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Full Length Research Paper

Escherichia Coli that Produces Extended-Spectrum Beta-Lactamases Showed a Great Diversity of Clones and Plasmids in Spanish Chicken Meat, According to Comparative Phylogenomics

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From farm to fork, there is a greater chance of coming into contact with bacteria resistant to antibiotics because animal food items are significant sources of zoonotic agents. Therefore, using a One Health approach, our goal was to identify and thoroughly characterize E. coli from the poultry industry that produces Extended-Spectrum Beta-Lactamase (ESBL). 48 samples of chicken meat were gathered from 16 locations in La Rioja (Northern Spain) between December 2021 and March 2022. The disk-diffusion method was used to evaluate antibiotic susceptibility testing. When plated on MacConkey-agar, 40 E. coli isolates were found in 33 out of 48 chicken meat samples examined (68.8%). Furthermore, six ESBL-E. coli (6/48, 12.5%) were isolated by Whole-Genome Sequencing on MacConkey-agar supplemented with cefotaxime. Clones and ESBL genes were found to be highly diverse, including ST1140-E/blaCTX-M-32 (n=1), ST752-A/blaTEM-52 (n=1), ST117-B2/blaCTX-M-1/blaSHV-12 (n=2), ST10-A/blaSHV-12 (n=1), and ST223-B1/blaSHV-12 (n=1). The blaSHV-12/blaCTX-M-1/blaCTX-M-32 genes were detected on three IncI1-plasmids (pST3-CC3) in two genetic environments: i) IS26-smc-glpR-blaSHV-12-IS26; and ii) wbuC-blaCTX-M-32/blaCTX-M-1-ISEcp1. An IS4-mediated composite transposon flanked a P1-like phage-plasmid that carried the blaTEM-52 gene. An IncHI2 plasmid contained additional genes that conferred resistance to aminoglycosides, chloramphenicol, and sulphonamides, in addition to a blaSHV-12 gene flanked by an IS26-mediated composite transposon. Our six genomes were mapped with publicly available genomes (n=2588) related to the STs found in order to analyze the cross-sectoral relatedness of our ESBL-E. coli isolates. This showed that one of our genomes (X3078-ST117) showed strong similarities (34–40 allelic differences) with a small number of genomes belonging to ST117 from the poultry sector in Germany and the USA. This investigation showed that chicken meat in Spain still contains a significant percentage of ESBL-E. coli. Furthermore, the IncI1-blaCTX-M-1-32/blaSHV-12 plasmids and the ST117 clone may be examples of effective clones and plasmids that have been modified for the chicken host.

Key words: AMR, Poultry, One health, Genetics Integrons, Transfer technique.

INTRODUCTION

Spain now ranks second in the EU for poultry meat production, which has increased significantly since 2010 due to the perception of a healthier product and a cheaper source of protein (Eurostat Agricultural Production - livestock and meat 2023). From farm to fork, the risk of exposure to antimicrobial-resistant bacteria (ARBs) is increased by poultry-derived products, which are significant

sources of zoonotic agents for humans (European Food Safety Authority EFSA and European Centre for Disease Prevention and Control ECDC, 2023; Vitas et al., 2018; Díaz-Jim´enez et al., 2020). Thus, one of the poultry industry's main challenges is limiting and stopping the spread of these germs throughout the food chain, which calls for constant observation (de Mesquita Souza Saraiva et al., 2022).

In chicken farms, antibiotics have been used as growth-promoting agents, prophylactics, and therapies (Manyi-Loh et al., 2018). The latter usage was prohibited in the EU and other countries in 2006, although it is still permitted in many others (OIE, 2022).

According to Vidovic and Vidovic (2020), the abuse of antibiotics caused selective pressure on the chicken microbiota, which favored the establishment and spread of ARB and produced reservoirs of antimicrobial resistance genes (ARGs). Importantly, the chicken microbiota naturally contains a number of zoonotic bacteria, including Salmonella spp., Escherichia coli, and Campylobacter spp. Transfers of ARGs to these (opportunistic) pathogens may compromise the effectiveness of antibiotics in animals and possibly in human medicine as well (Osman et al., 2018; Vidovic and Vidovic, 2020). Extended-Spectrum Beta-Lactamase (ESBL)-producing E. coli is a persistent problem in the poultry industry that requires a One Health approach to address (PRAN, 2019).

Prior research has examined the prevalence of ESBL-producing E. coli in poultry meat samples collected in Spain (Egea et al., 2012; Ojer-Usoz et al., 2013; Vitas et al., 2018; Díaz-Jimenez et al., 2020; Martínez-Laorden et al., 2023). None of them, however, carried out a comprehensive genomic investigation that included comparison with other publicly available genomes from human, animal, and environmental contexts on a worldwide basis. A comparative phylogenetic analysis and plasmid characterization of ESBL-producing E. coli isolates obtained from chicken meat samples in the La Rioja region were conducted in order to close this gap. Using Whole-Genome Sequencing (WGS), we characterized the clones (at the core-genome level) and the genetic factors that contribute to the spread of vitally important AMR genes at both large (plasmids) and small (transposons, integrons) scales. In order to prepare the way for upcoming One Health epidemiological studies with a broader focus, a comprehensive comparison of our ESBL-E. coli genomes with those that are publically accessible in databases was conducted to investigate potential connections between them.

2. Materials and methods

2.1. Isolation, identification, and sampling of bacteria

48 chicken meat samples (thigh, breast, and minced meat) were gathered from 16 locations (nine neighborhood stores and seven hypermarkets) in the La Rioja region of Northern Spain between December 2021 and March 2022. They were then sent to the microbiology lab for processing in sterile bags at 4°C. Sliced beef portions were cleansed with a sterile cotton swab under sterile circumstances and then put right into the Brain-Heart Infusion Broth, which was incubated at 37°C for the entire night. For the recovery of cefotaxime-resistant (CTXR) and non-CTXR E. coli, respectively, different aliquots were seeded on MacConkey (MC) agar plates treated with cefotaxime (MC-CTX; 2μg/mL) or not supplemented (MC). Up to eight presumed E. Coli colonies were randomly selected for each sample, and MALDI-TOF (Bruker Daltonik, Bremen, Germany) was used to confirm identification.

2.2. Antimicrobial susceptibility testing

The disk-diffusion method was used to test for susceptibility on Mueller-Hinton agar in accordance with the Clinical Laboratory Standard Institute 2023 criteria and clinical breakpoints (Clinical and Laboratory Standards Institute CLSI, 2023). Ampicillin, amoxicillin/clavulanate, ceftazidime, cefotaxime, cefoxitin, imipenem, nalidixic acid, ciprofloxacin, gentamicin, amikacin, tobramycin, chlor-amphenicol, trimethoprim/sulphamethoxazole, and tetracycline were among the antibiotic disks of human and/or veterinary interest that were tested. The double disc synergy test was used to verify ESBL screening. For quality control, E. Coli strain ATCC 25922 was included.

