Original Research

Microbiological risk assessment of turkey and chicken meat for consumer: significant differences regarding multidrug resistance, *mcr* or presence of hybrid aEPEC/ExPEC pathotypes of *E. coli*

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Abstract

To assess the microbiological risk for consumers, we propose a lab workflow based on six virulence/antimicrobial resistance (AMR) traits, and including a duplex PCR for the screening of extraintestinal pathogenic *E. coli* (ExPEC). This protocol was tested in 100 poultry meat products. The characterization of 323 isolates revealed that poultry meat is a rich phylogenetic source of *E. coli* phylogroups (A to G) and *Escherichia* clade I. Non-susceptible *E. coli* isolates to monobactams, 3rd-generation cephalosporins and/or fluoroquinolones, were present in 71% of the samples. Besides, 47% carried ≥ 2 different *E. coli* positive for ESBL, pAmpC or *mcr* genes. Isolates from 78% of the poultry meat exhibited ExPEC status, and 53% were carriers of isolates positive for the uropathogenic (UPEC) status. The sequence types (STs) identified in 86% of the samples belonged to the so-called ExPEC high-risk lineages, being 73% carriers of clonal groups identified in human infections of the same Health Area. Moreover, different human-associated clones co-occurred in same meat sample: ST131-B2 (CH40-22), ST648-F (CH4-58), ST93-A (CH11-neg) or ST95-B2 (CH38-27), ST354-F (CH88-58), ST155-B1 (CH4-neg). Globally, 84% of the meat samples posed ≥ 3 risks, including resistance genes, successful clones and virulence traits. Turkey meat showed significant higher rates concerning *mcr*-carriage or multidrug resistance; while the ExPEC status rate, or the presence of hybrid pathotypes such as the aEPEC/ExPEC O153:H10-A-ST10 (CH11-54), were associated with chicken origin ($P < 0.05$). In a "Farm to Fork Strategy", ExPEC should be clearly included in food surveillance.

Keywords

Escherichia coli, antimicrobial resistance (AMR), *mcr-1*, ESBL, ExPEC, ST131, poultry meat, risk assessment, One-Health

1. Introduction

A comprehensive assessment of antimicrobial resistance (AMR) in animals and the food chain is essential to reduce the burden of antimicrobial resistance in humans. However, food surveillance is considered commercially sensitive and so, the information derived from it is generally incomplete (Tacconelli et al., 2018). One-Health monitoring programs for AMR frequently include *Escherichia coli* as a sentinel (McEwen & Collignon, 2018). *E. coli* is a member of the normal intestinal microbiota of humans and other mammals (Kaper et al., 2004), whose presence in environmental samples, food, or water indicates recent faecal contamination or poor hygienic practices in food-processing facilities (Alonso et al., 2007; Odonkor & Ampofo, 2013).

In addition to its commensal role, *E. coli* can also act as a pathogen in a broad range of enteric diseases as well as extraintestinal infections (Kaper et al., 2004; Manges & Johnson, 2012). Traditionally, only enteric infections causing *E. coli* or diarrheagenic *E. coli* (DEC) have been accepted as foodborne pathogens. DEC has been classified into six major subgroups (pathotypes) based on specific mechanisms of virulence, namely, enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), diffuse adherent *E. coli* (DAEC) and Shiga toxin-producing *E. coli* (STEC) (Kaper et al., 2004; Nataro & Kaper, 1998). Since DEC can be distinguished from the commensal intestinal microbiota based on the associated virulence genes, it is relatively easy to detect or determinate the origin of the isolates. This is not as simple when extraintestinal pathogenic *E. coli* (ExPEC) are taken into account (Riley, 2020). No set of genes has been found to unequivocally characterise ExPEC and the different categories within a group defined by its isolation from infections outside the intestinal tract. Thus, the ExPEC group comprises: uropathogenic *E. coli* (UPEC), avian pathogenic *E. coli* (APEC) and neonatal meningitis *E. coli*

71 (NMEC) (Riley, 2014). Nevertheless, certain virulence traits were statistically
72 associated with the pathogenic potential of the isolates, which can be used in a
73 predictive way (Johnson et al., 2003; Spurbeck et al., 2012) together with the
74 identification of the so-called global extraintestinal lineages of *E. coli* such as ST131
75 (Manges et al., 2019; Riley, 2014). Apart from DEC and ExPEC, new hybrid
76 pathotypes have been reported since it happened the major outbreak of HUS in Europe
77 in 2011 by an EAEC/STEC O104:H4 (Cointe et al., 2020; Díaz-Jiménez et al., 2020b;
78 Mora et al., 2011).

79 While food, particularly poultry products, have been recently recognized as potential
80 reservoirs of ExPEC for humans (Jørgensen et al., 2019; Mellata et al., 2018; Mora et
81 al., 2010; 2013; Riley, 2020), food production animals are highlighted as the main cause
82 of the AMR increase, including resistance to colistin (Cyoia et al., 2018; García-Meniño
83 et al., 2018; García et al., 2018a). The rapid spread of extended-spectrum beta-
84 lactamases (ESBL) and multidrug resistance is in large part associated to ExPEC
85 successful lineages, such as the pandemic clonal groups ST131, ST38, ST405 or ST648
86 (Manges et al., 2019; Shaik et al., 2017; Yamaji et al., 2018a).

87 Based on the above considerations, the objectives of this study were the design of a lab
88 workflow for the comprehensive detection of foodborne pathogenic and antimicrobial-
89 resistant *E. coli*, including a rapid protocol for ExPEC screening, together with the
90 microbiological risk assessment for consumers via poultry meat consumption.

2. Materials and Methods

2.1. Meat sampling and *E. coli* recovery

Between September 2016 and September 2017, one hundred poultry meat products were acquired in eight points of sale in the city of Lugo (northwest Spain), including six supermarket chains (Table S1). The meat products were transported in an isothermal container and processed within the next two hours after collection. Of the 100 meat samples, 50 were chicken breast (24 packaged in a modified atmosphere and 26 freshly cut by the butcher at the moment of sampling), and another 50 were turkey meat (25 in modified atmosphere and 25 freshly cut). The lab method designed for this study was oriented to investigate i) the microbiological quality of poultry meat based on *E. coli* counts and ii) the prevalence of pathogenic and AMR foodborne *E. coli* (Fig. 1). For the latter (AMR and potential pathogens), the method was subdivided in six protocols (I to VI) based on a combination of selective media and incubation temperatures, whose bacterial growth was eventually screened by PCR for specific genetic targets. Through the characterization of the recovered isolates, the adequacy of each protocol was finally evaluated.

