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Presence of extended-spectrum β-lactamase in *Escherichia coli* strains isolated from beef, pork, and chicken meats sold in Recife, Brazil

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Abstract

This study aimed to identify extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* in chicken, beef, and pork meats sold in Recife, Pernambuco, Brazil. A total of 120 meat samples (40 of each type) were collected from supermarkets, butcher shops, and open-air markets across the city's eight health districts, using convenience sampling. The samples were processed in a microbiology laboratory, and *E. coli* was identified through selective isolation on eosin-methylene blue agar and biochemical tests. Phenotypic resistance was assessed using disk diffusion tests on Müller–Hinton agar with cefotaxime, ceftazidime, and ceftriaxone disks, followed by the double-disk synergy test to confirm ESBL production. Genotypic analysis was conducted by polymerase chain reaction to detect the *blaTEM* and *blaSHV* genes. Of the 40 *E. coli* isolates obtained, 34 (85%) exhibited phenotypic resistance, while 21 (52.5%) and 23 (57.5%) tested positive for the *blaSHV* and *blaTEM* genes, respectively. A higher prevalence of *blaSHV* was observed in pork samples (73.3%, 11/15), whereas *blaTEM* was more prevalent in beef (70%, 7/10). The presence of resistant bacteria in commercial meats highlights contamination risks in the production chain and underscores the need for surveillance and public awareness to protect human health.

Keywords: antimicrobial resistance; food safety; esbl; meat contamination; epidemiological surveillance.

Practical Application: Detection of extended-spectrum β-lactamase-producing *Escherichia coli* in meats highlights the risk of antimicrobial resistance.

1 INTRODUCTION

Bacteria from the Enterobacteriaceae family that produce extended-spectrum β-lactamases (ESBLs) are considered one of the most significant threats to antimicrobial resistance due to their rapidly increasing prevalence in recent years (Dahms et al., 2015; Li et al., 2007). The World Health Organization (WHO) has identified these bacteria as a critical priority group for the development and research of new antimicrobials (WHO, 2017).

ESBLs are enzymes that confer resistance to β-lactam antibiotics, including penicillins and cephalosporins. These resistance genes are often plasmid-borne, facilitating their horizontal transmission between bacterial species. In addition, these genes can be located on the bacterial chromosome, contributing to the persistence and spread of resistance (Berbers et al., 2023; Mostafa et al., 2020). ESBLs predominantly derive from the TEM and SHV families, which are commonly found in *E. coli*, *Klebsiella* spp., and *Proteus mirabilis*. Other variants, such as *CTX-M*, which exhibits a higher affinity for cefotaxime, have also been identified in *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. (Cantón et al., 2012).

The emergence of ESBL-producing *E. coli* strains is closely linked to the overuse of antibiotics in animal production and human self-medication (Mota et al., 2005). Bacterial contamination of food, such as raw meat, can occur at any stage of the production chain, involving pathogens like *E. coli*. In addition to causing food-borne illnesses, *E. coli* can facilitate the transmission of antimicrobial resistance genes (Carvalho et al., 2012; Luz et al., 2017).

E. coli, naturally present in the digestive tracts of humans and animals, is associated with both opportunistic infections and zoonotic diseases (Crespo-Piazuelo & Lawlor, 2021; Lee et al., 2012). Due to its wide distribution in both livestock and humans, along with its ability to persist in the environment, this bacterium has become a significant concern in public health, particularly regarding its capacity to develop antimicrobial resistance (Jang et al., 2017; Lim et al., 2010).

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Contamination of raw meat with multidrug-resistant *E. coli* strains represents a serious public health risk, as these products provide a favorable environment for bacterial proliferation (Húngaro et al., 2016). ESBL-producing strains are particularly concerning, especially in community-acquired urinary tract infections, where socioeconomic and environmental factors contribute significantly to their dissemination. Hospitals and wastewater systems also serve as key transmission routes. Addressing this resistance challenge requires a comprehensive One Health approach, which includes rational antibiotic use, improved hygiene practices, and environmental monitoring (Jia et al., 2021; Larramendy et al., 2021; Richelsen et al., 2020).

Given the public health impact of multidrug-resistant *E. coli* strains, it is crucial to understand their epidemiological and pathogenic profiles within the context of both human and animal health. Therefore, the aim of this study was to identify the occurrence of ESBL-producing *E. coli* isolates in chicken, beef, and pork samples sold in Recife, Pernambuco (PE), Brazil.

1.1 Relevance of the work

This study provides critical epidemiological data on extended-spectrum β-lactamase (ESBL)-producing Escherichia coli in beef, pork, and chicken sold in Recife, Brazil. The high prevalence of phenotypic resistance and the detection of blaTEM and blaSHV genes highlight the risk of antimicrobial resistance transmission through the food chain. These findings reinforce the need for surveillance, prudent antimicrobial use in animal production, and food safety policies aligned with the One Health approach. The results are directly applicable to public health strategies.