2.3. AMR gene and integron characterization in isolates of E. coli that are not ESBL

A collection of non-duplicate isolates, one isolate per sample and per antibiotic resistance pattern, was kept for additional study based on the phenotypic resistance profiles of E. coli isolated from MC plates. PCR and sequencing were used to identify the following AMR genes: β-lactams (blaTEM, blaCTX-M, blaSHV, and blaOXA); quinolones (qnrA, qnrB, qnrS, aac(6´)-Ib-cr, and qepA); gentamicin (aac(3)-II, aac(3)-III, and aac(3)-IV); tetracycline (tet(A), tet(B), tet(C), tet(D), and tet (E)); chloramphenicol (cmlA, catA1, and floR); and sul1, sul2, and sul3 (Jouini et al., 2007; Alonso et al., 2017a). Furthermore, the quinolone-resistant isolates' alterations in the amino acids of the ParC and GyrA proteins were examined by PCR-sequencing (Alonso et al., 2017a). PCR amplification of the integrase gene intI1 (for class 1 integrons) and intI2 (for class 2 integrons) was used to screen for the presence of integrons (Mazel et al., 2000). The entire array was acquired by using the "primer-walking" PCR technique to determine their genetic structure (Vinu´e et al., 2008a, 2008b).

2.4. Short-read sequencing of ESBL E. coli isolates

Genomic DNA was extracted using a NucleoSpin Microbial DNA extraction kit (Macherey-Nagel, Hoerdt, France) in

accordance with the manufacturer's instructions. A NovaSeq 6000 device (Illumina, San Diego, CA, USA) was used for sequencing, and Nex-tera XT technology was used for library preparation. Following sequencing, reads underwent quality trimming, de novo assembly using Shovill v1.0.4, and QUAST v5.0.2 quality assessment (Table S1). ABRicate v1.0.1 (https://github.com/tseemann/abricate) was used to infer resistance genes and replicon content, whereas MLSTFinder v2.0.4 (http://www.genomicepidemiology.org/), a CGE online tool, was used to determine sequence types (STs). SeroType Finder (http://www.genomicepidemiology.org/) was used to identify serotypes, and VirulenceFinder was used to identify virulence factors (VFs).

2.5. Phylogenetic analysis

Using the pyMLST workflow (https://github.com/bvalot/pyMLST), a core-genome alignment based on the concatenation of 2513 core genes was produced for phylogenetic studies. The threshold for closely related strains was separated into the following categories: >200 ADs, 51–100 ADs, 101–200 ADs, 21–50 ADs, 11–20 ADs, and 0–10 ADs. IQ-TREE v1.6.12 (model GTR+F+ASC+R5) was utilized to create a maximum likelihood phylogenetic tree using this alignment, and iTol v6 (http://itol.embl.de/itol.cgi) was used to view the finished tree.

2.6. Long-read sequencing, plasmid characterization, and transfer of ESBL E. coli isolates

The sodium azide-resistant E. coli J53 strain was used as the recipient cell for conjugation-based plasmid transfer in liquid media. On LB agar enriched with sodium azide (200 mg/L) and cefotaxime (2 mg/L), trans-conjugants were chosen. The number of transconjugants was divided by the number of donors to determine the conjugation frequencies. PlasmidFinder 2.0.1 was used to extract the plasmid content from the WGS data (http://www.genomicepidemiology.org/). When the ESBL gene was found on the same contig as the plasmid marker, plasmids containing the ESBL genes were allocated in silico. Plasmids containing the ESBL genes were identified using S1-PFGE gels (6V/cm for 20h at an angle of 120◦ at 14◦C with pulse times ranging from 1 to 30s) and Southern blot with suitable probes, as previously described, when in silico data revealed no co-occurrence on the same contig (Saidani et al., 2019). Furthermore, the ligation sequencing kit (SQK-LSK109) and the native barcode expansion kit (EXP-NBD104; Oxford Nanopore Technologies) were used for long-read sequencing. In order to produce the hybrid assemblies, sequencing was carried out on a MinION sequencer (SpotON Mk 1 R9) with a flow cell (FLO-MIN106D), assembled using Unicycler (Wick et al., 2017), and corrected with Pilon (Walker et al., 2014).

2.7. Accession numbers

Each and every genomic sequence was added to DDBJ/EMBL/GenBank with the BioProject accession number PRJNA1102028. Under accession numbers PP711258, PP711259, PP711261, and PP711260, respectively, the complete sequences of the plasmids pUR3078 (E. coli X3078), pUR3556 (E. coli X3556), pUR3561 (E. coli X3561), and pUR3081 (E. coli X3081) were added to the EMBL database.

3. Results

3.1. Prevalence of E. coli on chicken meat

33 of the 48 chicken meat samples that were analyzed had E. coli isolates recovered on MC agar plates (68.8%), resulting in a collection of 40 non-duplicate E. coli isolates (1–2 E. coli isolates/sample). Additionally, six samples (12.5%) from the breast and thigh, five from hypermarkets and one from a local store, had ESBL-producing E. coli isolates that were recovered using selective MC-CTX plates (Table S2).

3.2. Characterization of integrons and genetic characteristics of E. Coli isolates that do not produce ESBL

All 40 of the non-repetitive E. Coli isolates that were grown on non-selective plates did not develop ESBL. Resistance to tetracycline, chloramphenicol, kanamycin, and gentamicin was also found, although at lower rates (3%–15%). The highest resistance rates shown were for ampicillin, amoxicillin/clavulanate, nalidixic acid, ciprofloxacin, and trimethoprim/sulphamethoxazole (22.5%–50%) (Table 1).

Among the 40 E. Coli isolates that did not produce ESBL, a wide variety of AMR genes were found (Table 2). The blaTEM gene was primarily responsible for ampicillin resistance, while plasmid-mediated quinolone resistance genes aac(6′)-Ib-cr and qnrA, as well as amino acid alterations in the quinolone resistance detection region of the topoisomerases GyrA and ParC (S80I, D87N/S83I), were responsible for ciprofloxacin resistance. The aac(3)-II gene was present in every isolate that was resistant to gentamicin. The tet(A) or tet(B) genes mediated tetracycline resistance, and the cmlA gene was present in all isolates resistant to chloramphenicol. Furthermore, the dfrA1/dfrA12 genes and the sul1, sul2, and/or sul3 genes were present in SXT-resistant isolates (Table 2A). E (n = 14; 27.5%), A (n = 13, 25.5%), B1 (n = 8, 15.7%), C (n = 6, 11.8%), B2 (n = 4, 7.8%), and F (n = 4, 7.8%) were the phylogroups to which E. coli isolates belonged (Table S3).

