillin (Oxoid ®), 91 % (n =32/35) aztrenam (Oxoid ®), 97 % (n =34/35) amoxicillin/clavulanic acid (Oxoid ®) and 97 % (n=34/35) nalidixic acid (Oxoid ®). Regarding antibiotics groups it was presented *E.coli* 70 % of resistance to cefalosporines, 90 % to quinolones and 93 % to beta lactams (Figure 1). Regarding genes, they were identified fragments of the expected size, for the genes which codify for beta lactamase resistance it was identified 83 % of *AmpC* gene, 86 % of *blaCTXM* gene, 54 % of *blaSHV* gene and 57 % of *blaTEM* gene (Figure 2). Regarding genes which codify for quinolone resistance, it was identified 94 % of *qnrB* gene, 9 % of *qnrC* gene and 0 % of *qnrA* gene (Figure 2 and Table 1).

Figure 1: Antibiotics resistance groups

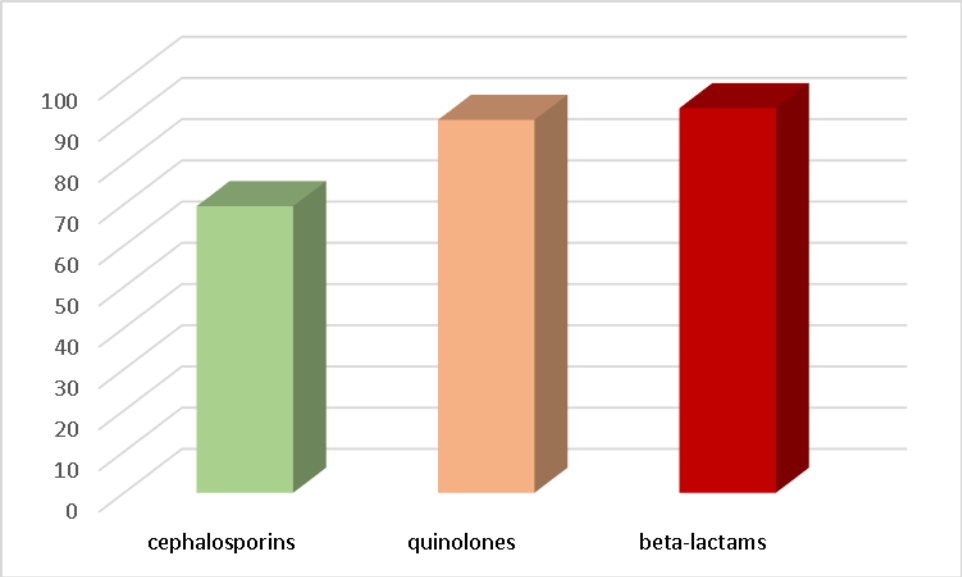
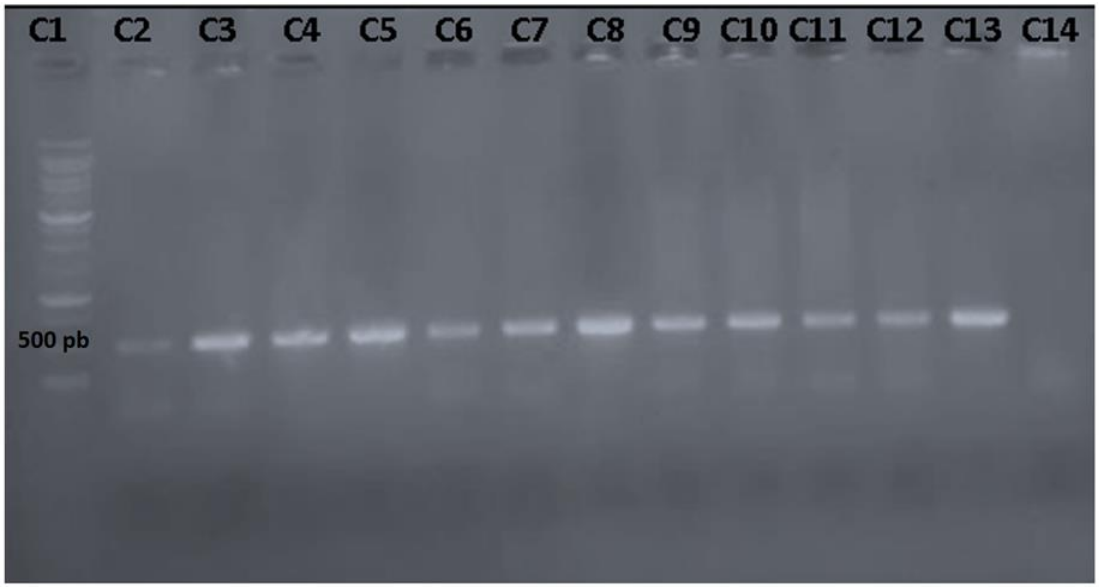


