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Characterization of ESBL-producing *Escherichia* spp. and report of an *mcr-1* colistin-resistance *Escherichia fergusonni* strain from minced meat in Pamplona, Colombia

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ABSTRACT

Foods of animal origin are increasingly considered a source of extended spectrum β-lactamase (ESBL) producing bacteria which can disseminate throughout the food chain and become a health concern for humans. This work aimed to evaluate the occurrence of ESBL-producing *Escherichia coli* in 100 retail minced meat samples taken in markets in Pamplona, Colombia. A total of 19 ESBL-producing isolates were obtained, 18 identified as *E. coli* and one as *E. fergusonii*. Fifteen isolates (78.9 %) carried $bla_{\text{CTX-M}}$ and bla_{TEM} genes, one (5.2 %) bla_{SHV} and bla_{TEM} genes, one isolate (5.2 %) carried $bla_{\text{CTX-M}}$ and one (5.2 %) bla_{SHV} alone. The majority of CTX-M-positive *E. coli* isolates carried the $bla_{\text{CTX-M-15}}$ gene (13 isolates), being the $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-2}}$, and $bla_{\text{CTX-M-8}}$ (one isolate each) also detected. Two SHV-positive isolates presented the $bla_{\text{SHV-5}}$ and $bla_{\text{SHV-12}}$ allele. The isolate identified as *E. fergusonii* was positive for $bla_{\text{CTX-M-65}}$ gene and *mcr*-1 gene. Sixteen isolates (84.2 %) belonged to phylogroups A and B1 and grouped together in the phylogenetic tree obtained by MLST; phylogroups E and F were also detected. Transfer of ESBL resistance was demonstrated for the *E. fergusonii* isolate. Whole genome sequencing of this isolate revealed the presence of plasmids carrying additional resistance genes. This investigation showed the high prevalence of ESBL-producing *E. coli* in retail samples of minced meat. Also, the isolation of a strain of *E. fergusonii* is an additional concern, as some resistance genes are located in mobile elements, which can be transmitted to other bacteria. These evidences support the increasing public health concern considering the spreading of resistance genes through the food chain.

1. Introduction

Antimicrobial resistance is one of the leading health problems worldwide, causing the drugs used to treat infections ineffective. The food chain is considered an important reservoir of antimicrobial resistant bacteria (ARB) and studies on the presence of ARB in food-producing environments and food products are considered a priority to stablish preventive measures (EFSA BIOHAZ Panel, 2021; Murray et al., 2022).

The production of extended-spectrum beta-lactamases (ESBLs), which confer resistance to a variety of β-lactams (including penicillins, cephalosporins up to fourth generation, and monobactams, but usually not to the carbapenems or the cephamycins), is a mechanism of resistance to antibiotics, particularly for members of the order *Enterobacterales*. ESBL-genes can be spread by mobile elements and transmitted through the food chain, and this has been exacerbated by the excessive use of this class of antibiotics in human and animal therapy. The most frequent ESBLs in *Enterobacterales* belong to the TEM, SHV and CTX-M enzymes (EFSA BIOHAZ Panel, 2011; Liebana et al., 2013).

Foods of animal origin are regularly contaminated with ESBL-producing bacteria and are considered a reservoir of antibiotic-resistance genes and there are reports providing circumstantial evidence that ESBL-producing *E. coli* can be associated with its transmission from food to humans (EFSA BIOHAZ Panel, 2011). ESBL-producing bacteria are spread worldwide, though there are differences in the distribution of major types between animal groups and geographical

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regions (Ewers et al., 2012). Resistance to third generation cephalosporins was frequently found in *Salmonella* spp. and *E. coli* isolates in poultry farms in Colombia (Castellanos et al., 2017; Donado-Godoy et al., 2015), and it has been reported the isolation of ESBL-producing Enterobacteriaceae from bulk tank milk (Vásquez-Jaramillo et al., 2017), but there are no data available on the contamination of beef meat.

The aim of the study was to characterize ESBL-producing isolates obtained from retail minced meat samples taken in the city of Pamplona (Colombia), studying their antimicrobial susceptibility and their phylogenetic relationships, as well as the genomic analysis of a colistin-resistant isolate.