One isolate had two class 1 integrons (intI1-dfrA1-aadA1-qacEΔ1-sul1 and intI1-dfrA12-orfX-aadA1-qacEΔ1-sul1) each (Table 2B), whereas two isolates had the classical class 2 integron associated with Tn7 (intI2-dfrA1-sat2-

aadA1-orfX-ybfA-ybfB-ybgA-tnsE-tnsD-tnsC-tnsB-tnsA) (Table 2C).

3.3. Characterisation of blaESBL-carrying E. coli isolates

On MC-CTX selective plates, six ESBL-producing E. coli isolates were gathered, one for each sample. Two isolates had extra genes that gave them resistance to tetracyclines and amino-glycosides (X3560) or to sulphonamides and amino-glycosides (X3556), making them multi-drug resistant. Resistance to imipenem and cefoxitin was not found (Table 3).

ST1140-E/blaCTX-M-32 (n = 1), ST752-A/blaTEM-52 (n = 1), ST117-B2/blaCTX-M-1 (n = 1), ST117-B2/blaSHV-12 (n = 1), ST10-A/blaSHV-12 (n = 1), and ST223-B1/blaSHV-12 (n = 1) were the ST-phylogroups/ESBL gene combinations that were found in the six ESBL-producing E. coli isolates (Table 3). These STs' phylogenetic analysis showed that the two isolates from the lineage ST117 (obtained from two distinct institutions) were not clonally related since they diverged by 135 ADs (Table S4).

3.3.1. IncI plasmids

In the ensuing genetic context, three IncI-plasmids were discovered to carry an ESBL gene: the novel IS26-smc-glpR-blaSHV-12-IS26 (IncI1/pST3), ISEcp1-blaCTX-M-1-wbuC (IncI1/pST3), and ISEcp1-blaCTX-M-1/32-wbuC (IncI2) (Fig. 1a-b, Fig. S1a-b). The two IncI1/pST3 plasmids were fully identified and covered by the replication, transfer, and leading region (Fig. S2), and 81%–97% of the plasmids had 99.96%–100% coverage with pJ31 (accession no. CP053788.1), pEC7 (CP053679.1), and pEC1 (CP053560.1), pESBL-283 (CP008736.1), or pEC161_1 (KU932032.1). A few insertions and deletions suggested recombination between the plasmids. The estimated size of the IncI2-blaCTX-M-32-carrying plasmid was 90 kb, which is comparable to the sizes of IncI1-plasmids (100–110 kb) that have been described. The conjugation frequencies of all IncI plasmids ranged from 2.6/3.1×10—6 for the IncI1-plasmids and 5.1×10—4 for the IncI2-plasmid.

Their adaptability modules, on the other hand, showed greater variability (Fig. 1, Fig. S2). Both the pUR3081 (12,672 bp) and pUR3078 plasmids displayed a sizable adaptation module (9,516 bp) between the replication area and the parA-parM partition genes (Fig. 1a-b) and the yacA type II toxin-antitoxin system (leading region) (Fig. 1). With the exception of the genetic content of the adaptability module (AMR genes and proteins with known functions), the adaptability modules of the various IncI1/I2 plasmids shown 100% similarity with the transfer, leading, and replication regions.

3.3.2. Phage plasmid

A P1-like phage-plasmid (107,992 bp) containing the blaTEM-52 gene demonstrated 83% to 99.7% coverage/identity with other E. coli genes previously reported in broiler farms and human infections (accession no. OY757102.1, OY754380.1, CP134391.1). Our ΔP1-plasmid displayed some conserved regions as the original one (accession no. AF234172 and AF234173) (Fig. 1c): the res-mod (restriction-modi- fication protein), the IS1-associated genes (isaA, insB, insA, and isaB), the encoding holing and tail-fiber related protein (lydC, cin, Sv', U′, U, and S), the super immunity-linked function (simC, simB, and simA), the possibly replication-linked function (rlfA, rlfB, and pmgF), the plasmid replication (repA and upfA), the tellurite/colicin resistance/inhibition of cell divi- sion (tciA, tciB, and tciC), and the SOS putative morphogenetic function (pmgT, pmgU, pmgV, upfM, upfN, upfO, hot, lxr, and humD). The blaTEM-52 and its flanking module composed by a transposon framed by IS4 were the only components of the adaptability module (Table 3).

Furthermore, upstream of the blaTEM-52 gene (183 bp), a transposon derived from Tn3 that contained solely the elements involved in its trans-position (tnpA, tnpR) was inserted (Fig. S1c).

3.3.3. IncHI2 plasmid

A sizable IncHI2/pST3 plasmid (225,327 bp) containing several AMR genes harbored one of the three blaSHV-12 genes (Fig. 1d, Table 3). A Tn21-derived transposon with an unusual class 1 integron linked to sul3 (Fig. 2a) was detected inside its adaptability module (34,056 bp), suggesting the sequential acquisition of AMR genes (amino-glycosides, chloramphenicol, and sulphonamides). A ΔTn1721 was then incorporated in its structure, bordering downstream the blaSHV-12 (Fig. S2d) and retaining just the parts involved in its transposition (tnpR, tnpA) and the terminal inverted repeat IRR and IRL (Fig. 2). Remarkably, this transposon generated from Tn21 was identical to one that had been previously discovered in the digestive tract of nestling white storks in Spain (pUR5279 plasmid, Fig. 2). The conjugation frequency of our conjugative IncHI2 plasmid was 4.2×10—5 (Table 3).

3.4. Cross-sectoral phylogenetic comparisons of the STs detected in ESBL E. coli

52 genomes, including the cecal content of pigs (n = 5), broilers (n = 2), dairy cows (n = 7), veal calves (n = 14), as well as retail chicken (n = 18), retail pork (n = 1), retail turkey (n = 1), and retail veal (n = 4), were mapped to the public genomes approved in the "EU AMR monitoring in livestock and meat" project (Brouwer et al., 2023) (accession no. PRJNA885502) (Fig. 3). According to these findings, three genomes associated with ST117 that were discovered in retail chicken in the Netherlands displayed allelic differences

between 53 and 56 with our chicken meat genomes and more distant ADs with those associated with retail veal (125–138 ADs) or broiler/veal calf cecal content (Tables S5–S6). Throughout this investigation, no significant connections (200 ADs) between ST10-associated genomes were found (Table S6).

Second, by comparing our genomes to all publically accessible genomes at NCBI linked to ST10 (n = 2130), ST117 (n = 341), ST223 (n = 81), ST752 (n = 19), and ST1140 (n = 17), we expanded the One Health analysis (Fig. 4, Fig. S3). Regarding ST10, we found that the genomes from chicken farms in China (n = 4), Paraguay (n = 1), Italy (n = 1), and Spain (n = 1) had commonalities (57–129 ADs), some of which were linked to human infections in Argentina (n = 1), Saudi Arabia (n = 1), and China (n = 1). Furthermore, close ties were observed between genomes taken from alpacas in the USA (n = 1), pigs in China (n = 1), and sewage in Japan (n = 1) (Table S5, Table S7, Fig. 4a-b). Our genome (X3078) showed narrow linkages (34–40 ADs) with the following: i) eight genomes previously reported from German fattening broiler farms; ii) one recovered from chicken breast (USA); iii) one from Pakistani chickens; and iv) one from a human infection in Lithuania. ST117 is a widely distributed lineage in the poultry industry (Table S5, Table S8, Fig. 4c-d).