Briefly, 25 g of each meat sample were aseptically cut and homogenized (2 min in a stomacher) with 225 ml of Buffer Peptone Water (BPW; ApplyChem Panreac). From the homogenate, 1 ml was plated into a 3M Petrifilm™ Select *E. coli*, which was examined after incubation 24 h/44 ºC for the *E. coli* counts following manufacturer's instructions. Then, the homogenized meat samples were incubated 6 h/37 ºC, from which 1 ml was inoculated in duplicate into 9 ml MacConkey Lactose broth (Oxoid) tubes, growth for 18-24 h at 37 ºC and 44 ºC, respectively. Finally, different selective agar media (protocols I to VI) were inoculated from the MacConkey Lactose broth tubes (Fig. 1). The protocols I to IV were meant for the detection of potentially

pathogenic *E. coli* (carriers of diarrheagenic or extraintestinal virulence traits): protocol I. MacConkey Lactose agar (ML) (Oxoid), 18-24 h/37 ºC; protocol II. MacConkey Sorbitol agar enriched with tellurite and cefixime (MSTC) (Oxoid), 18-24 h/37 ºC; protocol III. ML, 18-24 h/44 ºC; protocol IV. MSTC, 18-24 h/44 ºC. Additionally, ESBL-producing *E. coli* were screened by means of CHROMID® ESBL agar plates (bioMérieux) in protocol V, while the protocol VI screened carbapenemase-producing *E. coli* in CHROMID® CARBA SMART plates (bioMérieux).

As shown in Fig. 1, the confluent growth of plates I to IV and the pooled colonies recovered from I to VI and plated on Tryptone Soy Agar (TSA) (Oxoid) were analyzed by PCR for specific virulence factors (VF) associated with the DEC pathotypes EPEC (*eae*, *bfpA*), STEC (*stx1*, *stx2*, *eae*) and EAEC (*aaiC*, *aggR*). Likewise, specific VF linked to the pathogenic potential of extraintestinal pathogenic *E. coli* (ExPEC status) and uropathogenic *E. coli* (UPEC status) were tested (Table S2). The ExPEC status was assigned to isolates positive for ≥ 2 of these five markers (*papAH*, *sfa/focDE*, *afa/draBC*, *kpsM II* and *iutA*) (Johnson et al., 2003), while the UPEC status was assigned to isolates positive for ≥ 3 of these four markers (*chuA*, *fyuA*, *vat* and *yfcV*) (Spurbeck et al., 2012). For those isolates exhibiting ExPEC and/or UPEC status, other extraintestinal VF were analyzed to complete their characterization (Table S2). The O25b subtype (*rbfO25b*) associated with the clonal group ST131 was also screened by PCR (Clermont et al., 2008) and positive isolates were confirmed by multilocus sequence typing (MLST). PCR amplification of the β-D-glucuronidase-encoding gene (*uidA*) was routinely used to specifically identify *E. coli* (Gómez-Duarte et al., 2010) (Tables S2). Additionally, isolates suspected of being *E. coli* but *uidA* negative were identified by MALDI-TOF MS (Bruker Daltonik, Bremen, Germany) in duplicated; a reliable result (at the species level) was only considered if the score obtained was higher

than 2. All the isolates recovered in this study were stored at room temperature in nutrient broth (Difco™) with 0.75% nutrient agar (Difco™) for further characterization.

2.1.1. Rapid detection of *E. coli* conforming ExPEC status in meat samples

We designed a duplex PCR for a rapid and effective recovery of isolates with ExPEC status. Previous results indicated that >95% of the isolates present in meat and conforming ExPEC status were carriers of both *iutA* and *KpsM* II genes; furthermore, 100% of them were carriers of at least one of those genes (Herrera, 2015). Using these targets, the duplex PCR amplifies a fragment of 272 bp with the kpsII f and r primers described elsewhere for *KpsM* II (Johnson & Stell, 2000), and 441 bp of *iutA*. For the latter, we designed the new primer "iutA-Al f" 5'GCCGGAGCTGTCTCCGGCGG 3´ within the locus tag "NRG857 30235" of *iutA* from the GenBank CP001856 genomic sequence, which was used with the previously aer-1152r primer described by Johnson *et al*. (Johnson et al., 1997) (Fig. 2). For a 25 µl PCR reaction, the amplification mix includes 12.5 µl of NZYTaq 2x Green MasterMix (2.5 U), 0.6 µl of 20µM of KpsM II primers, 1 µl of 20 µM of *iutA* primers, and 5 µl of sample DNA. The PCR conditions were validated with DNA pools of negative and positive isolates for one or both genes, as well as with individual colonies (Fig. 2).

2.2. *E. coli* typing

The phylogenetic relatedness of the *E. coli* population recovered from the poultry meat was determined by means of the phylogroup, sequence type (ST), clonotype (CH) and serotype assignment as described elsewhere (Díaz-Jiménez et al., 2020b). In brief, the Clermont method (Clermont et al., 2013; 2019) recognizes eight phylogroups (A, B1, B2, C, D, E, F, G) belonging to *E. coli sensu stricto* and also discriminates those belonging to *Escherichia* cryptic clades. The MLST was performed following Achtman's scheme based on seven genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*)

(Wirth et al., 2006) (Table S3). The CHs were established based on the internal 469-nucleotide (nt) and 489-nt sequence of the *fumC* (allele obtained from MLST) and *fimH* genes, respectively (Weissman et al., 2012) (Table S3). The isolates confirmed as ST31 were characterized for their virotypes according to the scheme defined by Dahbi et al. (2014), based on the presence or absence of certain extraintestinal VF (*afa/draBC*, *afa* operon FM955459, *iroN*, *sat*, *ibeA*, *papG II*, *papG III*, *cnf1*, *hlyA*, *cdtB*, *kpsM II-K1*, *-K2 and -K5*) (Table S2). The collection was also investigated by PCR for specific *bla* genes using the TEM, CIT, SHV, CTX-M-1 and CTX-M-9 group-specific primers, and further sequencing, as well as for the *mcr* genes (1 to 5) as previously described (Díaz-Jiménez et al., 2020b) (Table S4).

