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Proceeding Paper

Highest Priority Critically Important Antimicrobial Resistant *Escherichia coli* and *Salmonella* spp. Isolated from Pork and Chicken Meat from Argentina †

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Abstract: Between June and September 2023, a total of 80 meat samples from pork and chicken were collected from 16 retail markets in La Plata, Argentina. Eighty-four Highest Priority Critically Important Antimicrobial-resistant *Escherichia coli* and two *Salmonella* spp. were isolated. Resistance to ciprofloxacin and to cefotaxime was observed in 65 and 49 E. coli respectively. Seventy-five *E. coli* isolates were multidrug resistant. Fourteen *E. coli* isolated from chicken meat showed resistance to three of the HPCIA. Resistance to 3rd generation cephalosporin was associated with blaCTX-M. The chances of finding HPCIA resistant-*E. coli* are 15 times more in chicken meat than in pork.

Keywords: highest priority critically important antimicrobial; *Escherichia coli*; *Salmonella*; pork; chicken meat; ESBL; AmpC

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1. Introduction

The emergence and dissemination of antimicrobial resistance (AMR) is of worldwide public health concern. To support this goal, the World Health Organization (WHO) in 2005 first developed the List of Critically Important Antimicrobials (CIA). The list categorizes antimicrobial classes authorized in humans and animals based on the importance of the antimicrobial class in human medicine and the contribution of non-human use to the risk of transmitting AMR to humans. WHO Medically Important Antimicrobial List is systematically updated. Cephalosporins (3rd, 4th generation), quinolones, polymyxins, and phosphonic acid derivatives are authorized for human and animal use and are categorized in the class as Highest Priority Critical Important (HPCIA) [1].

Resistant bacteria can be transmitted through the food chain with the consumption of raw foods or possibly through the consumption of inadequately cooked food, by cross-contamination with other food, or indirectly through the environment [2]. Pork and chicken can serve as reservoirs of antimicrobial resistance, which can be monitored using *Escherichia coli* as an indicator bacteria and *Salmonella* as a zoonotic pathogen. In 2022, pork and chicken meat consumption in Argentina was 16.76 kg/inhabitant/year [3] and 45.5 kg/inhabitant/year [4] respectively.

This study aimed to determine the presence of the "highest priority critically important antimicrobial" resistant-*E. coli* and *Salmonella* spp. from pork and chicken meat at retail markets in La Plata, Buenos Aires, Argentina.

2. Materials and Methods

2.1. Retail Markets Sampling

Between June and September 2023, were purchased meat samples from 16 retail markets randomly selected in La Plata, Buenos Aires, Argentina. Were collected a total of 80 meat samples, 48 and 32 from pork and chicken meat, respectively.

2.2. Sample Processing

Briefly, 25 g of each meat sample was mixed with 225 mL of buffered peptone water followed by incubation overnight at 37 °C. Enriched cultures (30 µL) were inoculated on Mac Conkey agar plates supplemented with 2 mg/L of cefotaxime or 0.5 mg/L ciprofloxacin (HCl salt) followed by incubation at 37 °C for 18 h. Presumptive *E. coli* colonies were selected for biochemical identification and those confirmed to be *E. coli* were subcultured and preserved at −20 °C. One colony was picked per plate, though, rarely, if colonies had clearly different morphologies, up to two colonies were picked, one representing each colony type.

Isolation of *Salmonella* spp. was performed according to ISO 6579-1:2017 [5].

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was evaluated by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [6], except for colistin for which resistance was evaluated as growth or not on Müeller–Hinton screening agar plates containing 3 mg/mL colistin. Isolates were considered multidrug resistant (MDR) when were resistant to ≥1 agent in >3 antimicrobial categories [7].

2.4. Molecular Characterization of Beta-Lactamases Resistance Genes

PCR was performed to detect common ESBL and plasmidic AmpC β-lactamase genes, and specific PCR was also used to discriminate between blaCTX-M-2, blaCTX-M-1/15, blaCTX-M-8/25, and blaCTX-M-9/14 groups [8].