We only found weak similarities (117–195 ADs) between the genomes of the least common ST, ST223, and those of human (n = 1) and animal (n = 1) infections in the USA and wastewater (n = 1) in Brazil (Table S5, Table S9, Fig. S3a-b). Lastly, there were no discernible similarities between the ST752 and ST1140 (84–499 ADs); however, the ST752 (11/19) was clearly associated with the avian clade (Table S5, Tables S10–11, Fig. S3c-f).

4. Discussion

E. coli isolates were found in 68.8% of the chicken meat samples in this investigation, indicating possible contamination during slaughter, meat processing, or transportation. Consistent with other research, these E. coli isolates showed significant resistance rates to quinolones, sulphonamides, and narrow-spectrum β-lactams (Jammoul and El Darra, 2019; Pena et al., 2010). In the poultry industry, resistance to first-line therapies is making it possible to use fluoroquinolones or third/fourth-generation cephalosporins for second-line treatments, which are vital for human medicine. As a result, humans may contract resistant germs by way of the food chain.

From 62.5% in 2007 to 93.3% in 2010 (Egea et al., 2012), the prevalence of retail poultry meat in Spain contaminated with ESBL-producing E. coli isolates rose significantly before a 2018 investigation found a nearly 50% decrease (Vitas et al., 2018). Our findings imply that the implemented procedures on the management and use of antimicrobials in chicken farming are producing favorable effects, however they should be verified by a larger-scale investigation. Although the percentage of E. coli that produces ESBL is still high, these are positive findings. Ceftiofur is widely used in commercial chicken flocks to treat one-day-old chicks and prevent early mortality linked to E. coli in turkeys and broilers. This may be the cause of this, as it encourages the emergence and spread of ESBL producers in the gastrointestinal tract of these birds (de Mesquita Souza Saraiva et al., 2022). Although to a lesser degree, ceftiofur is still used in livestock in the majority of EU nations, including Spain. These findings highlight the need to implement comparable policies to those already in place in Australia, where ceftiofur restrictions were implemented concurrently with the elimination of ESBL-producing bacteria from meat samples (Abraham et al., 2019).

The prevalence of blaCTX-M-14 and blaSHV-12, which were simultaneously appearing in Spanish and European hospitals (Coque et al., 2008; Diestra et al., 2008), was reported in the first investigation of ESBL-producing E. coli in the poultry sector, conducted in Spanish chicken farms (Briņas et al., 2003). From 44% to 94% in Spain, Sweden, and the Netherlands, according to numerous studies conducted in the past few decades, ESBL-producing E. coli has been found in poultry and food-derived products (Leverstein-van Hall et al., 2011; Bo¨rjesson et al., 2013; Martínez-Alvarez et al., 2022). It is primarily encoded by the blaCTX-M-1 gene in Sweden, blaSHV-12 in Spain, and blaTEM-52 in the Netherlands. Our ESBL-E. coli isolates' genomes revealed a great deal of genetic lineage variability. Isolates were not clonal, however only ST117 was found twice. Although ST117 was initially linked to avian hosts and the poultry industry (Mora et al., 2012), it is currently also regarded as a high-risk clone linked to septicemia or urinary tract infections in humans. According to Martínez-Alvarez et al. (2022), ST117 is one of the most commonly reported clones in the poultry industry in Spain. It typically carries the blaCTX-M-1 and blaSHV-12 genes. In the poultry industry in various European nations, it has also been discovered to carry additional ESBL genes, such as blaCTX-M-14/blaCTX-M-15 (Apostolakos et al., 2020) or plasmidic AmpC genes like blaCMY-2 (Roedel et al., 2021). A common clone, ST10 has been found in the environment, in clinical settings (where it has been linked to invasive human illnesses), and in animals, including chickens (Day et al., 2019).

Particularly in Enterobacterales, mobile genetic elements play a crucial role in the transmission of ARGs from human to environmental sources. A range of blaESBL genes, including the blaSHV-12, blaCTX-M-1, blaCTX-M-32, and blaTEM-52 genes, were also present in our isolates that were obtained from chicken meat. These genes were carried by several plasmids.

As has been frequently reported in chickens (García-Fernández et al., 2008; Bortolaia et al., 2010), pigs/meat (Alonso et al., 2017a, 2017b; Martínez-A´lvarez et al., 2024), and even migratory birds (Martínez-A´lvarez et al., 2023), the IncI1-type plasmids carried the blaSHV-12 and blaCTX-M-1 genes. Alonso et al., 2017a, 2017b; Martínez-Alvarez et al., 2023, 2024) reported a slightly different genetic environment for blaSHV-12 because we found it upstream of the gene flanked by the transcriptional regulator glpR (IS26-

smc-glpR-blaSHV-12-IS26) instead of the common deoR. The flanking regions (ISEcp1-blaCTX-M-1/32-wbuC) of the blaCTX-M genes were found to be highly conserved and consistent with earlier findings (Coque et al., 2008; Diestra et al., 2008; Tian et al., 2011; Martínez-Alvarez et al., 2023).

It's interesting to note that we discovered the blaSHV-12 gene on an IncHI2/pST3 plasmid. Both the clinical scale IncX3 plasmids (Liakopoulos et al., 2018) and the livestock and derivative products IncI1 plasmids (Alonso et al., 2017a, 2017b; Martí-nez-Alvarez et al., 2024) have extensive documentation of this gene. A recent investigation on livestock in Germany also found that the blaSHV-12 was co-harboring the bla-VIM-1 gene on an IncHI2 plasmid. This study confirmed that IncHI2 plasmids containing blaSHV-12 (Pauly et al., 2021), which were first found in the environment, are huge (> 200 kbp) and have a significant ability to propagate several AMR genes. Remarkably, the adaptability module included a sizable

The genetic structure of ΔTn21 with a Tn1721 implanted is the same as that recently reported in Spanish nestling storks (Martínez-Alvarez et al., 2023).

We also discovered an unusual phage-plasmid that carries the blaTEM-52 gene. This ESBL gene has never been discovered in E. coli on a phage-plasmid, but it has been previously identified in clinical isolates and/or production animals from Canada, Portugal, France, Greece, the Netherlands, Germany, Belgium, Great Britain, Croatia, and Japan with various plasmid locations (Cloeckaert et al., 2007; Bielak et al., 2011). Overall, in accordance with the results reported, this gene may be found on a variety of plasmid replicons, most likely as a result of its interaction with Tn3 elements. The Tn3-blaTEM-52 element may have been transported both horizontally and clonally once it was inserted into the plasmid backbone (Bielak et al., 2011). But only one gene was included in our phage plasmid's resistance module and several conserved areas that were involved in the plasmid's replication, stability, and maintenance.