The susceptibility testing was performed using the disc-diffusion assay and including ampicillin (AMP), amoxicillin-clavulanic acid (AMC), cefuroxime (CXM), ceftazidime (CAZ), cefotaxime (CTX), cefoxitin (FOX), aztreonam (ATM), imipenem (IPM), gentamicin (GEN), tobramycin (TOB), amikacin (AMK), fosfomycin (FOF), colistin (CST), doxycycline (DOX), chloramphenicol (CHL), nitrofurantoin (NIT), cotrimoxazole (SXT), ciprofloxacin (CIP), nalidixic acid (NAL) and tigecycline (TGC). Furthermore, MICs for colistin (CST) were manually obtained by broth microdilution for *mcr*-positive colonies. All methods and results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (2020). Multidrug-resistant (MDR) isolates were defined according to Magiorakos *et al*. criteria, as those showing acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

2.3. Microbiological risk assessment

In order to evaluate the microbiological risk exposure for consumers, we performed an assessment based on the food-risk definition described by Díaz-Jiménez et al. (2020a).

In the present study, each meat sample was qualified between zero (lowest) to six (highest) in association with the following microbiological parameters, considered as summative risks when happened: i) *E. coli* counts > 500 cfu/g of poultry meat. We took as reference the Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. This Regulation establishes that for meat preparations at the end of the manufacturing process, and using *E. coli* as an indicator of recent fecal contamination, the limits considered are "m = 500 and M = 5000" cfu/g to recommend improvements in production hygiene and improvements in selection and/or origin of raw materials. ii) The recovery of *E. coli* resistant to antimicrobials of categories A ("Avoid") or B ("Restrict") (European Medicines Agency, 2020). iii) The recovery of ≥ 2 different isolates of *E. coli* positive for typically plasmid-borne ESBL, AmpC (pAmpC) or *mcr* resistance genes. iv) The identification of high-risk lineages of *E. coli* associated with human extraintestinal pathologies (Flament-Simon et al., 2020b; 2020c; Mamani et al., 2019; Manges et al., 2019; Yamaji et al., 2018b). v) The isolation of *E. coli* conforming ExPEC status (Johnson et al., 2003). vi) The isolation of *E. coli* conforming UPEC status (Spurbeck et al., 2012).

2.4. Statistical analysis

Differences within groups were analyzed by a two-tailed Fisher's exact test. *P* values < 0.05 were considered statistically significant.

3. Results

We designed a lab workflow to assess the microbiological risk for consumers, which was tested in 100 poultry meat products. Ninety-two of the 100 samples (46 of chicken and 46 of turkey) were positive for the presence and recover of *E. coli* isolates. From those, 323 isolates fulfilled the criteria stated in the Material and Methods section and

constituted the collection of study (163 *E. coli* from chicken and 160 from turkey). Per protocol, 137 (42.4%) *E. coli* were recovered from protocol V, 86 (26.6%) from protocol II, 79 (24.5%) from protocol I, 16 (5.0%) from protocol IV and 5 (1.5%) from protocol III. None carbepenemase-producing isolate was recovered in this study, which were specifically searched in the protocol VI. The screening of VF associated with DEC pathotypes determined the presence of aEPEC (*eae*-positive, *bfp*-negative), but no STEC or EAEC. The screening of VF associated with the ExPEC and UPEC status, *rfbO25b*, *bla* and *mcr* were also positive as it is detailed below.

3.1. Microbiological quality of the poultry meat

As noted above, 92% of the meat samples were positive for *E. coli* isolation in 3M Petrifilm™ Select *E. coli*. While 27 samples showed < 10 CFU of *E. coli* per g, 43 showed ≥ 50 CFU/g with significant differences regarding meat origin (28 of 50 turkey *vs* 15 of 50 chicken; $P = 0.015$) (Table 1). Besides, five of the 100 samples obtained "not satisfactory" *E. coli* counts (> 500), if we take as reference the Commission Regulation (EC) No 2073/2005 for meat preparations at the end of the manufacturing process. In this Regulation, the limits to recommend improvements in selection and/or origin of raw materials are “m = 500 and M = 5000” cfu/g. Finally, similar levels of contamination were observed for the two packaging systems (modified atmosphere and freshly butchered) ($P > 0.05$) (Table 1).

3.2. Evaluation of protocols I to V

The method designed here was thought to detect *E. coli* potentially pathogenic for humans (diarrheagenic, extraintestinal and MDR), using different media, temperatures of incubation and specific genetic targets (named as protocols I to VI within the method). As summarized in Table 2 and Table S5, we evaluated the adequacy of this method through the characterization of 323 *E. coli* and the assessment of six virulence

241 traits. We found that the protocols I and II (ML and MLST incubated at 37 ºC,
242 respectively) were the most effective for the recovery of isolates satisfying the ExPEC
243 and UPEC status. In detail, of the 150 isolates from 78 different meat samples that
244 satisfied the ExPEC status, 118 (78.7%) from 71 samples were recovered in plates of
245 protocols I and II. Likewise, of the 83 isolates positive for UPEC status from 53
246 individual samples, 69 (83.1%) recovered in 47 samples come from plates of protocols I
247 and II. The protocol V (CHROMID® ESBL agar plates 37 ºC) was key for the recovery
248 of ESBL or pAmpC-producing *E. coli*. In fact, of 155 ESBL/pAmpC-producing *E. coli*
249 isolated from 78 samples, 137 (88.4%) isolates and 76 samples were detected in
250 protocol V. Although most *mcr* isolates were from protocols I and II, with 10 of 13
251 (76.9%) *mcr E. coli* recovered in six of the seven positive meat samples, they were also
252 isolated in plates of protocols IV and V (two and one isolate, respectively). Of the 323
253 isolates analyzed here, 253 *E. coli* recovered from 88 meat samples were MDR
254 according to Magiorakos et al. (2012). MDR isolates were mostly those 137 (54.1%)
255 ESBL/pAmpC-producing *E. coli* recovered from 76 meat samples (protocol V), and 100
256 *E. coli* from protocols I and II. Finally, the screening by PCR of the *rbfO25b* associated
257 with the clonal group ST131 allowed the detection of 13 isolates; 12 (92.3%) from nine
258 samples were recovered in plates of protocols I and II (Table 2, Table S5) (Fig. S1).
259 Regarding meat origin, we found a significant higher prevalence of isolates with ExPEC
260 status in chicken meat (58% *vs* 42% in turkey), while MDR and *mcr-1* isolates were
261 more prevalent within *E. coli* of turkey origin (53.4% *vs* 46.6% in chicken and 92.3% *vs*
262 7.7% in chicken, respectively) ($P < 0.05$) (Table 2) (Fig. S2).
263 In summary, the microbiological method applied in this study showed high prevalence
264 rates of ExPEC and UPEC status, ESBL/AmpC enzymes, *mcr-1* gene, MDR, or
265 *rbfO25b* gene (positive isolates present in 78%, 53%, 78%, 7%, 88% and 10% of the