2. Materials and methods

2.1. Sample collection and processing

One hundred retail samples of ca. 200 g of minced beef meat were purchased in 60 different stores in the city of Pamplona (Colombia) between 2018 and 2019 and were always from different batches. Eighty samples were prepared and grinded on demand and the remaining 20 samples were minced in advance and displayed to sell in the store. After collection, samples were immediately transported to the laboratory in an insulated cooler.

Samples of 10 g were aseptically weighed and cultured in 90 mL of tryptone soya broth (TSB, Oxoid, UK) at 37 °C for 24 h. After incubation, 700 μL were taken and mixed with glycerol to a final concentration of 40 % and stored at −30 °C.

2.2. Microbiological analysis

One hundred μL of the frozen stock were inoculated into 5 mL of TSB and cultured at 37 °C for 18 h. An aliquot of this enrichment culture was streaked onto Chromagar ESBL (Chromagar, France) and incubated at 37 °C for 24 h. Suspected coliform bacteria (dark pink to reddish and metallic blue colonies were picked and cultured to purify. All the isolates were preserved at −80 °C in TSB plus 40 % glycerol for further characterization (Alegría et al., 2020).

2.3. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) identification of isolates

The isolates were grown on TSA for 16–24 h at 37 °C. Colony material was collected with a sterile pipette tip and smeared as a thin film on a MALDI target plate. After air drying, each sample was overlaid with 0.5 μL of the matrix solution (α-Cyano-4-hydroxycinnamic acid, CHCA) and allowed to dry. Spectra were acquired with the MALDI Biotyper system (Bruker Daltonik, Germany) and compared with the reference database (Bruker Daltonik).

2.4. Antimicrobial resistance characterization

Phenotypic screening of ESBL production was carried out by combination disk test using both cefotaxime and ceftazidime disks, alone and in combination with clavulanic acid (Condalab, Spain). MAST D72C AmpC and ESBL detection kit (MAST group, UK) was used for ESBL confirmation. The determination of the Minimum Inhibitory Concentration (MICs) for cefotaxime, ceftazidime, imipenem and colistin was performed using the broth microdilution method according to EUCAST (https://www.eucast.org/ast_of_bacteria/mic_determination/). *Klebsiella pneumoniae* CCUG 45421, *K. pneumoniae* CCUG 56233 and *E. coli* CCUG 70662 were used as positive control for ESBL, carbapenemases and colistin resistance and *E. coli* ATCC 25922 was used as non-resistant control strain.

2.5. PCR detection and characterization of virulence factors and resistance genes

The virulence factors genes *stx*1, *stx*2 and *eae* were determined by PCR as already described (Álvarez-Suárez et al., 2015). PCR detection of bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ genes was done using the primers and conditions described by Monstein et al. (2007). Control strains from a previous work were used as positive controls (Alegría et al., 2020). Subtyping of CTX-M and SHV groups was carried out by sequencing and comparison with AMRFinderPlus tool available from the National Center for Biotechnology Information (NCBI) server. The presence of colistin-resistance gene *mcr*-1 was checked by PCR using *E. coli* CCUG 70662 as positive control (Liu et al., 2016).

2.6. Molecular typing of isolates

Phylogenetic groups were determined by the quadruplex PCR method (Clermont et al., 2013). Multilocus sequence typing (MLST) was carried out by amplifying and sequencing seven conserved housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*), as recommended by the pubMLST Database (https://pubmlst.org/). PCR products were sequenced in a MegaBACE 500 sequencer (Amersham Biosciences, USA). Raw sequences were visually reviewed and edited using the Chromas Lite 2.1 software (Technelysium, Australia) and aligned with the ClustalW algorithm of the MEGA software (Tamura et al., 2021). Each gene locus was assigned an allele number and a sequence type (ST) was determined for each isolate according to the allele profile. Grouping of isolates into clonal complexes was done with the Burst algorithm implemented in the pubMLST platform. Concatenated sequences of the seven housekeeping genes were aligned and the phylogenetic tree was obtained using the UPGMA method, with the distances estimated by the Kimura 2-parameter model and a bootstrapping of 1000 replications using MEGA software (Tamura et al., 2021).