2.5. Statistical Analysis

Generalized linear models (GLM) with binomial distribution were fitted and validated. The type of sample (pork/chicken meat) was used as a fixed effect predictor variable. The proportion of isolates obtained in both types of meat per establishment was evaluated. Likewise, it was studied whether there were differences in the proportion of isolates resistant to Highest Priority Critical Important Antimicrobials obtained for each type of sample analyzed. Both the effects of errors and the goodness of fit to the proposed models and assumptions were tested. The degree of significance was set at $p < 0.05$. Statistical analysis was performed with R software (R Core Team, 2020).

3. Results

All retail markets were positive for at least one resistant *E. coli*. Of the total samples processed, at least one resistant *E. coli* isolate was obtained in 63.75% (51/80). From 43.7% (21/48) of the pork samples and 93.75% (30/32) of chicken meat, 84 resistant *E. coli*, 34 and 50 were obtained, respectively. Two *Salmonella* spp. were isolated from chicken meat.

Figure 1 shows the distribution of *E. coli* resistant to HPCIA by retail market.

Retail market	Sample positive to resistant-*E. coli* Total	Pork	Chicken meat	Resistant-*E. coli* isolates Total	Pork	Chicken meat	Resistant-*E. coli* to HPCIA (n° pork-n° chicken meat) CTX	CIP	FOS	CTX, CIP	CIP. FOS	CTX, FOS	CTX, CIP, FOS
1	5	3/3	2/2	7	3	4	1 (0-1)	4 (2-2)	1 (1-0)	1 (0-1)			
2	3	1/3	2/2	4	1	3	1 (0-1)	1 (0-1)		1 (0-1)	1 (1-0)		
3	3	1/3	2/2	3	1	2		2 (0-2)				1 (1-0)	
4	4	2/3	2/2	4	2	2		2 (2-0)		2 (0-2)			
5	3	1/3	2/2	4	1	3		3 (1-2)		1 (0-1)			
6	5	3/3	2/2	10	6	4	4 (3-1)	3 (3-0)		1 (0-1)		2 (0-2)	
7	3	1/3	2/2	8	2	6		2 (1-1)		1 (1-0)	2 (0-2)	2 (0-2)	1 (0-1)
8	4	2/3	2/2	5	2	3	1 (0-1)	2 (1-1)			1 (1-0)		1 (0-1)
9	5	3/3	2/2	9	6	3	3 (1-2)	4 (3-1)		1 (1-0)			1 (1-0)
10	3	1/3	2/2	5	1	4				4 (1-3)			1 (0-1)
11	3	2/3	1/2	5	3	2		2 (2-0)				1 (1-0)	2 (0-2)
12	4	2/3	2/2	6	3	3	2 (1-1)	2 (1-1)		1 (1-0)			1 (0-1)
13	1	0/3	1/2	1	0	1	1 (0-1)						
14	2	0/3	2/2	5	0	5		2 (0-2)					3 (0-3)
15	3	1/3	2/2	4	1	3				1 (1-0)			3 (0-3)
16	3	1/3	2/2	4	2	2	1 (1-0)				2 (1-1)		1 (0-1)
	54/80 (67.5%)	**24/48 (50%)**	**30/32 (93.75%)**	**84**	**34 (40.5%)**	**50 (59.5%)**	**14 (6-8)**	**29 (16-13)**	**1**	**14 (5-9)**	**6 (3-3)**	**6 (2-4)**	**14 (1-13)**

HPCIA: Highest Priority Critical Important Antimicrobial. n°: amount of resistant *E. coli* isolates. CTX: cefotaxime. CIP: ciprofloxacin. FOS: fosfomycin.