meat samples, respectively). Importantly, the protocols I+II+V allowed the detection of around 85-90% of those positive samples (ML, MSTC and CHROMID® ESBL media incubated at 37 ºC), conforming an optimized workflow combination that would capture the greatest risk as analyzed here (Fig. S3).

3.3. *E. coli* characterization

The phylogenetic analysis revealed that the 323 *E. coli* isolates belonged to *Escherichia* clade I (8 isolates; 2.5%) and the eight phylogroups of *E. coli sensu stricto*: A (105 of 323; 32.5%), B2 (57; 17.6%), B1 (56; 17.3%), E (35; 10.8%), F (33; 10.2%), D (11; 3.4%), C (9; 2.8%), G (9; 2.8%). The isolates which exhibited ExPEC status were mainly of phylogroups B2, F, A and E (122 of 150; 81.3%), while the UPEC status appeared associated with phylogroups B2 and F (78 of 83; 94%). The ESBL producers, as well as MDR isolates, belonged mostly to phylogroups A, B1 and E, accounting for 127 of 155 (81.9%) and 179 of 253 (70.7%), respectively (Table 3) (Fig. S4).

MLST was performed for 272 representative isolates. As a result, 89 different STs were determined, including eight new (Fig. 3). However, 16 of those 89 STs detected in at least five isolates accounted for 153 of 272 isolates (56.2%): 10, 93, 95, 115,117, 131, 155, 355, 428, 648, 770, 752, 1158, 1485, 4243, 10740. And the most prevalent combination of STs and CHs revealed the following clonal groups: ST1485-F (CH231-58) (19 isolates); ST10-A (CH11-54) (12 isolates); ST93-A (CH11-neg) (11 isolates); ST752-A (CH11-24) (9 isolates); ST131-B2 (CH40-22) (8 isolates); ST117-G (CH45-97) (8 isolates); ST155-B1 (CH4-32) (7 isolates); ST355-B2 (24-154) (7 isolates); ST115-E (CH26-270) (7 isolates); ST770-clade I (CH116-552) (7 isolates); ST95-B2 (CH38-27) (6 isolates); ST1158-E (CH3-47) (6 isolates); ST648-F (CH4-58) (6 isolates); ST10740-B2 (1544-9) (5 isolates); and ST4243-D (3-1002) (5 isolates) (Table S6). Besides, different clones could be distinguished within these prevalent clonal

groups by serotype. In fact, serotyping showed high heterogeneity with 184 different O:H antigen combinations and only five serotypes determined for > 5 isolates in the characterized collection: O83:H42 (14 isolates); O25:H4 (12); O2:H5 (8); O2:HNM (7) and O5:H10 (6).

An important finding in this study was the high prevalence of meat samples with isolation of hybrid pathotypes aEPEC/ExPEC (19% of samples and 22 isolates). Table 4 shows the characterization of the 22 aEPEC/ExPEC, which were all positive for the *eae*-beta1 intimin, belonged to phylogroup A and mostly to the CC10. Besides, four of the 22 were ESBL/pAmpC producers.

The study of susceptibility showed that only 13 out of 323 isolates were susceptible to all the antibiotics tested. On the contrary, 258 (79.9%) were multidrug resistant, with acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012) (Table 5). The highest prevalence was against ampicillin (82.7%), nalidixic acid (74.0%), ciprofloxacin (71.8%), doxicicline (65.9%) and cefotaxime (46.7%). We found that turkey isolates exhibited significant higher rates of resistance against ampicillin, amoxicillin-clavulanic acid, ceftazidime, cefotaxime, aztreonam, doxycycline, chloramphenicol, sulfamethoxazole-trimethoprim, as well as in the number of MDR ($P < 0.05$). Chicken isolates only showed a significant difference in gentamicin resistance (Table 5). While the disc diffusion method for colistin resistance gave non-susceptible values only for six *mcr*-positive isolates out of the 323, the broth microdilution method, performed only for the *mcr*-bearing *E. coli*, showed CMI values of ≥ 2 μg/mL for 11 of 13 *mcr*-positive isolates.

Seventy-eight meat samples (39 from chicken and 39 from turkey) were carriers of 153 different ESBL/pAmpC-producing isolates, of which 108 (70.6%) carried bla_{SHV}: $bla_{\text{SHV-12}}$ (107 isolates) and $bla_{\text{SHV-2}}$ (1 isolate). Besides, 39 (25.5%) were positive for