Genomic DNA for Pulse Field Gel Electrophoresis (PFGE) was prepared following the protocol proposed by PulseNet (https://www.cdc.gov/pulsenet/index.html). DNA digestion with *Xba*I and PFGE were carried out as already described (Álvarez-Suárez et al., 2016). Comparison of PFGE profiles was done with the GelCompar 6.6 software (Applied Maths, Belgium). Similarities were obtained using the Dice coefficient at 0.5 % optimization and 1.5 % tolerance and a dendrogram was constructed with the Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering method.

2.7. Plasmid analysis and conjugation assays

Plasmid analysis and conjugation assays were performed with one *Escherichia fergusonii* isolate. Plasmid DNA was isolated by the alkaline lysis method (Birnboim and Doly, 1979) and inspected by PCR for the detection of $bla_{CTX\text{-}M}$ and *mcr-1* genes as described above.

Transfer of resistance genes was assayed in liquid medium using streptomycin-resistant *E. coli* CECT 670 as the recipient. Donor isolate was cultured until logarithmic phase of growth and mixed with recipient in a 1:1 ratio in LB broth and the mixture was incubated at 37 °C for 4 h. Transconjugants were selected on Sorbitol MacConkey (SMAC, Oxoid) agar plates supplemented with cefotaxime (32 mg/L) or colistin (2 mg/L) plus streptomycin (32 mg/L). Putative transconjugants were verified by PCR and MALDI-TOF identification as described above.

2.8. Whole genome sequencing

Whole genome sequencing of an *E. fergusonii* isolate was carried out. Genomic DNA was extracted using Illustra bacteria genomicPrep Mini Spin Kit (GE Healthcare, US) following the manufacturer instructions for gram-negative bacteria. The amount of DNA obtained was quantified by Qubit fluorometer (Thermo Fisher Scientific, US). A sequencing library

was created with a Nextera DNA XT kit (Illumina, USA), and DNA was sequenced using a NovaSeq sequencer (Illumina), obtaining 150 b.p. pair-end reads. The raw sequences were trimmed, assembled and annotated using PATRIC web platform (Wattam et al., 2017). VirulenceFinder (Joensen et al., 2014), CARD (Alcock et al., 2020) and PlasmidFinder (Carattoli et al., 2014) were used to find virulence factors, antibiotic resistance genes and plasmids, respectively.

3. Results

3.1. Isolation and identification of isolates

A total of 19 isolates resembling *E. coli* in Chromagar ESBL plates were obtained (19 %; only one isolate was picked from every suspect minced meat sample). MALDI-TOF analysis identified 18 isolates as *Escherichia coli* and one as *Escherichia fergusonii*. All the isolates were ESBL-producers as shown by combination disk test and MAST D72C detection kit. All the isolates were resistant to cefotaxime (MIC >2 mg/L) with MIC ranging from 32 to >256 μg/mL and all but one (94.7 %) were resistant to ceftazidime (MIC >4 mg/L) with MIC ranging from 4 to 32 μg/mL (Table 1). All the isolates were sensitive to imipenem and colistin, though one isolate (24_JM, *E. fergusonii*) presented a MIC for colistin of 2 mg/L, which is the breakpoint established by EUCAST to considered the presence of an acquired resistance mechanism (EUCAST, 2021). The virulence factors genes analysed were not detected among the isolates.

3.2. Characterization of antibiotic resistance genes and phylogenetic analysis

Fifteen isolates (78.9 %) carried $bla_{\text{CTX-M}}$ and bla_{TEM} genes, one (5.2 %) bla_{SHV} and bla_{TEM} genes, one isolate (5.2 %) carried $bla_{\text{CTX-M}}$ and one (5.2 %) bla_{SHV} alone (Table 1). The isolate identified as *E. fergusonii* (24_JM) was positive for $bla_{\text{CTX-M}}$ gene and *mcr*-1 gene. The majority of CTX-M-positive isolates carried the $bla_{\text{CTX-M-15}}$ gene (13 isolates), the remaining four isolates carried $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, and $bla_{\text{CTX-M-65}}$ (one isolate each). Two SHV-positive isolates presented the $bla_{\text{SHV-5}}$ and $bla_{\text{SHV-12}}$ allele (Table 1).