Figure 2: Gel de electroforesis de gen bla*CTMX*



C1= 1Kb, C2= Positive control, C3= Mx1, C4= Mx2, C5=Mx3, C6= Mx4, C7= Mx5, C8=Mx6, C9= Mx7, C10= Mx8, C11= Mx9, C12= Mx10, C13= Mx11, C14= negative control.

Table 1: Results of the amplified genes

Sample	***Ampc***	***blaCTX-M***	***blaSHV***	***blaTEM***	***qnrA***	***qnrB***	***qnrS***	**CRO**	**CTX**	**FEP**	**AMC**	**CIP**	**NOR**	**PRL**	**ATM**
1	P	P	P	P	N	N	N	R	I	I	R	R	S	I	I
2	P	P	N	P	N	N	N	I	R	S	R	I	I	R	R
3	P	P	P	P	N	P	P	R	R	R	R	R	R	R	R
4	N	N	N	N	N	P	P	S	I	S	R	R	R	R	R
5	P	P	P	N	N	P	N	R	R	R	R	R	R	R	R
6	P	P	P	P	N	P	N	R	I	R	R	I	I	R	R
7	P	P	P	N	N	P	N	S	I	S	R	R	R	R	R
8	P	P	N	P	N	P	N	R	R	R	R	R	R	R	R
9	P	P	N	P	N	P	N	R	R	R	R	R	R	R	R
10	N	N	P	N	N	P	N	R	R	R	R	R	R	R	R
11	P	N	N	P	N	P	N	I	R	I	R	R	I	R	I
12	P	P	N	P	N	P	N	S	S	S	R	I	R	R	R
13	P	P	P	N	N	P	N	I	R	R	R	R	R	R	R
14	P	P	N	P	N	P	N	R	R	R	R	R	R	R	R
15	P	P	P	N	N	P	N	R	R	I	R	R	R	R	R
16	P	N	N	P	N	P	N	S	S	S	R	R	R	R	R
17	P	P	N	N	N	P	N	S	S	S	R	R	R	I	R
18	P	P	P	N	N	P	N	I	R	I	R	R	R	R	R
19	P	P	P	N	N	P	N	S	R	R	R	R	R	R	R
20	P	P	P	P	N	P	P	R	R	R	R	R	R	R	R
21	P	P	P	N	N	P	N	R	R	R	R	R	R	R	R