Integrons have been commonly found in isolates from a variety of sources, including healthy individuals (Vinue´ et al., 2008a), hospitalized patients (Vinue´ et al., 2008b), and food animals (Casella et al., 2015). Some of them, like class I (dfrA1-aadA1; dfrA12-orfX-aadA2) or class 2 integrons (dfrA1-sat2-aadA1; sat2-aadA1) (Machado et al., 2008; Soufi et al., 2009, 2011), are widely distributed. In this case, integrons with distinct gene cassette arrangements were also found, particularly on transposons (Tn21) and/or plasmids (IncI1, IncF, IncHI2), which correspond to earlier reports in various ecosystems (Alonso et al., 2017a, 2017b; Martínez-A´lvarez et al., 2023, 2024).

The selection of distinct genetic backgrounds that are likely to persist and, thus, be spread in this environment and/or along the food supply chain may be associated with the risk of transmission through ingestion of chicken meat contaminated with ESBL-producing E. coli (EFSA Panel on Biological Hazards (BIOHAZ) et al., 2021, 2022, 2024). Here, we discovered extremely close cluster links (between 34 and 40 ADs) between our ST117 genomes and those of the public ST117 genomes associated with the poultry industry (poultry meat in the USA, fattening farms in Germany or Pakistan), as well as in one instance with a human clinical isolate that was reported in Lithuania. These results may be explained by the fact that this genetic lineage gained widespread recognition in the livestock industry after first appearing in the therapeutic setting and then quickly expanding into the animal kingdom. Furthermore, this mobility of cattle or meat products, or even just the movement of human populations, has favored its extensive diffusion because Spain is one of the EU's top exporters of meat.

Our results demonstrate that certain isolates are better suited to the chicken host, underscoring the need to use containment strategies at every stage of the food traceability chain in order to significantly lower the risk of transmission from the "environment to the bedside." To reduce the risk of infections from raw meat that are caused by resistant Campylobacter spp. or Salmonella spp., as well as ESBL-producing E. coli, good practice guidelines should be established during the meat processing process and in our own homes. Future research should also look into food traceability globally to see how daily cleaning, disinfection, processing, and handling practices affect the persistence and spread of ARBs and ARGs. Additionally, this study indicated that the successful spread of clones, ESBL genes, and their carrying plasmids through chicken flesh can transcend borders because of the import/export of poultry goods on a European and international level.

Ethical approval

Not required.

Declaration of competing interest

The authors state that none of the work described in this study could have been influenced by any known competing financial interests or personal relationships.

Data availability

Data will be made available on request.

References

Abraham, S., O'Dea, M., Sahibzada, S., Hewson, K., Pavic, A., Veltman, T., Abraham, R., Harris, T., Trott, D.J., Jordan, D., 2019. Escherichia coli and Salmonella spp. isolated from Australian meat chickens remain susceptible to critically

important antimicrobial agents. PloS One 14 (10), e0224281. https://doi.org/10.1371/journal. pone.0224281.

Alonso, C.A., Gonza´lez-Barrio, D., Ruiz-Fons, F., Ruiz-Ripa, L., Torres, C., 2017a. High frequency of B2 phylogroup among non-clonally related fecal Escherichia coli isolates from wild boars, including the lineage ST131. FEMS Microbiol. Ecol. 93 (3) https:// doi.org/10.1093/femsec/fix016.

Alonso, C.A., Michael, G.B., Li, J., Somalo, S., Simo´n, C., Wang, Y., Kaspar, H., Kadlec, K.,

Torres, C., Schwarz, S., 2017b. Analysis of blaSHV-12-carrying Escherichia coli clones and plasmids from human, animal and food sources. J. Antimicrob. Chemother. 72 (6), 1589–1596. https://doi.org/10.1093/jac/dkx024.

Apostolakos, I., Feudi, C., Eichhorn, I., Palmieri, N., Fasolato, L., Schwarz, S., Piccirillo, A., 2020. High-resolution characterisation of ESBL/pAmpC-producing Escherichia coli isolated from the broiler production pyramid. Sci. Rep. 10, 11123. https://doi.org/10.1038/s41598-020-68036-9.

Bielak E, Bergenholtz RD, Jørgensen MS, Sørensen SJ, Hansen LH, Hasman H. Investigation of diversity of plasmids carrying the blaTEM-52 gene. J. Antimicrob. Chemother. 2011 Nov;66(11):2465–74. doi:https://doi.org/10.1093/jac/dkr331.

Bo¨rjesson, S., Egerv¨arn, M., Lindblad, M., Englund, S., 2013. Frequent occurrence of extended-spectrum beta-lactamase- and transferable ampc beta-lactamase-producing Escherichia coli on domestic chicken meat in Sweden. Appl. Environ. Microbiol. 79 (7), 2463–2466. https://doi.org/10.1128/AEM.03893-12.

Bortolaia, V., Guardabassi, L., Trevisani, M., Bisgaard, M., Venturi, L., Bojesen, A.M., 2010. High diversity of extended-spectrum beta-lactamases in Escherichia coli isolates from Italian broiler flocks. Antimicrob. Agents Chemother. 54 (4), 1623–1626. https://doi.org/10.1128/AAC.01361-09.

Brin˜as, L., Moreno, M.A., Zarazaga, M., Porrero, C., Sa´enz, Y., García, M., Dominguez, L.,

Torres, C., 2003. Detection of CMY-2, CTX-M-14, and SHV-12 beta-lactamases in Escherichia coli fecal-sample strains from healthy chickens. Antimicrob. Agents Chemother. 47 (6), 2056–2058. https://doi.org/10.1128/AAC.47.6.2056-2058.2003.

Brouwer, M.S.M., Zandbergen Van Essen, A., Kant, A., Rapallini, M., Harders, F., Bossers, A., Wullings, B., Wit, B., Veldman, K.T., 2023. Implementation of WGS analysis of ESBL-producing Escherichia coli within EU AMR monitoring in livestock and meat. J. Antimicrob. Chemother. 78 (7), 1701–1704. https://doi.org/10.1093/ jac/dkad158.

Casella, T., Rodríguez, M.M., Takahashi, J.T., Ghiglione, B., Dropa, M., Assunç˜ao, E., Nogueira, M.L., Lincopan, N., Gutkind, G., Nogueira, M.C., 2015. Detection of blaCTX- M-type genes in complex class 1 integrons carried by Enterobacteriaceae isolated from retail chicken meat in Brazil. Int. J. Food Microbiol. 197, 88–91. https://doi.org/ 10.1016/j.ijfoodmicro.2014.12.001.

Clinical and Laboratory Standards Institute (CLSI), 2023. Performance standards for antimicrobial susceptibility testing. In: CLSI Supplement M100-Ed33. National Committee for Clinical Laboratory Standards, USA, 33rd ed.