The *E. coli* isolates belonged to phylogroups A (10 isolates, 52,6 %), B1 (six isolates, 31,6 %) and E and F (one isolate each). Twelve isolates were assigned to a sequence type (ST10-A, ST101-B1, ST-11630-B1, ST1771-A (two isolates), ST226-A, ST4204-A, ST457-F, ST7059-*E. fergusonii*, ST7285-B1, ST-7353-A, ST8072-A) and seven isolates showed an allelic profile not included in the pubMLST database but closed to defined ST complex (ST10-related-A, three isolates; ST1196-related-B1, three isolates; ST350-related-E, one isolate) (Table 1).

In spite of the diversity of STs, the phylogenetic tree obtained from the concatenated sequences showed a clear separation of the different phylogroups, with a defined cluster including the isolates belonging to phylogroups A and B1 and the isolate of phylogroup E and separated branches for phylogroup F and *E. fergusonii* isolate (Fig. 1) in agreement with the genetic structure of the genus *Escherichia* (Clermont et al., 2013; Tenaillon et al., 2010). Four clonal groups were detected, including isolates 3_JM and 5_JM (ST1196-related-B1), 8 J and 9_JM (ST1771-A), 11_JM and 16_JM (ST10-related-A) and 1_JM and 15_JM (ST10-related-A).

PFGE analysis of *E. coli* isolates revealed 15 different profiles (Fig. 2), with three non-typable isolates (2_JM, 8_JM and 9_JM). Only three isolates (3_JM, 4_JM and 5_JM) showed a close relationship, which was already demonstrated by MLST phylogenetic analysis (Figs. 1 and 2).

3.3. Transfer of antimicrobial resistance

Plasmid DNA was purified from the *E. fergusonii* isolate and $bla_{\text{CTX-M-65}}$ and *mcr*-1 gene were detected by PCR. Conjugation experiments demonstrated the transfer of $bla_{\text{CTX-M-65}}$ gene to the recipient strain, which resulted in resistance to cefotaxime. Transfer of *mcr*-1 gene was not successful.

3.4. Genomic features of E. fergusonii 24_JM

Whole genome sequencing of *E. fergusonii* 24_JM showed a genome of 4,792,652 bp, with a G + C content of 49.98 % and a coverage of 600×. PlasmidFinder revealed five plasmids, two of them belonging to Inc1 and one to Inc2 incompatibility type, as well as plasmids pHAD28 and pO111. The Inc2 plasmid carried a quinolone-resistance gene *qnrB10*. Besides $bla_{\text{CTX-M-65}}$ and *mcr-1* genes, other resistance genes identified were $bla_{\text{TEM-1}}$, *fosA3*, which confers resistance to fosfomycin, tetracycline-resistance gen *tetC*, and two aminoglycoside modifying enzyme genes, *aph(4)-Ia* and *aac(3)-IV*; which were related to insertion sequence ISEc59. Virulence factors related to diarrheagenic *E. coli* were not found by genomic analysis.

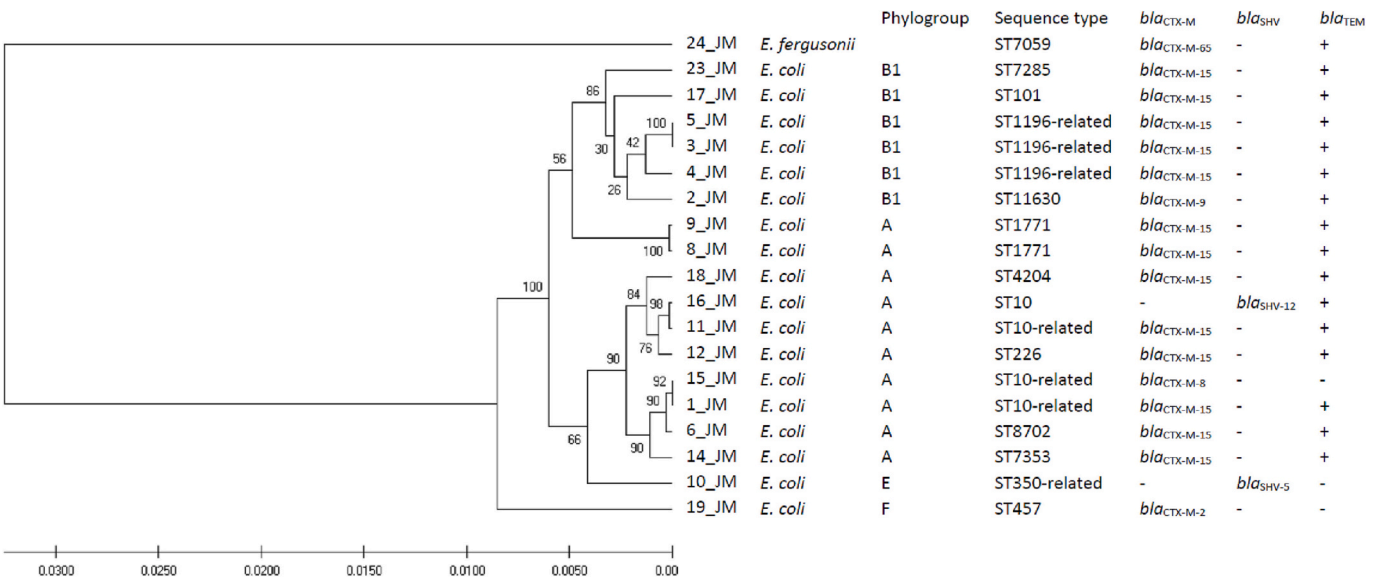
The whole genome sequence of *E. fergusonii* 24_JM can be found in the NCBI Bioproject PRJNA892292.