Figure 1. Distribution of HPCIA-resistant *E. coli.*

The proportion of chicken samples positive for HPCIA resistant-*E. coli* was significantly higher than that obtained in those from pigs (p-value < 0.05) (Figure 2). In this sense, the chances of finding HPCIA resistant-*E. coli* are 15 times more in chicken meat than in pork [OR chicken/pig = 15; CI (3.58; 62.78)] (Table 1)

Likewise, the proportion of *E. coli* isolates resistant to the following combinations of HPCIA was evaluated: CTX-CIP/CTX-FOS/CTX-FOS-CIP, obtained from pork and chicken. Only for this last combination of antibiotics was it significantly higher for chicken meat compared to pork (p < 0.05), while the other two combinations of antibiotics did not present differences between both types of meat.

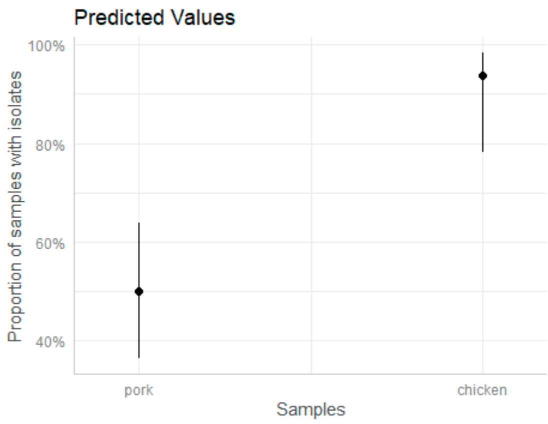

Figure 2. Proportion of samples that presented isolates according to the type of meat (pork/chicken).

Table 1. Results obtained for the proportion of samples that presented isolates, according to the type of meat (pork/chicken).

	prop		
Predictors	*Odds Ratios*	*CI*	*p*
Sample [chicken]	15.00	3.58 – 62.77	**<0.001**
Sample [pork]	0.07	0.01 – 0.31	**0.001**

prop: proportion of samples with isolates; CI = confidence intervals; *p*: *p*-value.

All the isolates (100%) were susceptible to the polymyxin colistin, the carbapenems meropenem and imipenem, and nitrofurantoin. The rates of resistance of the *E. coli* isolates were as follows: ampicillin, 91.7%; tetracycline, 78.6%; ciprofloxacin, 77.4%; cefotaxime, 58.3%; chloramphenicol, 48.8%; amoxicillin/clavulanic acid, 47.6%; sulfamethoxazole-trimethoprim, 45.2%; fosfomycin, 32.1%; cefepime, 25%; gentamicin, 22.6%; ceftazidime, 8.3% and cefoxitin, 4.8%. Fourteen *E. coli* isolated from chicken meat showed resistance to three of the HPCIA cefotaxime/cefepime, ciprofloxacin, and fosfomycin.

Figure 3 shows the percentage of *E. coli* isolates resistant to important antimicrobials discriminated by pork and chicken meat.

A high diversity of resistance profiles was observed. Moreover, 75 isolates (89%) were categorized as MDR. Twenty-four *E. coli* isolates showed resistance to four antibiotic classes; and 20 strains were resistant to five antibiotic classes.

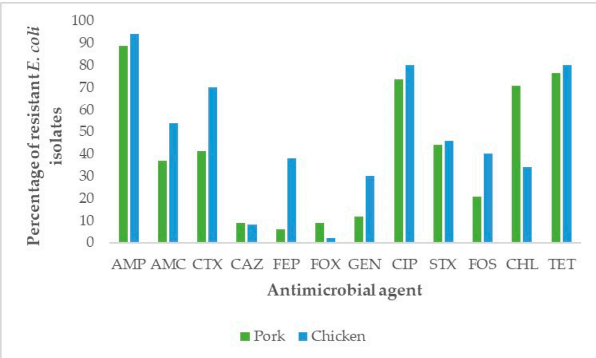

Figure 3. Percentage of *E. coli* isolates resistant to important antimicrobials.

Salmonella spp. isolates were sensitive to ampicillin, gentamicin, nalidixic acid, ciprofloxacin, fosfomycin, trimethoprim-sulfamethoxazole, chloramphenicol, and azithromycin. The isolates showed resistance to tetracycline.