Cloeckaert, A., Praud, K., Doublet, B., Bertini, A., Carattoli, A., Butaye, P.,Imberechts, H., Bertrand, S., Collard, J.M., Arlet, G., Weill, F.X., 2007. Dissemination of an extended-spectrum-beta-lactamase blaTEM-52 gene-carrying IncI1 plasmid in various Salmonella enterica serovars isolated from poultry and humans in Belgium and France between 2001 and 2005. Antimicrob. Agents Chemother. 51 (5),

1872–1875. https://doi.org/10.1128/AAC.01514-06.

Coque, T.M., Baquero, F., Canton, R., 2008. Increasing prevalence of ESBL- producing Enterobacteriaceae in Europe. Eurosurveillance 13, 19044. https://doi.org/10.2807/ ese.13.47.19044-en.

Day MJ, Hopkins KL, Wareham DW, Toleman MA, Elviss N, Randall L, Teale C, Cleary P, Wiuff C, Doumith M, Ellington MJ, Woodford N, Livermore DM. Extended-spectrum β-lactamase-producing Escherichia coli in human-derived and food chain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study. Lancet Infect. Dis. 2019 Dec;19(12):1325–1335. doi:https://doi. org/10.1016/S1473-3099(19)30273-7.

Díaz-Jim´enez, D., García-Menin˜o, I., Ferna´ndez, J., García, V., Mora, A., 2020. Chicken

and Turkey meat: consumer exposure to multidrug-resistant Enterobacteriaceae

including mcr-carriers, uropathogenic E. Coli and high-risk lineages such as ST131. Int. J. Food Microbiol. 16 (331), 108750. https://doi.org/10.1016/j. ijfoodmicro.2020.108750.

Diestra, K., Coque, T.M., Miro´, E., Oteo, J., Nicolau, C.J., Campos, J., Moya´, B.,

Curiao, T., Pe´rez-Va´zquez, M., Canto´n, R., Oliver, A., Navarro, F., Matas, L., Gime´nez, M., Sanfeliu, I., Prats, G., Ruiz, M., Vila, J., Domínguez, M., Aznar, J., 2008. Caracterizacio´n y epidemiología molecular de betalactamasas de espectro extendido en Escherichia coli y Klebsiella pneumoniae en once hospitales espan˜oles. Enferm. Infecc. Microbiol. Clin. 26, 404–410. https://doi.org/10.1157/13125636.

EFSA Panel on Biological Hazards (BIOHAZ), Koutsoumanis, K., Allende, A., A´lvarez-Ordo´n˜ez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Argüello, H., Berendonk, T., Cavaco, L.M., Gaze, W., Schmitt, H., Topp, E., Guerra, B., Lie´bana, E., Stella, P., Peixe, L., 2021. Role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain. EFSA J. 19 (6), e0665 https://doi.org/10.2903/j.efsa.2021.6651.

EFSA Panel on Biological Hazards (BIOHAZ), Koutsoumanis, K., Allende, A., A´lvarez-Ordo´n˜ez, A.,

Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Argüello-Rodríguez, H., Dohmen, W., Magistrali, C.F., Padalino, B., Tenhagen, B.A., Threlfall, J., García-Fierro, R., Guerra, B., Lie´bana, E., Stella, P., Peixe, L., 2022. Transmission of antimicrobial resistance (AMR) during animal transport. EFSA J. 20 (10), e07586 https://doi.org/10.2903/j.efsa.2022.7586.

EFSA Panel on Biological Hazards (BIOHAZ), Koutsoumanis, K., Allende, A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Nonno, R., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Fox, E., Gosling, R.B., Gil, B.M., Møretrø, T., Stessl, B., da Silva Felício, M.T., Messens, W., Simon, A.C., Alvarez-Ordo´n˜ez, A., 2024. Persistence of microbiological hazards in food and feed production and processing environments. EFSA J. 22 (1), e8521 https://doi.org/10.2903/j.efsa.2024.8521.

Egea, P., Lo´pez-Cerero, L., Torres, E., Go´mez-S´anchez Mdel, C., Serrano, L., Navarro S´anchez-Ortiz, M.D., Rodriguez-Ban˜o, J., Pascual, A., 2012. Increased raw poultry meat colonization by extended-spectrum beta-lactamase-producing Escherichia coli in the south of Spain. Int. J. Food Microbiol. 159 (2), 69–73. https://doi.org/ 10.1016/j.ijfoodmicro.2012.08.002.

European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC), 2023. The European Union one health 2022 Zoonoses report. EFSA J. 21 (12), e8442 https://doi.org/10.2903/j.efsa.2023.8442.

Eurostat. European Commission 2022-2023. Agricultural production - livestock and meat - Statistics Explained. https://ec.europa.eu/eurostat/statistics-explained/index.php? oldid=427096#Meat_production.

García-Ferna´ndez, A., Chiaretto, G., Bertini, A., Villa, L., Fortini, D., Ricci, A., Carattoli, A., 2008. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum beta-lactamases in Escherichia coli and Salmonella of human and animal origin. J. Antimicrob. Chemother. 61 (6), 1229–1233. https://doi.org/10.1093/jac/ dkn131.

Jammoul, A., El Darra, N., 2019. Evaluation of antibiotics residues in chicken meat samples in Lebanon. Antibiotics 8, 69. https://doi.org/10.3390/antibiotics8020069.

Jouini, A., Vinu´e, L., Slama, K.B., Sa´enz, Y., Klibi, N., Hammami, S., Boudabous, A., Torres, C., 2007. Characterization of CTX-M and SHV extended-spectrum beta- lactamases and associated resistance genes in Escherichia coli strains of food samples in Tunisia. J. Antimicrob. Chemother. 60 (5), 1137–1141. https://doi.org/10.1093/ jac/dkm316.

Leverstein-van Hall, M.A., Dierikx, C.M., Cohen Stuart, J., Voets, G.M., van den Munckhof, M.P., van Essen-Zandbergen, A., Platteel, T., Fluit, A.C., van de Sande-Bruinsma, N., Scharinga, J., Bonten, M.J., Mevius, D.J., National ESBL surveillance group, 2011. Dutch patients, retail chicken meat, and poultry share the same ESBL genes, plasmids, and strains. Clin. Microbiol. Infect. 17 (6), 873–880. https://doi. org/10.1111/j.1469-0691.2011.03497.x.

Liakopoulos, A., van der Goot, J., Bossers, A., Betts, J., Brouwer, M.S.M., Kant, A., Smith, H., Ceccarelli, D., Mevius, D., 2018. Genomic and functional characterisation of IncX3 plasmids encoding blaSHV-12 in Escherichia coli from human and animal origin. Sci. Rep. 8 (1), 7674. https://doi.org/10.1038/s41598-018-26073-5.