2 MATERIAL AND METHODS

2.1 Sample collection and bacterial isolation

A total of 120 fresh meat samples, comprising 40 samples each of beef, pork, and chicken, were collected from commercial establishments (supermarkets, butcher shops, and open-air markets) in the eight health districts of Recife, PE (Figure 1), using non-probabilistic convenience sampling (Sampaio, 1998).

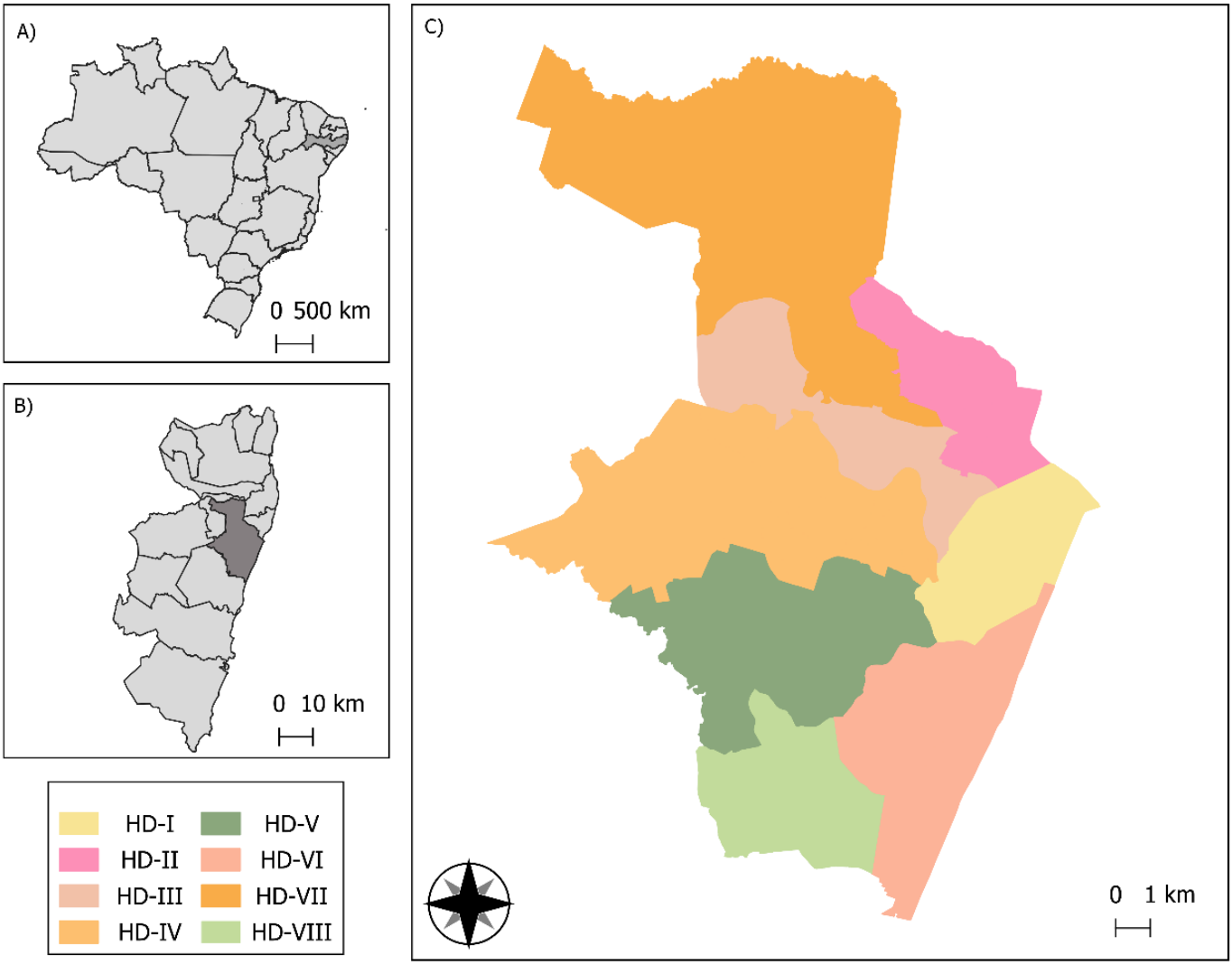

Figure 1. Health districts (HDs) of the city of Recife PE.

The samples were labeled and transported to the laboratory in thermal boxes with reusable ice packs and were refrigerated until processing, which was conducted on the same day. Each of the 120 meat samples was processed aseptically, starting with the weighing of 25 ± 0.2 g from each sample. These were transferred to pre-labeled sterile stomacher bags containing 225 mL of 0.1% peptone water for homogenization for 60 s. The samples were then incubated for 18–24 h at 37 °C under aerobic conditions in a microbiological incubator (Brasil, 2022).

