

Highest priority critically important antimicrobial resistant *Escherichia coli* and *Salmonella* spp. isolated from pork and chicken meat from Argentina

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Introduction

The World Health Organization (WHO) developed the Medically Important Antimicrobial List 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 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.

This study aimed to determine the presence of the "Highest Priority Critical Important Antimicrobial" resistant *E. coli* and *Salmonella* spp. from pork and chicken meat at retail markets in La Plata, Buenos Aires, Argentina.

Materials and methods

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.

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.

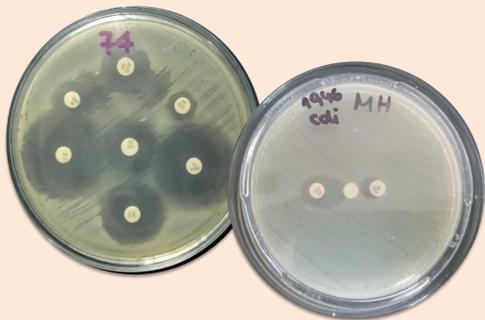


Molecular characterization of beta-lactamases resistance genes

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.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was evaluated by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines, except for colistin for which resistance was evaluated as growth or not on Müller–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.



Results

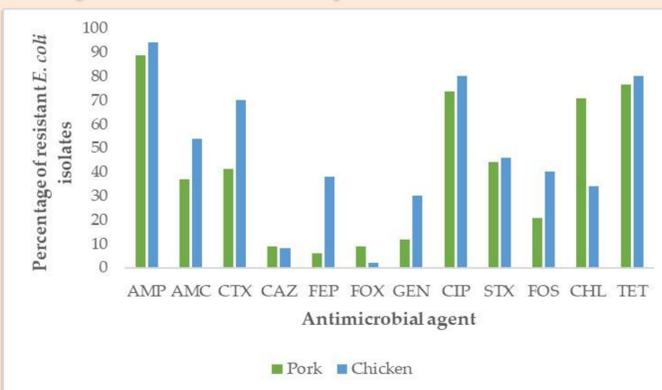
All retail markets were positive for at least one resistant *E. coli*. Of the total samples processed, at least one resistant *E. coli* isolate was obtained in 63.75% (51/80). From 43.7% (21/48) of the pork samples and 93.75% (30/32) of chicken meat, 84 resistant *E. coli*, 34 and 50 were obtained, respectively.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

Distribution of HPCIA-resistant *E. coli*

Retail market	Sample positive to resistant- <i>E. coli</i>		Resistant- <i>E. coli</i> isolates		Resistant- <i>E. coli</i> to HPCIA (n° pork ^a /chicken meat)							
	Total	Chicken meat	Total	Chicken meat	CTX	CIP	FOS	CTX, CIP	CIP, 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 (0-1)		1 (1-0)		
4	4	2/3	2/2	4	2	2	2 (0-2)	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 (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)	1 (1-0)	
10	3	1/3	2/2	5	1	4	4 (3-1)	1 (0-1)				
11	3	2/3	1/2	5	3	2	2 (2-0)	1 (1-0)		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)	1 (1-0)			3 (0-3)	
16	3	1/3	2/2	4	2	2	1 (1-0)	2 (1-1)			1 (0-1)	
51/80	21/48	30/32	84	34	50	11 (6-8)	20 (16-13)	1	14 (8-9)	6 (3-9)	4 (2-4)	14 (1-13)
	63.75%	80%	93.75%	84	(80.5%)	31 (6-8)	20 (16-13)	1	14 (8-9)	6 (3-9)	4 (2-4)	14 (1-13)

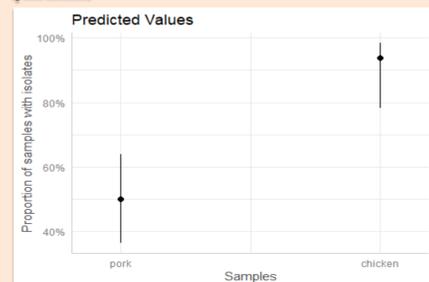
HPCIA: Highest Priority Critical Important Antimicrobial; n°: amount of resistant *E. coli* isolates; CTX: cefotaxime; CIP: ciprofloxacin; FOS: fosfomicin.

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



The chances of finding HPCIA resistant-*E. coli* are 15 times more in chicken meat than in pork. The proportion of *E. coli* isolates resistant to the combinations of HPCIA CTX-FOS-CIP was significantly higher for chicken meat compared to pork.

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



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

Predictors	prop		
	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

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

Beta-lactamases genes	Pork	Chicken meat
blaTEM	16	8
blaCTX-2	1	1
blaCTX-M-15	1	3
blaCTX-M-2	1	9
blaCTX-M-8/25	1	0
blaCTX-M-9/14	3	0
blaTEM, blaCTX-M-2	0	4
blaTEM, blaCTX-2	1	2
blaCTX-M-2, blaCTX-2	0	1
blaTEM, blaCTX-M-15	4	10
blaTEM, blaCTX-M-9/14	1	0
blaTEM, blaCTX-M-8/25	0	2
blaCTX-M-2, blaCTX-M-8/25	0	1
blaTEM, blaCTX-M-15, blaCTX-M-2	0	1
blaTEM, blaCTX-M-9/14, blaCTX-M-8/25	0	1
blaCTX-M, blaCTX-2	1	0
blaCTX-M	0	1

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

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Conclusion

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.

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

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