316 *bla*$_{\text{CTX-M}}$: *bla*$_{\text{CTX-M-1}}$ (14), *bla*$_{\text{CTX-M-14}}$ (6), *bla*$_{\text{CTX-M-15}}$ (5), *bla*$_{\text{CTX-M-32}}$ (9), *bla*$_{\text{CTX-M-9}}$ (3)
317 and two were not-typeable (NT) (2). In addition, six isolates (3.9%) from different meat
318 samples were carriers of *bla*$_{\text{TEM-52}}$. We also recovered two *bla*$_{\text{CMY-2}}$ isolates from one
319 turkey sample (Table 6).
320 The *mcr* (1 to 5) screening resulted in 13 *mcr-1.1.* isolates recovered from seven
321 samples. As shown in Table S7, the isolates belonged to different phylogroups (A, B1,
322 B2, D) and STs (10, 69, 101, 140, 155, 212, 522, 744, 853). To highlight three samples
323 that carried more than one different *mcr*-positive clone. The MIC values for colistin
324 were of 4 μg/mL (11 isolates), 2 and 1 μg/mL (1 isolate each).
325 The screening of *rbfO25b* gave as a result 13 positive isolates from 10 meat samples.
326 MLST typing confirmed 12 as ST131 from nine meat samples. The remaining one
327 belonged to ST1011 and phylogroup E. The virulence profile of the ST131 isolates
328 conformed virotype D (*ibeA* carriers) with subtypes: D4 (10 isolates; *ibeA*, *kpsM II-K1*
329 positive), D1 (1 isolate; *ibeA*, *cdtB*, *kpsM II-K5*) and D-not typeable (1 isolate). The 13
330 ST131 exhibited two clonotypes: CH40-22 (7 isolates) and CH40-neg (5 isolates)
331 (Table S8).

332 **3.4. Risk assessment**

333 The Table 6 summarizes the risk assessment of the 100 poultry meat samples analyzed
334 in this study. We took into account the summative presence of events, based on the six
335 microbiological parameters described in section 2.3. The results determined that the
336 majority (92%) of meat samples were positive for any of those parameters, with 61%
337 positive for ≥ 4 risks and 84% for ≥ 3 risks.
338 Per parameter, there was evidence of non-susceptible *E. coli* against monobactams, 3$^{\text{rd}}$-
339 generation cephalosporins and/or fluoroquinolones in 71% of the meat samples.
340 Besides, 47% of the samples showed presence of ≥ 2 different isolates of *E. coli*

positive for ESBL, pAmpC or *mcr* genes. *E. coli* isolates belonging to STs/CCs identified as global ExPEC high-risk lineages were present in 86% of the samples and, what is more important, 73% showed carriage of the same clones as those determined within clinic human isolates of our Health Area. Besides, the isolates from 78% of the samples exhibited ExPEC status, and 53% were carriers of isolates positive for UPEC status. Finally, five samples showed "not satisfactory" *E. coli* counts (> 500 cfu/g).

4. Discussion

We aimed to develop a standardized protocol to assess exposure risk via food to drug-resistance genes and *E. coli* strains potentially pathogenic to humans. To the best of our knowledge, this would be the first study that reports a comprehensive typing of the *E. coli* isolates per food sample, which, on the other side, helped us to show the relevance of our proposal. In previous studies, we had observed the genetic similarity between isolates of certain ExPEC clonal groups recovered from poultry and human pathologies (Mora et al., 2009; 2010; 2013). We had also demonstrated close genomic relatedness between isolates of a hybrid MDR aEPEC/ExPEC O153:H10-A-ST10 (CH11-54) from different sources, including avian farm, chicken meat and human diarrheagenic samples (Díaz-Jiménez et al., 2020b). We had found, in another study, a short distance of less than 55 SNPs on the core genome comparison between a human and an avian isolates of ST131 subclade B3 (Flament-Simon et al., 2020a). Other authors also investigated the genomic overlap between avian pathogenic *E. coli* (APEC) and human ExPEC of the specific ST95, and found that certain ExPEC clones may indeed have the potential to cause infection in both poultry and humans (Jørgensen et al., 2019). For those evidences, and in agreement with Riley (2020), we claim the need of looking at ExPEC genotypes to elucidate their role as extraintestinal foodborne pathogen.

366 The selective media, genetic targets and virulence traits of the protocol proposed here
367 are based on results from previous studies. In relation to DEC targets, we included
368 clinically important *E. coli* for humans, and potentially prevalent in poultry meat. Thus,
369 the analysis of all DEC pathotypes in 200 poultry samples showed that none of the 200
370 meat samples was positive for EIEC or ETEC (Herrera, 2015). Nor were these
371 pathotypes relevant within the diarrheagenic stools of patients of our Health Area (Mora
372 et al., 2011). Also in the study of Herrera (2015), we had isolated ESBL-producing *E.*
373 *coli* in 45.5% of the samples by means of ML and MLST. Subsequently, we proved that
374 the CHROMID® ESBL medium is essential for the rapid and accurate recovery of
375 ESBL-producing isolates (Díaz-Jiménez et al., 2020a). We performed here the selective
376 characterization of ESBL-producing *E. coli* as indicator of drug-resistance gene
377 exposure via food, due to being by far the most prevalent species isolated in
378 CHROMID® ESBL (77%). Taken into account the presence of other ESBL-producers,
379 the global rate of positive samples would be 82% (Díaz-Jiménez et al., 2020a). To
380 assess exposure risk to ExPEC, we used the virulence traits which are statistically
381 associated with the pathogenic potential of causing extraintestinal infections,
382 conforming the ExPEC status (Johnson et al., 2003); and then, those specifically linked
383 to uropathogenic isolates, conforming the UPEC status (Spurbeck et al., 2012). The
384 duplex PCR based on *iutA* and *KpsM II* genes on ML and MLST was essential for the
385 accurate screening of the isolates with ExPEC status, as well as for the recovery of those
386 with UPEC status, since most of the latter also satisfies the ExPEC status (but not the
387 other way around). As a result, we found worrying prevalence rates of positivity for the
388 ExPEC and the UPEC status (78% and 53%, respectively). There are few comparable
389 data available. Two studies on AMR and ExPEC in retail foods performed in
390 Minneapolis (1999-2000 and 2001-2003), found a prevalence of 35.7% and 46% of