After incubation, the samples were plated on Levine eosin-methylene blue (EMB) agar and incubated at 37 °C for 24 h in a bacteriological incubator. Colonies were evaluated based on their macroscopic characteristics and by Gram staining. Suspected colonies (greenish sheen and Gram-negative) were transferred to 3-mL Eppendorf® tubes containing EC broth and were incubated again at 37 °C for 24 h.

Subsequently, biochemical tests were performed to identify the colonies, following the methods described by Silva et al. (2011). Isolates with the following biochemical characteristics were classified as *E. coli*: acid slant/acid butt in Triple Sugar Iron (TSI), negative hydrogen sulfide production, positive glucose fermentation with gas production in the Durham tube, positive indole, positive motility, negative urease, positive methyl red test, negative Voges–Proskauer test, and negative citrate utilization (Koneman et al., 2018).

2.2 DNA Extraction from E. coli *isolates*

All isolates confirmed as *E. coli* were cultured on EMB agar to obtain enough pure colonies for DNA extraction. Thermal DNA extraction was performed following the procedure described by Kyselková et al. (2015). The extracted DNA was then quantified, and its purity was assessed using spectrophotometry (Multiskan Go, Thermo Scientific) with absorbance readings at 260 nm. DNA concentrations were adjusted to 100 ng/μL.

2.3 Phenotypic identification of ESBL production

All colonies morphologically and biochemically identified as *E. coli* were subjected to phenotypic resistance testing using the disk diffusion method on Müller-Hinton agar plates. Disks containing the following antimicrobials were used: cefotaxime (30 μg), ceftazidime (30 μg), and ceftriaxone (30 μg).

Initially, the isolates were suspended in sterile saline solution to a turbidity equivalent to 0.5 on the McFarland scale, followed by inoculation onto the plates and distribution of the antimicrobial disks. The plates were incubated at 37 °C for 24 h. Isolates with inhibition zone diameters equal to or smaller than 21 mm for cefotaxime, 23 mm for ceftriaxone, and/or 22 mm for ceftazidime were selected for confirmatory ESBL production tests.

The phenotypic confirmation of ESBL production was performed using the double-disk synergy test (DDST), according to the European Committee on Antimicrobial Susceptibility Testing guidelines (EUCAST, 2017), for the detection of resistance mechanisms in epidemiologically important isolates. Disks containing cephalosporins (cefotaxime, ceftazidime, and ceftriaxone) and amoxicillin-clavulanic acid were used.

After a 24-h incubation period, the results were interpreted, with ESBL production considered positive if there was an increase in the zone of inhibition around any cephalosporin disk or if an expansion toward the disk containing clavulanic acid was observed.

2.4 Genotypic analysis of β*-lactam resistance*

The genotypic analysis of β-lactam resistance was performed by detecting the *blaTEM* and *blaSHV* genes, which are responsible for ESBL production in *E. coli* strains. Primers such as SHV-F and SHV-R were used for the amplification of the *blaSHV* gene, and primers such as TEM-F and TEM-R were used for the amplification of the *blaTEM* gene (Carvalho et al., 2012; Faúla, 2016; Silva et al., 2011).

The conventional polymerase chain reaction (PCR) reaction mixture (12.5 μL) contained: 2X GoTaq® G2 Green Master Mix, forward and reverse primers for the *blaSHV* gene (1 pmol/μL) or forward and reverse primers for the *blaTEM* gene (5 pmol/μL), ultrapure water, and 2.5 μL of DNA. The conditions for both reactions were as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 60°C for 30 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 4 min.

Klebsiella pneumoniae ATCC 700603 was used as a positive control for *blaSHV*, and *E. coli* ATCC 35218 was used as a positive control for *blaTEM*. *E. coli* ATCC 25922 was used as a negative control.

After PCR amplification, 10 μL of the reaction products was subjected to electrophoresis for 40 min at 100 V on a 1.5% agarose gel stained with BlueGreen, visualized, and photographed using an ultraviolet transilluminator.

3 RESULTS AND DISCUSSION

A total of 40 *E. coli* isolates were identified from the meat samples: 10 from beef, 15 from pork, and 15 from chicken, following morphological and biochemical characterization. Phenotypic resistance testing revealed that 34 out of the 40 isolates (85%) were phenotypically positive for ESBL production, while 6 isolates (15%) were negative.

ESBL-producing bacteria from the Enterobacteriaceae family are considered one of the greatest threats to antimicrobial resistance, due to their rapid global spread (Van Hoek et al., 2011). The WHO has classified ESBL-producing bacteria as a critical priority for the development of new antimicrobials (WHO, 2017). The presence of ESBL-producing bacteria in food-producing animals has become an increasing concern for public health, primarily due to the horizontal transfer of resistance genes between bacterial strains via plasmids. Additionally, these bacteria often coexist with other resistance determinants, contributing to the increased frequency of multidrug resistance (Dahms et al., 2015; Li et al., 2007).