Machado, E., Coque, T.M., Canto´n, R., Sousa, J.C., Peixe, L., 2008. Antibiotic resistance integrons and extended-spectrum {beta}-lactamases among Enterobacteriaceae strains recovered from chickens and swine in Portugal. J. Antimicrob. Chemother. 62 (2), 296–302. https://doi.org/10.1093/jac/dkn179.

Manyi-Loh, C., Mamphweli, S., Meyer, E., Okoh, A., 2018. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules 23 (4), 795. https://doi.org/10.3390/molecules23040795.

Martínez-A´lvarez, S., Sanz, S., Olarte, C., Hidalgo-Sanz, R., Carvalho, I., Ferna´ndez-Ferna´ndez, R., Campan˜a-Burguet, A., Latorre-Ferna´ndez, J., Zarazaga, M., Torres, C., 2022. Antimicrobial resistance in Escherichia coli from the broiler farm environment, with detection of SHV-12-producing strains. Antibiotics (Basel) 11 (4), 444. https:// doi.org/10.3390/antibiotics11040444.

Martínez-A´lvarez, S., Cha^tre, P., Cardona-Cabrera, T., François, P., Sa´nchez-Cano, A.,Ho¨fle, U., Zarazaga, M., Madec, J.Y., Haenni, M., Torres, C., 2023. Detection and genetic characterization of blaESBL-carrying plasmids of cloacal Escherichia coli isolates from white stork nestlings (Ciconia ciconia) in Spain. J Glob Antimicrob Resist. 34, 186–194. https://doi.org/10.1016/j.jgar.2023.07.011.

Martínez-A´lvarez, S., Cha^tre, P., François, P., Abdullahi, I.N., Simo´n, C., Zarazaga, M., Madec, J.Y., Haenni, M., Torres, C., 2024. Unexpected role of pig nostrils in the clonal and plasmidic dissemination of extended-spectrum beta-lactamase-producing Escherichia coli at farm level. Ecotoxicol. Environ. Saf. 8 (273), 116145 https://doi. org/10.1016/j.ecoenv.2024.116145.

Martínez-Laorden, A., Arraiz-Ferna´ndez, C., Gonz´alez-Fandos, E., 2023. Microbiological quality and safety of fresh Turkey meat at retail level, including the presence of ESBL-producing Enterobacteriaceae and methicillin-Resistant S. Aureus. Foods 12 (6), 1274. https://doi.org/10.3390/foods12061274.

Mazel, D., Dychinco, B., Webb, V.A., Davies, J., 2000. Antibiotic resistance in the ECOR collection: integrons and identification of a novel aad gene. Antimicrob. Agents Chemother. 44 (6), 1568–1574. https://doi.org/10.1128/AAC.44.6.1568-1574.2000.

de Mesquita Souza Saraiva, M., Lim, K., DFM, do Monte, PEN, Givisiez, LBR, Alves, de Freitas Neto, O.C., Kariuki, S., Júnior, A.B., CJB, de Oliveira, Gebreyes, W.A., 2022. Antimicrobial resistance in the globalized food chain: a one health perspective applied to the poultry industry. Braz. J.

Microbiol. 53 (1), 465–486. https://doi.org/10.1007/s42770-021-00635-8.

Mora, A., Lo´pez, C., Herrera, A., Viso, S., Mamani, R., Dhabi, G., Alonso, M.P., Blanco, M., Blanco, J.E., Blanco, J., 2012. Emerging avian pathogenic Escherichia coli strains belonging to clonal groups O111:H4-D-ST2085 and O111:H4-D-ST117 with high virulence-gene content and zoonotic potential. Vet. Microbiol. 156 (3–4), 347–352. https://doi.org/10.1016/j.vetmic.2011.10.033.

O.I.E. Annual Report on Antimicrobial Agents Intended for Use in Animals. In: World Organization for Animal Health, 6th edition, 2022. https://www.woah.org/app/uploads/2022/06/a-sixth-annual-report-amu-final.pdf.

Ojer-Usoz, E., Gonza´lez, D., Vitas, A.I., Leiva, J., García-Jalo´n, I., Febles-Casquero, A., Escolano, Mde L., 2013. Prevalence of extended-spectrum β-lactamase-producing Enterobacteriaceae in meat products sold in Navarra, Spain. Meat Sci. 93 (2), 316–321. https://doi.org/10.1016/j.meatsci.2012.09.009.

Osman, K.M., Kappell, A.D., Elhadidy, M., ElMougy, F., El-Ghany, W.A.A., Orabi, A., Mubarak, A.S., Dawoud, T.M., Hemeg, H.A., Moussa, I.M.I., Hessain, A.M., Yousef, H.M.Y., 2018. Poultry hatcheries as potential reservoirs for antimicrobial-resistant Escherichia coli: a risk to public health and food safety. Sci. Rep. 8 (1), 5859. https://doi.org/10.1038/s41598-018-23962-7.

Pauly, N., Hammerl, J.A., Schwarz, S., Grobbel, M., Meemken, D., Malorny, B., Tenhagen, B.A., K¨asbohrer, A., Irrgang, A., 2021. Co-occurrence of the blaVIM-1 and blaSHV-12 genes on an IncHI2 plasmid of an Escherichia coli isolate recovered from German livestock. J. Antimicrob. Chemother. 76 (2), 531–533. https://doi.org/10.1093/jac/dkaa436.

Pena, A., Silva, L.J.G., Pereira, A., Meisel, L., Lino, C.M., 2010. Determination of fluoroquinolone residues in poultry muscle in Portugal. Anal. Bioanal. Chem. 397 (6), 2615–2621. https://doi.org/10.1007/s00216-010-3819-0.

PRAN Plan Nacional Frente a la Resistencia a los Antibio´ticos 2019–2021 [(Accessed on 30 October 2020)]. Available online. http://www.resistenciaantibioticos.es/es/system/files/field/files/pran_2019-021_0.pdf?file=1&type=node&id=497&force=0.

Roedel, A., Vincze, S., Projahn, M., Roesler, U., Rob´e, C., Hammerl, J.A., Noll, M., Al

Dahouk, S., Dieckmann, R., 2021. Genetic but no phenotypic associations between biocide tolerance and antibiotic resistance in Escherichia coli from German broiler fattening farms. Microorganisms 9 (3), 651. https://doi.org/10.3390/ microorganisms9030651.

Saidani, M., Messadi, L., Mefteh, J., Chaouechi, A., Soudani, A., Selmi, R., Daˆaloul-Jedidi, M., Ben Chehida, F., Mamlouk, A., Jemli, M.H., Madec, J.Y., Haenni, M., 2019. Various Inc-type plasmids and lineages of Escherichia coli and Klebsiella pneumoniae spreading blaCTX-M-15, blaCTX-M-1 and mcr-1 genes in camels in Tunisia. J. Glob. Antimicrob. Resist. 19, 280–283. https://doi.org/10.1016/j. jgar.2019.05.007.