Principally, resistance to 3rd and 4th generation cephalosporin was associated with *bla*$_{CTX-M}$ genes. Table 2 describes the distribution of beta-lactamase resistance genes found in pork and chicken meat.

Table 2. Distribution of beta lactamase resistance genes found in pork and chicken meat.

Beta-Lactamases Genes	Pork	Chicken Meat
bla$_{TEM}$	16	8
bla$_{CMY-2}$	1	1
bla$_{CTX-M-1/15}$	1	3
bla$_{CTX-M-2}$	1	9
bla$_{CTX-M-8/25}$	1	0
bla$_{CTX-M-9/14}$	3	0
bla$_{TEM}$, *bla*$_{CTX-M-2}$	0	4
bla$_{TEM}$, *bla*$_{CMY-2}$	1	2
bla$_{CTX-M-2}$, *bla*$_{CMY-2}$	0	1
bla$_{TEM}$, *bla*$_{CTX-M-1/15}$	4	10
bla$_{TEM}$, *bla*$_{CTX-M-9/14}$	1	0
bla$_{TEM}$, *bla*$_{CTX-M-8/25}$	0	2
bla$_{CTX-M-2}$, *bla*$_{CTX-M-8/25}$	0	1
bla$_{TEM}$, *bla*$_{CTX-M-1/15}$, *bla*$_{CTX-M-2}$	0	1
bla$_{TEM}$, *bla*$_{CTX-M-9/14}$, *bla*$_{CTX-M-8/25}$	0	1
bla$_{CTX-M}$, *bla*$_{CMY-2}$	1	0
bla$_{CTX-M}$	0	1

4. Discussion

In Argentina, the published data related to the presence of resistant-*E. coli* in pigs and poultry were carried out in farm or slaughterhouse. In relation to the resistance profiles and enzymes involved, the results obtained from chicken meat are related to those observed by other authors [9]. A similar situation was not observed with pork [8,10].

Although our results agree with those shown by Clemente et al. [11], regarding the higher frequency of *E. coli* resistant to 3rd generation cephalosporins and fluoroquinolones observed in chicken meat, our isolation rate of ESBL/AmpC *E. coli*-producers exceeds what they reported. In this work, 27% (13/48) of the pork samples and 72% (23/32) were positive, contrasting with 10.5% and 30.3% respectively.

Dominguez et al. [9] reported CTX-M-2 cefotaximase was the main mechanism responsible for third generation cephalosporins resistance, observed in *E coli* from avian systems in Argentina. Our results partially agree with this information since the presence of CTX-M-2 cefotaximase and CTX-M-1/15 cefotaximase was observed in equal parts. CTX-M-1 and CTX-M-15 are the leading ESBL-producing Enterobacterales associated with animal and human infection, respectively, and are an increasing antimicrobial resistance global health concern. Faccone et al. [10] and Gómez et al. [8] reported that the main mechanism of resistance to 3rd generation cephalosporin was mainly associated with CTX-M, with those grouped as CTX-M-8/25.

It is important to highlight that by municipal provision, in retail markets, meat from different origins (pork, chicken, beef) must be separated. This would explain the fact that resistant-*E. coli* isolates from pork and chicken meat from the same place are not similar.

5. Conclusions

The observed results do not refute the hypothesis proposed, "pork and chicken meat obtained from retail markets in La Plata City are contaminated with Highest Priority Critical Important resistant-*E.* coli". The presence of resistant-*E. coli* in pork and chicken meat is a source of multiple resistance genes associated with clones epidemiologically relevant to public health.

These are the first data obtained from pork and chicken meat from retail markets in La Plata City. Complementary studies are necessary to determine the totality of resistance genes carried by these resistant-*E. coli* isolates. The information that will be obtained will allow proposing intervention strategies that will reduce the risk of cross-contamination.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Resistance phenotype and beta-lactamases resistance genes in *Escherichia coli* isolated from pork and chicken meat in La Plata, Buenos Aires, Argentina.

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