In the genotypic analysis, aimed at identifying the presence of the *blaTEM* and *blaSHV* genes, 57.7% (21/40) of the isolates tested positive for the *blaTEM* gene and 52.5% (21/40) harbored

the *blaSHV* gene, while 22.5% (9/40) were negative for both genes. The identification of these resistance genes represents a significant health risk, as ESBL-producing bacteria are capable of hydrolyzing broad-spectrum β-lactam antibiotics, such as third-generation cephalosporins and monobactams, which are commonly used in both human and veterinary medicine (Hawkey & Jones, 2009).

The analysis of resistance gene prevalence across meat types revealed the presence of both *blaTEM* and *blaSHV* genes in beef, pork, and chicken meat samples (Table 1). A higher prevalence of *blaSHV* was observed in pork (11/15; 73.3%) compared to beef (7/10; 70%) and chicken (3/15; 20%). In contrast, the *blaTEM* gene was most frequently detected in beef (7/10; 70%), followed by chicken (9/15; 60%) and pork (7/15; 46.7%). Additionally, isolates positive for both the *blaSHV* and *blaTEM* genes were found in beef, pork, and chicken meats, further highlighting the widespread distribution of these resistance genes across different meat types.

The widespread occurrence of *E. coli* isolates harboring ESBL genes in meat samples underscores the significant risk posed by the dissemination of antimicrobial resistance through the food chain. The coexistence of multiple resistance genes within the same isolates further complicates treatment options and highlights the urgency for continued surveillance and the implementation of stringent antimicrobial use policies in food production systems.

The presence of ESBL-producing *E. coli* expressing resistance genes to β-lactam antimicrobials in meats sold and consumed in Recife, PE, indicates the contamination of the meat by this pathogen. This contamination can originate at various stages of the production chain, including animal confinement, transportation, slaughter, or even during commercialization. Additionally, transmission can occur between animals or between humans and animals, often due to poor handling practices (Sharma et al., 2018). The adaptability of these multidrug-resistant bacteria has increased at an alarming rate, raising a global concern. Importantly, antimicrobial resistance is not limited to pathogenic bacteria; there is also a growing prevalence of resistance among non-pathogenic bacteria within the intestinal microbiota of humans and animals, with both genotypic and phenotypic expression (Woerther et al., 2013).

Some isolates identified in our study exhibited a high potential for multidrug resistance to β-lactam antimicrobials, as they expressed both phenotypic and genotypic resistance traits. This poses serious public health implications, including prolonged hospital stays due to the reduced efficacy of β-lactam antibiotics against these potentially life-threatening bacteria. Furthermore, there is a significant risk of the dissemination and transfer of these multidrug-resistant genes to non-pathogenic bacteria in the intestinal tract of healthy animals, exacerbated by the widespread use of β-lactam antimicrobials in animal production.

To fully understand the dynamics of the dissemination and transfer of multidrug resistance, it is essential to study and monitor these phenotypic and genotypic characteristics not only in the microbiota of sick animals but also in healthy ones (Lentz, 2022; Woerther et al., 2013; WHO, 2022). Although the Ministry of Agriculture, Livestock, and Food Supply (MAPA) established Normative Instruction nº 60 on December 20, 2018, to enforce microbiological control in pig and beef carcasses in slaughterhouses, this measure does not fully address the issue. While it regulates the control of *E. coli* and other Enterobacteriaceae, the instruction is limited to pig and beef carcasses (Brasil, 2018).

We emphasize the importance of expanding these regulations to other production systems, such as poultry, to ensure more comprehensive control of antimicrobial-resistant bacteria.

Table 1. The phenotypic and genotypic resistance profile of *Escherichia coli* isolated from meats commercialized in the city of Recife PE.

Type of meat	Isolated (n)	Resistance profile					
		Phenotypic		Genotypic			
				blaSHV		*blaTEM*	
		AF	RF	AF	RF	AF	RF
Beef	10	9	90	7	70	7	70
Pork	15	14	93.3	11	73.3	7	46.7
Chicken	15	12	80	3	20	9	60

AF: absolute frequency; RF: relative frequency.

4 CONCLUSIONS

This study reports the occurrence of ESBL-producing *E. coli* in meats sold in Recife, PE. The detection of bacteria with phenotypic resistance profiles and harboring resistance genes has serious implications for consumer health. It underscores the need for enhanced epidemiological surveillance and the implementation of health education programs. These initiatives should include guidelines for producers, food handlers, and consumers to raise awareness about the health risks associated with this contamination and to promote best practices in food production and handling.

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