4. Discussion

In the present work we aimed to study the prevalence and characterization of ESBL-producing *E. coli* in samples of minced meat. Our results showed that 19 % of the samples carried extended spectrum

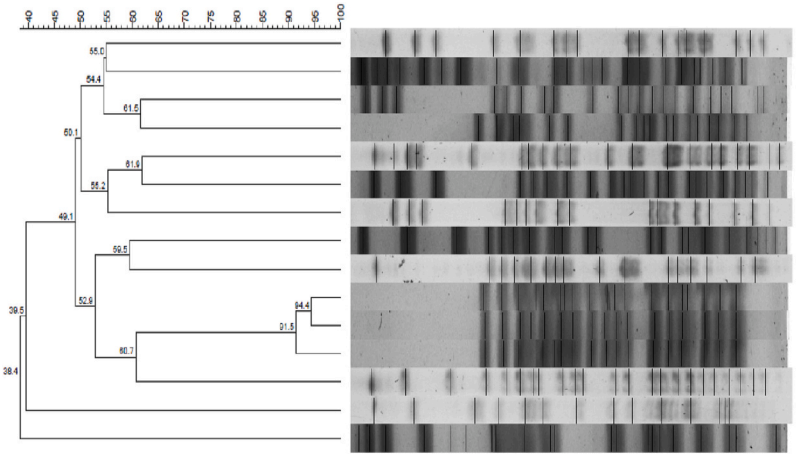
Table 1
Isolate identification, MICs (mg/L) of antimicrobial agents, identified ESBL genes and phylogenetic characterization of ESBL-producing *Escherichia* spp.

Isolate	MALDI-TOF identification	MIC CTX	MIC CAZ	MIC COL	$bla_{\text{CTX-M}}$	bla_{SHV}	bla_{TEM}	Sequence type	Phylogroup
1_JM	*Escherichia coli*	>256	16	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST10-related	A
2_JM	*Escherichia coli*	>256	8	<0.5	$bla_{\text{CTX-M-9}}$	–	+	ST11630	B1
3_JM	*Escherichia coli*	>256	32	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST1196-related	B1
4_JM	*Escherichia coli*	>256	32	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST1196-related	B1
5_JM	*Escherichia coli*	>256	32	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST1196-related	B1
6_JM	*Escherichia coli*	>256	16	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST8702	A
8_JM	*Escherichia coli*	>256	32	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST1771	A
9_JM	*Escherichia coli*	>256	32	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST1771	A
10_JM	*Escherichia coli*	4	16	<0.5	–	$bla_{\text{SHV-5}}$	–	ST350-related	E
11_JM	*Escherichia coli*	>256	16	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST10-related	A
12_JM	*Escherichia coli*	>256	32	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST226	A
14_JM	*Escherichia coli*	>256	16	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST7353	A
15_JM	*Escherichia coli*	64	0,5	<0.5	$bla_{\text{CTX-M-8}}$	–	–	ST10-related	A
16_JM	*Escherichia coli*	32	32	<0.5	–	$bla_{\text{SHV-12}}$	+	ST10	A
17_JM	*Escherichia coli*	>256	16	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST101	B1
18_JM	*Escherichia coli*	>256	32	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST4204	A
19_JM	*Escherichia coli*	>256	4	<0.5	$bla_{\text{CTX-M-2}}$	–	–	ST457	F
23_JM	*Escherichia coli*	>256	16	<0.5	$bla_{\text{CTX-M-15}}$	–	+	ST7285	B1
24_JM	*Escherichia fergusonii*	>256	8	2	$bla_{\text{CTX-M-65}}$	–	+	ST7059	