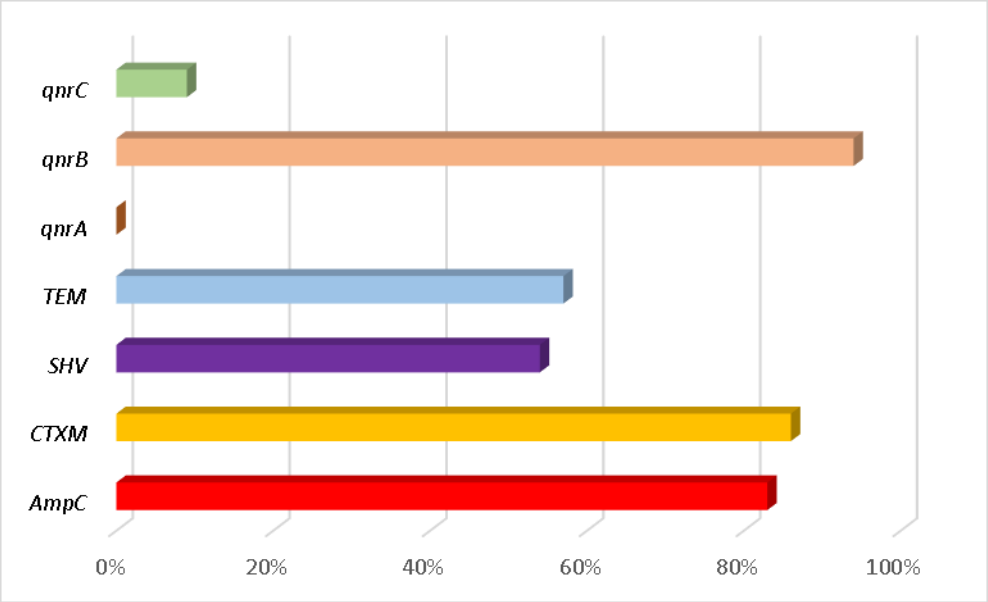
22	P	N	P	N	N	P	N	S	S	S	R	R	R	R	R
23	P	P	N	N	N	P	N	R	R	R	R	R	R	R	R
24	P	P	P	P	N	P	N	R	R	R	R	R	R	R	R
25	P	P	P	P	N	P	N	R	R	R	R	R	R	R	R
26	P	P	N	P	N	P	N	R	R	R	R	R	R	R	R
27	N	N	N	N	N	P	N	R	R	R	R	R	R	R	R
28	P	P	P	P	N	P	N	R	R	R	R	R	R	R	R
29	P	P	N	P	N	P	N	R	R	R	R	R	R	R	R
30	P	P	P	P	N	P	N	R	R	R	R	R	R	R	R
31	N	N	N	N	N	P	N	I	R	R	R	R	R	R	R
32	P	P	N	P	N	P	N	S	R	R	R	R	R	R	R
33	N	P	P	P	N	P	N	R	R	R	R	R	R	R	R
34	P	P	P	P	N	P	N	R	R	R	R	R	R	R	R
35	N	N	N	N	N	P	N	R	R	R	R	R	R	R	R

P= positive; N= negative; R= resistant; S= sensitive; I= intermediate.
CRO= ceftriaxone; CTX= cefotaxime; FEP= cefepime; AMC= amoxycillin/clavulanic acid. CIP= ciprofloxacin; NOR= norfloxacin; PRL= piperacillin; ATM= aztreonam.

Discussion

From susceptibility profiles, it can be noticed the strains presented multiresistence, taking into account that these ones had resistance to more than four antibiotics; 49 % presented resistance to all the antibiotics, these results generate a great concern. In South Korea, they found resistance to even eleven antibiotics, including ciprofloxacin[12]. Yurong *et al* obtained similar results when finding resistance to more than five antimicrobial agents; in which, ciprofloxacin and levofloxacine stand out[13]. Regarding antibiotic groups, it excels that 93 % presented resistance to beta-lactams, followed by quinolones in 90 % and cephalosporins in 70 % (Figure 3); antimicrobials which are employed for daily use of bacterial infections in humans. Similar reports were made in Korea with resistance to ampicilin (75 %), followed by tetracycline (69 %) and ciprofloxacin (65 %)[14]. While Varga *et al*[15] identified resistance to beta-lactams, sulfonamides and tetracyclines in poultry. In Colombia, bacteria resistant to multiple drugs such as ceftiofur, enrofloxacin, nalidixic acid and tetracycline, were isolated from the meat of poultry from independent stores and from a distribution center of the main chain, which generates an alarm for the health entities of the country[16].