391 ExPEC contamination in poultry meat, respectively (Johnson et al., 2005a; 2005b). The
392 media used here, ML and MSTC, inoculated with the MacConkey Lactose broth
393 (growth for 18-24 h at 37 ºC), together with the specific PCR on confluents and pools of
394 colonies, probably explains the significant differences with the US findings. In those,
395 the virulence traits associated to ExPEC status were investigated on a selection of
396 colonies obtained from a non-specific protocol (Johnson et al., 2005a; 2005b).
397 The finding here of aEPEC/ExPEC O153:H10-A-ST10 (CH11-54) *eae*-beta1 and
398 similar hybrids in 19% of the meat sampled, reinforces the role of poultry meat in their
399 maintenance and transmission. The prevalence and implication of hybrid pathotypes of
400 *E. coli* in food and infections are probably underestimated since there is no systematic
401 search of them. Recently, we described the hybrid MDR aEPEC/ExPEC of the clonal
402 group O153:H10-A-ST10 (CH11-54) found within different surveillance studies (2005-
403 2015), and the close genomic relatedness between isolates of human and animal origin
404 belonging to it. This hybrid has been circulating in our region within different hosts,
405 including wildlife, and seems implicated in human diarrhea via meat transmission and
406 in the spreading of ESBL genes. Furthermore, we found genomic evidence of a related
407 hybrid in at least one other country (Díaz-Jiménez et al., 2020b). Interestingly, Flament-
408 Simon et al. (2020c) detected a hybrid EAEC/ ExPEC isolate O153:HNT-A-ST10
409 (CH11-54) among 96 *E. coli* implicated in UTIs and other extraintestinal human
410 infections in the Hospital of Beaujon (Clichy, Paris) in 2016. Lindstedt et al. (2018)
411 reported that a high frequency (> 93%) of routinely submitted faecal *E. coli* isolates
412 from Norwegian hospitals (2012-2013), previously characterized as DEC, harbored
413 ExPEC virulence factors. In view of our and other author´s findings, we believe that
414 hybrid *E. coli* isolates should be monitored as a pre-warning of altered virulence
415 capabilities.

In addition to O153:H10-A-ST10 (CH11-54), other human-associated clonal groups characterized in our own Health Area (Flament-Simon et al., 2020b; 2020c) were determined in 73% of our meat samples (as detailed in Table 6). What is more, around 25% of the meat samples showed co-occurrence of two or more different human-associated ExPEC clones. To highlight, the concomitant presence in four meat samples of isolates belonging to the pandemic clonal group O25b:H4-B2-ST131 (subclones CH40-22 and CH40-neg), together with others such as ST648-F (CH4-58); or a turkey meat sample (T40) with the co-occurrence of the human-associated ExPEC clones ONT:H9-A-ST744 (CH11-54), O153:H34-F-ST354 (CH88-58), ST141-B2 (CH52-14), together with *mcr-1.1*-positive ST140-B2 isolates.

Within the 323 isolates analyzed in this study, we found representatives of the eight phylogroups of *E. coli* and of the *Escherichia* clade I, being the phylogroup A the most prevalent (32.5%), followed by phylogroups B1 and B2 (around 17.5% each) and phlogroups E and F (around 10.5% each). This would be a close picture of the *E. coli* population present in poultry farming and meat products, based on the comprehensive method performed here. Previous data showed that if we only take a representative *E. coli* recovered from ML into consideration, the phylogroups A and B1 would account for around 30% each, and B2 for 6%; while considering only ESBL-producing *E. coli*, the figures would be 40.1%, 29.2% and 2.2.%, respectively (Díaz-Jiménez et al., 2020a). Similar distribution to the latter was observed within 84 ESBL-producing *E. coli* recovered from 52 avian farms in our region (39.3% A, 33.3% B1, 3.5% B2) (García et al., 2018b).

It is outstanding here, the high prevalence of meat samples with carriage of *E. coli* exhibiting the UPEC status (53%). The 83 isolates recovered from positive samples belonged to phylogroups B2, F and G (68.7%, 25.3% and 6%, respectively). Within the

22 STs established for the 83 meat isolates, we found some of the most prevalent in UPEC human collections, such as ST95-B2, ST131-B2 and ST141-B2 (Flament-Simon et al., 2020c). In concordance with the referenced study, we observed that the 22 isolates belonging to STs 95, 131 or 141 of our study conformed to the UPEC status. The relevant presence of isolates belonging to phylogroups F and G within poultry meat was mostly due to the clones ST648-F (CH4-58), ST1485-F (CH231-58) and ST117-G (CH45-97), which were also in the human clinic collection, but especially within the ESBL-producing *E. coli* (Flament-Simon et al., 2020b). Isolates belonging to the phylogroup F seems to be of particular significance as they have been reported as extraintestinal pathogens of companion animals, food-producing animals and humans. Further, specific F lineages such as CC648 or CC354 are resistant to fluoroquinolones (FQ) and/or extended-spectrum cephalosporins, and are increasingly associated with extraintestinal pathologies (Abreu-Salinas et al., 2020; Johnson et al., 2017; Vangchhia et al., 2016). On the other hand, the phylogroup G has been recently defined as a group intermediate between the F and B2 phylogroups. CC117 is its most prevalent G lineage, whose isolates commonly possess many traits associated with extraintestinal virulence and exhibit multidrug resistance. Epidemiologic data suggest that CC117 is a poultry-associated lineage that appears also established in humans and cause extraintestinal diseases (Clermont et al., 2019). In the present study, we recovered nine ST117 isolates, all of them MDR, seven were ESBL producers (3 CTX-M-1 and 4 SHV-12) and five were positive for the UPEC status.

We also recovered in this study eight isolates belonging to *Escherichia* clade I (ST770, 7 isolates; ST4994, 1 isolate). The eight isolates exhibited the ExPEC status and five were ESBL producers (2 CTX-M-9 and 3 SHV-12). Although ST770 *Escherichia* clade I is infrequently reported, it has been associated with $bla_{\text{CTX-M-1}}$ carriage in poultry in

the Netherlands and Switzerland (Dierikx et al., 2013; Vogt et al., 2014). It has been also associated with pAmpC production, specifically CMY-2, isolated from rooks wintering in Czechia and from broilers in Sweden (Börjesson et al., 2013; Jamborova et al., 2015). Recently, we recovered *bla*$_{\text{CTX-M-14}}$-carrying ST770 isolates from five healthy dogs of our region (Abreu-Salinas et al., 2020). But importantly, ST770 isolates have been also found implicated in UTI cases: in a dog in Argentina by an *mcr*-1 and *bla*$_{\text{CTX-M-2}}$ isolate, and in a patient in Spain (Rumi et al., 2019; Valverde et al., 2009).