		Phylogroup	Sequence type	bla_{CTX-M}	bla_{SHV}	bla_{TEM}
24_JM	*E. fergusonii*		ST7059	$bla_{CTX-M-65}$	-	+
23_JM	*E. coli*	B1	ST7285	$bla_{CTX-M-15}$	-	+
17_JM	*E. coli*	B1	ST101	$bla_{CTX-M-15}$	-	+
5_JM	*E. coli*	B1	ST1196-related	$bla_{CTX-M-15}$	-	+
3_JM	*E. coli*	B1	ST1196-related	$bla_{CTX-M-15}$	-	+
4_JM	*E. coli*	B1	ST1196-related	$bla_{CTX-M-15}$	-	+
2_JM	*E. coli*	B1	ST11630	$bla_{CTX-M-9}$	-	+
9_JM	*E. coli*	A	ST1771	$bla_{CTX-M-15}$	-	+
8_JM	*E. coli*	A	ST1771	$bla_{CTX-M-15}$	-	+
18_JM	*E. coli*	A	ST4204	$bla_{CTX-M-15}$	-	+
16_JM	*E. coli*	A	ST10	-	bla_{SHV-12}	+
11_JM	*E. coli*	A	ST10-related	$bla_{CTX-M-15}$	-	+
12_JM	*E. coli*	A	ST226	$bla_{CTX-M-15}$	-	+
15_JM	*E. coli*	A	ST10-related	$bla_{CTX-M-8}$	-	-
1_JM	*E. coli*	A	ST10-related	$bla_{CTX-M-15}$	-	+
6_JM	*E. coli*	A	ST8702	$bla_{CTX-M-15}$	-	+
14_JM	*E. coli*	A	ST7353	$bla_{CTX-M-15}$	-	+
10_JM	*E. coli*	E	ST350-related	-	bla_{SHV-5}	-
19_JM	*E. coli*	F	ST457	$bla_{CTX-M-2}$	-	-

Fig. 1. UPGMA tree obtained from the phylogenetic analysis of the combined nucleotide sequences of the seven housekeeping genes. Bootstrapping values are shown in branch nodes.



Isolate	Phylogroup	Sequence type	bla_{CTX-M}	bla_{SHV}	bla_{TEM}
11_JM	A	ST10-related	$bla_{CTX-M-15}$	-	+
19_JM	F	ST457	$bla_{CTX-M-2}$	-	-
15_JM	A	ST10-related	$bla_{CTX-M-8}$	-	-
17_JM	B1	ST101	$bla_{CTX-M-15}$	-	+
16_JM	A	ST10	-	bla_{SHV-12}	+
23_JM	B1	ST7285	$bla_{CTX-M-15}$	-	+
6_JM	A	ST8702	$bla_{CTX-M-15}$	-	+
10_JM	E	ST350-related	-	bla_{SHV-5}	-
18_JM	A	ST4204	$bla_{CTX-M-15}$	-	+
3_JM	B1	ST1196-related	$bla_{CTX-M-15}$	-	+
5_JM	B1	ST1196-related	$bla_{CTX-M-15}$	-	+
4_JM	B1	ST1196-related	$bla_{CTX-M-15}$	-	+
12_JM	A	ST226	$bla_{CTX-M-15}$	-	+
14_JM	A	ST7353	$bla_{CTX-M-15}$	-	+
1_JM	A	ST10-related	$bla_{CTX-M-15}$	-	+

Fig. 2. Dendrogram of PFGE profiles of 15 typable *E. coli* isolates.

β-lactamases-resistant *Enterobacteriaceae* of the genus *Escherichia*, the majority of them belonging to the species *E. coli* and one identified as *E. fergusonii*. It must be taken into account that this figure can be underestimated, due to the fact that only one colony per sample was selected for characterization.