Figure 3: Genes prevalence



Within resistance it is necessary to confirm the phenotypes of resistance by means of PCR identifying the genes which codify it, for beta-lactamases they can be found the next genes *blaTEM*, *blaSHV* , *blaCTX* and *Amp-C*[17]; and for fluoroquinolones the genes are *qnrA* , *qnrB* and *qnrC*[11]*;* from the profiles previously analyzed, it can be noticed similarity to what other authors reported. Researches made in Brazil, exposed that the isolations which present the genes *blaCTX-M-2* or *blaCMY-2* tend to accumulate resistance to a higher rate of non-betalactamic antimicrobials[4]. In China, the genes which predominated in isolations were *blaCTXM* and *blaTEM*, likewise they found variants of *blaCMY*; as long as *blaSHV* was not identified[13]; as well as in studies made in Pakistan[18], while for the current research, it was presented in a 57 %. Alonso *et.al* refer that the dissemination of *blaSHV* can occur by horizontal transfer, mainly caused by plasmids, which could facilitate the dissemination of this gene[19]. Regarding *AmpC*, in the United Kingdom, they were analyzed imported chicken finding un 23 % of this gene, as well as they were identified mutations of this one[20]. However, in countries like Ecuador they obtained a high prevalence of the *blaCTXM* gene, results that differ from those obtained in the study[21].

The use of antibiotics as growing promoters in animals generates a great concern due to a spreading of resistant bacteria, since chicken has an easy commercialization. In the present study it is noticed that 26 % of the strains presented the four genes, 46 % three genes, 14 % two genes, 3 % one gene and 4 % no gene; Molecular biology techniques have a great relevance, because through them, they are confirmed the resistance phenotypes by punctual mutations in the target genes in susceptible bacteria[22].

The *qnr* genes are mediated by plasmids, transmissible by conjugation that relates to their potential for circulation. The primer has been reported in 1998 and since then five types of qnr genes (*qnrA*, *qnrB*, *qnrS*, *qnrC*, and *qnrD*) have been reported, containing more than 30 alleles[23]. The animals can act as reservoirs for a series of zoonotic infections, which can be transmitted to humans by direct contact or through the food chain[24]. Kilani *et al* in animal samples, 17.6 % identified *qnr*-type genes, as well as genes for beta-lactamases, which is why it is similar to what was identified in the present study[25]. Clemente *et al* detected in *E. coli* isolations the gene *gyrA* in food producer animals which expressed in a whole the gene *blaCMY-2*[26]. In the research made by Montes *et al* they reported only 1 % of the gene *gyrB* and the gene *gyrA* 0%[11], while in Quito, 36 % of isolations of Broiler chicken in a poultry the gene *qnrB*. Results similar to those reported in Brazil, in which they were able to identify variants of the *gyr* gene in isolates from food and humans, observing a reduced susceptibility to ciprofloxacin[27]. The results obtained in the present research about the presence of genes which codify for resistance to quinolines is high regarding the other researches made by other authors, different studies have found that genes *qnr* are highly distributed in *E. coli* isolated of healthy humans, domestic and farm animals[9].

Conclusions and implications

The coexistence of genes which codify for antibiotics resistance is a serious problem that requires vigilance. In light of this situation they must generate control strategies to avoid spreading through food chain, since chicken is one of the most available foods within market basket. These results reflect the resistance found mainly for antimicrobials that act by inhibiting wall synthesis and protein synthesis, such as cephalosporins and gentamicin respectively, showing evidence of the theory of the production of extended-spectrum beta-lactamases mechanism that may be plasmid mediated, which represents an emerging resistance problem. The limitations of this study include a sampling bias, since only one farm was worked, in addition to having no stool samples. Therefore, this study could overestimate the frequency of resistance by samples coming from birds that may have already been treated with antimicrobials.

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Ethical standards compliance

Every procedure was made taking into account the institutional and national research committee and Helsinky declaration of 1964 and its subsequent ammendments or similar ethical standars. This study was approved by the local ethical committee.

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Conflict of interests

Authors declare there is not any conflict of interests.

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