Globally, we found significant differences regarding meat origin. Thus, turkey meat showed worse microbiological quality (56% of turkey samples with *E. coli* counts > 50 cfu/g *vs* 30% of chicken), higher rates of multidrug resistance and higher rates of *mcr*-carriage. These differences are probably associated with a longer fattening period and so, with a longer exposition to antibiotics. There are also different reports suggesting that poultry production systems alternative to the conventional broiler production are associated with reduced frequency of antibiotic-resistant *E. coli* among the commensal gut microbiota, posing a lower risk to the environment and the consumer (Davis et al., 2018; Pesciaroli et al., 2020). Davis et al. (2018) found that the resistance prevalence varied by meat type and was higher among *E. coli* isolates from turkey for the majority of antibiotics tested compared to chicken meat.

5. Conclusion

The finding that more than 80% of the poultry meat samples posed ≥ 3 risks including resistance genes, virulence traits, and human-associated pathogenic clones of *E. coli* means that consumers are highly exposed to those threats. To which extend poultry participates in the human microbiota composition and extraintestinal pathologies such as MDR UTIs needs deep elucidation. But first it is necessary the implementation of a

systematic AMR surveillance in food, together with the monitoring of ExPEC and DEC, which would enable effective food safety interventions under both "farm to fork strategy" and "One-Health perspective". Based on our observations, we propose an optimized workflow combination. The microbiological method (pre-enrichment, enrichment in ML broth, and inoculation onto ML/MSTC/CHROMID® ESBL), followed by the screening of six virulence/AMR traits, and including a duplex PCR for the screening of ExPEC, would estimate the greatest risk for consumers.

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Table 1

Association of E. coli counts with meat origin (chicken vs turkey) and packaging (modified atmosphere vs freshly butchered)

[1] CFU / g *E. coli*	Poultry meat n = 100	Chicken meat n = 50	Turkey meat n = 50	*P* two-tailed value	Modified atmosphere n = 49	Freshly butchered n = 51	*P* two-tailed value
< 10	27	16 (32%)	11 (22%)	0.367	10 (20.4%)	17 (33.3%)	0.179
10-49	30	19 (38%)	11 (22%)	0.125	15 (30.6%)	15 (29.4%)	1.000
50-500	38	14 (28%)	24 (48%)	0.063	22 (44.9%)	16 (31.4%)	0.216
> 500	5	1 (2%)	4 (8%)	0.322	2 (4.1%)	3 (5.9%)	1.000
≥ 50	43	15 (30%)	28 (56%)	**0.015**	24 (49%)	19 (37.3%)	0.419

Note: [1] CFU: colony forming units.

Statistically significant differences ($P < 0.05$) are highlighted in bold.

800 **Table 2**

801 *Association of virulence traits with protocols and meat origin for the E. coli collection (N = 323)*

Virulence trait	Protocol I ML 37 ºC	Protocol II MLST 37 ºC	Protocol III ML 44 ºC	Protocol IV MLST 44 ºC	Protocol V CHROMID® 37 ºC	Chicken origin	Turkey origin	[7]*P* two tail Chicken vs Turkey
[1] ExPEC status (%) N = 150	57 (38)	61 (40.7)	4 (2.7)	10 (6.6)	18 (12)	87 (58)	63 (42)	**0.014**
[2] UPEC status (%) N = 83	32 (38.6)	37 (44.6)	1 (1.2)	7 (8.4)	6 (7.2)	47 (56.6)	36 (43.4)	0.205
[3] ESBL/ pAmpC producer (%) N = 155	7 (4.5)	9 (5.8)	0	2 (1.3)	137 (88.4)	71 (45.8)	84 (54.2)	0.119
[4] *mcr-1* carrier (%) N = 13	4 (30.8)	6 (46.1)	0	2 (15.4)	1 (7.7)	1 (7.7)	12 (92.3)	**0.001**
[5] MDR (%) N = 253	48 (19.0)	52 (20.6)	3 (1.2)	13 (5.1)	137 (54.1)	118 (46.6)	135 (53.4)	**0.010**
[6] *rbfO25b* (%) N = 13	6 (46.1)	6 (46.1)	1 (7.7)	0	0	10 (76.9)	3 (23.1)	0.086
No. isolates per protocol and meat origin	79	86	5	16	137	163	160	-

802

Note: [1] No. of isolates conforming ExPEC status (Johnson *et al*., 2003).

[2] No. of isolates conforming status UPEC (Spurbeck *et al*., 2012).

[3] No. of ESBL/pAmpC-producing *E. coli*.

[4] No. of isolates carriers of the *mcr-1* gene.

[5] No. of MDR isolates according to Magiorakos *et al*. (2012) criteria.

[6] No. of *rbfO25b*-positive isolates: O25b subtype associated with the clonal group ST131 screened by PCR (Clermont *et al*., 2008).

[7] Statistically significant differences ($P < 0.05$) highlighted in bold.

Table 3

Association of virulence traits with phylogroup distribution for the E. coli collection (N = 323)

Virulence trait	Phylogroup A	Phylogroup B1	Phylogroup B2	Phylogroup C	Phylogroup D	Phylogroup E	Phylogroup F	Phylogroup G	Clade I
[1]ExPEC status (%) N = 150	24 (**16**)	7 (4.7)	51 (**34**)	3 (2)	8 (5.3)	16 (**10.7**)	31 (**20.7**)	2 (1.3)	8 (5.3)
[2]UPEC status (%) N = 83	0	0	57 (**68.7**)	0	0	0	21 (**25.3**)	5 (**6**)	0
[3]ESBL/ pAmpC producer (%) N = 155	63 (**40.6**)	44 (**28.4**)	5 (3.2)	4 (2.6)	3 (1.9)	20 (**12.9**)	4 (2.6)	7 (4.5)	5 (3.2)
[4]*mcr-1* carrier (%) N = 13	6 (**46.2**)	4 (**30.8**)	2 (**15.4**)	0	1 (7.7)	0	0	0	0
[5]MDR (%) N = 253	91 (**36**)	55 (**21.7**)	20 (7.9)	8 (3.2)	6 (2.4)	33 (**13**)	25 (9.9)	9 (3.6)	6 (2.4)
[6]*rbfO25b* (%) N = 13	0	0	12 (**92.3**)	0	0	1 (7.7)	0	0	0
No. isolates per phylogroup (%) (N = 323)									
	105 (32.5)	56 (17.3)	57 (17.6)	9 (2.8)	11 (3.4)	35 (10.8)	33 (10.2)	9 (2.8)	8 (2.5)

Note: [1] No. of isolates conforming ExPEC status (Johnson *et al*., 2003).