Foods of animal origin are regularly reported as carriers of ESBL-producing *E. coli*. The prevalence in beef is lower than in poultry meat (Castellanos et al., 2017; Costa et al., 2009; Díaz-Jiménez et al., 2020; Egea et al., 2012; Randall et al., 2017); several studies reported a prevalence in beef meat between 10 and 20 % (Adefioye et al., 2021; Carattoli, 2008; Clemente et al., 2021; EFSA BIOHAZ Panel, 2011; Randall et al., 2017), which are in accordance with the results obtained in this study. Few data are available on the prevalence of ESBL-producing *Enterobacteriaceae* in the cattle sector in Colombia; Vásquez-Jaramillo et al. (2017) reported a 3.3 % frequency of ESBL-producing Enterobacteriaceae in bulk tank milk, in agreement with other studies conducted in bulk tank milk reporting a prevalence between 0 and 9 % (Geser et al., 2012; Odenthal et al., 2016; Skočková et al., 2015; Sudarwanto et al., 2015).

The majority of the isolates (17/19) carried two of the three ESBL genes analysed, mainly $bla_{CTX-M-15}$ and bla_{TEM} (13/19). The $bla_{CTX-M-15}$ gene is predominant in human isolates worldwide, usually associated whit clonal group ST131 and phylogenetic group B2 (Nicolas-Chanoine et al., 2014) and is increasingly reported in food animals (Adefioye et al., 2021; Coppola et al., 2020; EFSA BIOHAZ Panel, 2011). The isolates characterized in this work belonged to phylogroups A and B1, and were assigned to a diversity of sequence types (Table 1), none of the of clinical relevance, suggesting a commensal origin. Moreover, virulence factors related to intestinal pathogenic *E. coli* (pathotypes STEC and EPEC) were not detected in the isolates, in accordance with other reports, which find few intestinal virulence factors among environmental and food isolates of ESBL-producing *E. coli* (Müller et al., 2016).

The detection of $bla_{CTX-M-8}$ is of particular interest, as it is an emergent prevalent variant already reported in poultry in Colombia (Castellanos et al., 2017) and widely disseminated in the cattle sector in South America (Ferreira et al., 2014; Palmeira et al., 2020). $bla_{CTX-M-2}$ and $bla_{CTX-M-9}$ genes, which were detected in two *E. coli* isolates, are regularly reported in food of animal origin (Adefioye et al., 2021; Alegría et al., 2020; Bevan et al., 2017; Cantón et al., 2008; EFSA BIOHAZ Panel, 2011; Ferreira et al., 2016). The $bla_{CTX-M-65}$ gene has been detected in the *E. fergusonii* isolate and is a CTX-M enzyme regularly reported in strains of *E. coli* isolated from food animals in China (Rao

et al., 2014) and South America (Riccobono et al., 2015), where is also spread through a multidrug-resistant clone of *Salmonella enterica* serovar Infantis causing diarrhea in children (Granda et al., 2019). The presence of ESBL-positive *E. fergusonii* in minced meat is not unexpected, as it has been demonstrated that it is readily isolated from food animals (Tang et al., 2022). The isolate showed high resistance to cefotaxime (Table 1). Furthermore, the strain is also resistant to colistin, carrying the *mcr*-1 gene. Strains of *E. fergusonii* carrying both $bla_{\text{CTX-M-65}}$ and a colistin-resistance gene were isolated from food animals in China and in Europe (Clemente et al., 2021; Tang et al., 2022), thus our results added new evidence to the role of *E. fergusonii* as and emergent reservoir of antimicrobial genes in the food chain (Ferreira et al., 2016; Galetti et al., 2019; Tang et al., 2022).

Two isolates carried bla_{SHV} genes and were identified as $bla_{\text{SHV-5}}$ and $bla_{\text{SHV-12}}$ respectively (Table 1), which are among the prevalent variants found in food producing animals and associated with different species of *Enterobacteriaceae* (Liakopoulos et al., 2016). They have been reported in isolates obtained in the poultry chain in Colombia (Castellanos et al., 2017). As expected, the isolates carrying $bla_{\text{CTX-M}}$ genes were more resistant to cefotaxime than the isolates with bla_{SHV} genes (Table 1) (Cantón and Coque, 2006; EFSA BIOHAZ Panel, 2011).