Soufi, L., Abbassi, M.S., S´aenz, Y., Vinue´, L., Somalo, S., Zarazaga, M., Abbas, A., Dbaya, R., Khanfir, L., Ben Hassen, A., Hammami, S., Torres, C., 2009. Prevalence and diversity of integrons and associated resistance genes in Escherichia coli strains from poultry meat in Tunisia. Foodborne Pathog. Dis. 6 (9), 1067–1073. https://doi.org/10.1089/fpd.2009.0284.

Soufi, L., Sa´enz, Y., Vinu´e, L., Abbassi, M.S., Ruiz, E., Zarazaga, M., Ben Hassen, A., Hammami, S., Torres, C., 2011. Escherichia coli of poultry food origin as a reservoir of sulphonamide resistance genes and integrons. Int. J. Food Microbiol. 144 (3), 497–502. https://doi.org/10.1016/j.ijfoodmicro.2010.11.008.

Tian, S.F., Chu, Y.Z., Chen, B.Y., Nian, H., Shang, H., 2011. ISEcp1 element in association with Bla(CTX-M) genes of E. Coli that produce extended-spectrum β-lactamase among the elderly in community settings. Enferm. Infecc. Microbiol. Clin. 29 (10), 731–734. https://doi.org/10.1016/j.eimc.2011.07.011.

Vidovic, N., Vidovic, S., 2020. Antimicrobial resistance and food animals: influence of livestock environment on the emergence and dissemination of antimicrobial resistance. Antibiotics (Basel). 9 (2), 52. https://doi.org/10.3390/antibiotics9020052.

Vinu´e, L., Lantero, M., S´aenz, Y., Somalo, S., de Diego, I., P´erez, F., Ruiz-Larrea, F., Zarazaga, M., Torres, C., 2008a. Characterization of extended-spectrum beta- lactamases and integrons in Escherichia coli isolates in a Spanish hospital. J. Med. Microbiol. 57 (Pt 7), 916–920. https://doi.org/10.1099/jmm.0.47723-0.

Vinu´e, L., S´aenz, Y., Somalo, S., Escudero, E., Moreno, M.A., Ruiz-Larrea, F., Torres, C., 2008b. Prevalence and diversity of integrons and associated resistance genes in faecal Escherichia coli strains of healthy humans in Spain. J. Antimicrob. Chemother. 62 (5), 934–937. https://doi.org/10.1093/jac/dkn331.

Vitas, A.I., Naik, D., P´erez-Etayo, L., Gonz´alez, D., 2018. Increased exposure to extended- spectrum β-lactamase-producing multidrug-resistant Enterobacteriaceae through the consumption of chicken and sushi products. Int. J. Food Microbiol. 23 (269), 80–86. https://doi.org/10.1016/j.ijfoodmicro.2018.01.026.

Walker, B.J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C.A., Zeng, Q., Wortman, J., Young, S.K., Earl, A.M., 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PloS One 9 (11), e112963. https://doi.org/10.1371/journal.pone.0112963.

Wick, R.R., Judd, L.M., Gorrie, C.L., Holt, K.E., 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13 (6), e1005595 https://doi.org/10.1371/journal.pcbi.1005595.

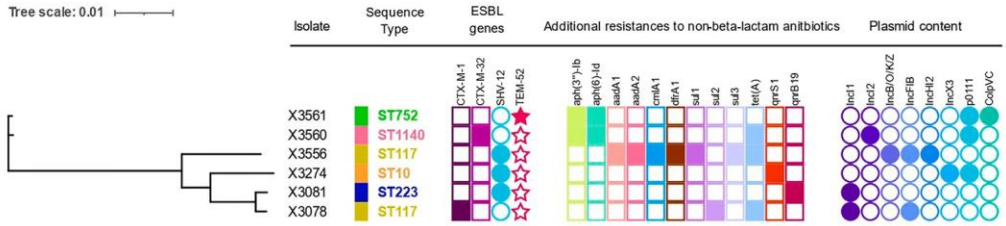
Table 1

Antimicrobial resistance rates of ESBL-*E. coli* isolates (obtained in CTX-supplemented MC media) and the non-ESBL-*E. coli* isolates (recovered in MC media).

Antibiotics	ESBL *E. coli* (N = 6)	non-ESBL *E. coli* (*N* = 40)
	N/%	N/%
Amoxicillin-clavulanate	2/33.3	14/35.0
Ampicillin	6/100	20/50.0
Cefotaxime	6/100	0/0.0
Ceftazidime	6/100	0/0.0
Ceftriaxone	6/100	0/0.0
Cefoxitin	0/0.0	0/0.0
Imipenem	0/0.0	0/0.0
Kanamycin	2/33.3	0/0.0
Streptomycin	1/16.7	0/0.0
Gentamicin	1/16.7	6/15.0
Chloramphenicol	1/16.7	3/7.5
Tetracycline	3/50.0	12/3.0
Trimethoprim/ sulphamethoxazole	2/33.3	9/22.5
Nalidixic acid	2/33.3	15/37.5
Ciprofloxacin	2/33.3	15/37.5

Table 3

Characteristics of the six *bla*$_{ESBL}$-harbouring *E. coli* isolates recovered in MC-CTX plates and their plasmids.



ID number	Sample/ Establishment[1]	ST –PhG[2]	*bla*$_{ESBL}$-carrying plasmid			
			ESBL genetic environment	Replicon type/ pMLST (Size)	Conjugation frequency	Other co-located resistance genes
X3560*	T/H-M48	1140-E	IS*Ecp1*-*bla*$_{CTX-M-32}$-*wbuC*	I2 (90 kb)	5.1×10^{-4}	*aph(3'')-Ib, aph(6)-Id, tet(A)*
X3561	B/H-M46	752-A	IS*4*-Tn*3*-*bla*$_{TEM-52C}$-IS*4*	P1-phage (110 kb)	-	-
X3556*	T/H-M45	117-B2	IS26-*bla*$_{SHV-12}$- *deoR*-Tn*1721*	HI2/pST3 (CC3) (225 kb)	4.2×10^{-5}	*sul3, qacL, aadA1, aadA2, cmlA1*, tet*ABCDEFWY*
X3274	T/H-M30	10-A	IS*26*-*bla*$_{SHV-12}$-*deoR*-IS*26*	X3 (50 kb)	3.8×10^{-6}	*qnrS1*
X3081	B/H-M40	223-B1	IS*26*-*smc*-*glpR*-*bla*$_{SHV-12}$-IS*26*	I1/pST3 (CC3) (100 kb)	2.6×10^{-6}	*qnrB19*
X3078*	T/RS-M12	117-B2	IS*Ecp1*-*bla*$_{CTX-M-1}$-*wbuC*	I1/pST3 (CC3) (100 kb)	3.1×10^{-6}	*sul2*

*Multidrug-resistant *E. coli* isolates
[1]T - Thig, B - Breast, H - Hypermarket, RS - Retail Shop
[2]Phylogroup