According to the phylogroups detected the majority of isolates (15 out of 18) are of commensal origin, belonging to phylogroups A and B1 (Table 1). One isolate (19_JM) was assigned to phylogroup F, which is closely related to phylogroup B2, the origin of most human clinical isolates (Clermont et al., 2013; Tenaillon et al., 2010) and has been detected in poultry, with an increasing tendency along the poultry production chain (Apostolakos et al., 2019).

Typing by MLST and PFGE revealed a high degree of genetic diversity among the isolates. Phylogenetic analysis of MLST results agreed with the genetic structure of the genus *Escherichia* (Clermont et al., 2013; Tenaillon et al., 2010). No concordance was observed between ST and PFGE, with only one clonal group detected by PFGE, which included isolates with similar STs, whereas some isolates belonging to the same ST showed diverse PFGE profile (Fig. 2). The isolates included in two of the clonal groups detected carried different resistant genes, thus in one clonal group isolate 11_JM carried $bla_{\text{CTX-M-1}}$ and 16_JM $bla_{\text{SHV-12}}$. In another clonal group, isolate 1_JM carried $bla_{\text{CTX-M-1}}$ and 15_JM $bla_{\text{CTX-M-8}}$ (Table 1). The results from MLST and PFGE suggested that the dissemination of ESBL-producing *E. coli* among the meat samples analysed is not derived from the spread of a specific clone, but facilitated by mobile genetic elements carrying resistance genes.

The genomic characteristics e of *E. fergusonii* 24_JM were similar to other available sequences of *E. fergusonii* (Lin et al., 2022; Liu et al., 2022). The Inc2 plasmid carried a quinolone-resistance gene *qnrB10*, which has been found associated with ESBL phenotype in clinical enterobacteria (Andres et al., 2013). Besides ESBL, *mcr*-1 and *qnrB10* genes, other resistance genes were identified: *fosA3*, which confers resistance to fosfomycin and has been demonstrated to co-disseminate with $bla_{\text{CTX-M-65}}$ in China (Hou et al., 2012; Yang et al., 2014), tetracycline-resistance gen *tetC*, and two aminoglycoside modifying enzyme genes, *aph(4)-Ia* and *aac(3)-IV,* also found in avian strains of *E. fergusonii* (Lin et al., 2022) and related to the insertion sequence ISEc59, which was detected in antibiotic-resistant strains of STEC isolated from foods in China (Shen et al., 2022). $bla_{\text{CTX-M-65}}$ gene was detected in plasmid DNA and transferred to a host strain by conjugation but genomic analysis failed to located the gene in a mobile element, probably due to the short contig sequence. Furthermore, *mcr-1* gene was amplified by PCR in plasmid DNA but not located in the mobile elements detected in the genomic analysis. A detailed analysis with long-read sequencing is planned and will resolve the plasmid composition of this strain.

The virulence factors investigated by PCR (*stx1*, *stx2* and *eae*) were not detected in the *E. fergusonii* isolate and no genes related to diarrheagenic *E. coli* were found by genomic analysis, but other authors have demonstrated the presence of the heat-labile enterotoxin (LT) gene of enterotoxigenic *E. coli* (ETEC) in a number of *E. fergusonii* strains isolated from healthy chicken feces (Oh et al., 2012).

5. Conclusions

This investigation showed the high prevalence of ESBL-producing *E. coli* in retail samples of minced meat. Also, the isolation of a resistant strain of *E. fergusonii* resistant to cephalosporins and colistin and carrying other resistance genes is an additional concern, as the resistance genes located in mobile elements can be horizontally transmitted to other bacteria. These evidences support the increasing public health concern considering the spreading of resistance genes through the food chain.

Ethical approval

Not required.

CRediT authorship contribution statement

Joana C.L. Martins: Investigation, Writing – review & editing. **Alberto Pintor-Cora:** Methodology, Investigation, Writing – review & editing. **Ángel Alegría:** Methodology, Investigation, Writing – review & editing. **Jesús A. Santos:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition. **Fanny Herrera-Arias:** Investigation, Conceptualization, Writing – review & editing.

Declaration of competing interest

There are no conflicts of interest among authors.

Data availability

Data will be made available on request.

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