[2] No. of isolates conforming UPEC status (Spurbeck *et al*., 2012).

[3] No. of ESBL/pAmpC-producing *E. coli*.

[4] No. of isolates carriers of the *mcr-1* gene.

[5] No. of MDR isolates according to Magiorakos *et al*. (2012) criteria.

[6] No. of *rbfO25b*-positive isolates: O25b subtype associated with the clonal group ST131 screened by PCR (Clermont *et al*., 2008).

[7] In bold, the most prevalent associations.

819 **Table 4**

820 *Characterization of the 22 isolates exhibiting hybrid pathotype aEPEC/ExPEC recovered from 19 meat samples*

E. coli counts [1] cfu /g	[2]Sample code	Protocol	[3] Clones	*eae* type	Virulence profile	[4]Antibioresistance profile	ESBL /pAmpC typing
40	Ch2	IV	ONT:H40-A-ST752-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iutA traT iss*	AMP, GEN, DOX, CIP, NAL	-
40	Ch3	II	O123:H34-A-ST752-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iutA traT*	AMP, GEN, TOB*, DOX, SXT, CIP*, NAL	-
40	Ch4	II	O123:H34-A-ST752-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iutA traT*	AMP, NIT*, CIP*, NAL	-
20	Ch5	II	O11:H40-A-ST752-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iutA traT iss*	DOX, CIP*, NAL	-
20	Ch5	II	O80:H26-A-ST165-CC189 *	β1	*fimH fimAvMT78 traT fyuA*	AMP, GEN, TOB*, CIP, NAL	-
10	Ch6	I	O145:H40-A-ST752-CC10 (CH11-24)	β1	*fimH24 traT*	CIP*, NAL	-
100	Ch7	II	ONT:HNT-A-ST19-CC10 (CH11-122)	β1	*fimH122 hlyF iucD iroN cvaC traT tsh, ompT iss chuA yfcV*	AMP, CIP*, NAL	-
70	Ch8	II	O132:H37-ST10-CC10 (CH11-24)	β1	*fimH24 traT*	CIP*, NAL	-
40	Ch10	V	O123:H34-A-ST752-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iutA traT*	AMP, CXM, CTX, FOX*, ATM*, CHL*, NIT*, CIP, NAL	CTX-M-1
10	Ch14	I	O153:H10-A-ST10-CC10 (CH11-54)	β1	*fimH54 fimAvMT78 traT fyuA*	-	-

<10	Ch16	II	O68:H51-A-ST10-CC10 (CH11-24)	β1	*fimH24 traT*	AMP, GEN, TOB*, NAL	-
20	Ch17	II	O153:HNM-A-ST10-CC10 (CH11-54)	β1	*fimH54 fimAvMT78 traT fyuA*	AMP, DOX, CHL	-
440	Ch18	IV	O153:H10-A-ST10-CC10 (CH11-54)	β1	*fimH54 fimAvMT78 traT fyuA*	AMP, GEN, DOX, CHL	-
40	Ch20	II	O11:HNT-A-ST752-CC10 (CH11-54)	β1	*fimH54 traT fyuA*	AMP, CIP*, NAL	-
40	Ch20	II	O123:H34-A-ST10-CC10 (CH11-54)	β1	*fimH54*	AMP*, CIP*, NAL	-
40	Ch22	II	O154:H51-A-STnew11-CC10 (23-823)	β1	*fimH823 fimAvMT78 traT usp*	AMP, DOX*, SXT, CIP, NAL	-
200	Ch24	V	O153:H10-A-ST10-CC10 (CH11-54)	β1	*fimH54 fimAvMT78 traT fyuA*	AMP, CFZ, CXM, CAZ*, CTX, ATM, GEN, DOX, CHL	CTX-M-32
20	Ch36	I	O153:H10-A-ST10-CC10 (CH11-54)	β1	*fimH54 papEF papC papG II traT fyuA*	AMP, DOX, CHL, CIP, NAL	-
<10	Ch40	I	O145:H40-A-ST752-CC10 (CH11-24)	β1	*fimH24 traT*	AMP, NAL	-
40	T18	II	O57:HNT-A-ST165-CC189 *	β1	*fimH fimAvMT78 hlyF iucD iroN cvaCtraT usp iss fyuA*	AMP, GEN, TOB, DOX, SXT, CIP, NAL	-
40	T47	II	O2:H40-A-ST10-CC10 (CH11-24)	β1	*fimH24 hlyF traT*	AMP, AMC, CFZ, CXM, CAZ, CTX, FOX, SXT, CIP, NAL	CMY-2
40	T47	II	O2:H40-A-ST10-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iroN cvaC traT iss*	AMP, AMC, CFZ, CXM, CAZ, CTX, FOX, DOX, SXT, CIP, NAL	CMY-2

Note: [1] cfu: colony forming units.

[2] Ch (chicken meat), T (turkey meat).

[3] Clone defined as combination of serotype-filogroup-Sequence Type-Clonal Complex (Clonotype); * CH not determined.

[4] Antimicrobial susceptibility tested by disc diffusion assay and interpreted according to the CLSI standard breakpoints (CLSI, 2020): ampicillin (AMP), amoxicillin-clavulanic acid (AMC), cefuroxime (CXM), ceftazidime (CAZ), cefotaxime (CTX), cefoxitin (FOX), aztreonam (ATM), gentamicin (GEN), tobramycin (TOB), doxycycline (DOX), chloramphenicol (CHL), sulfamethoxazole-trimethoprim (SXT), ciprofloxacin (CIP), nalidixic acid (NAL). Intermediate values are indicated with *.

828 **Table 5**

829 *Study of susceptibility for the for the E. coli collection (N = 323)*

[1] ATB	*E. coli* from chicken n = 163		*E. coli* from turkey n = 160		Total N = 323		Chicken *vs* turkey origin
	No. non-susceptible	%	No. non-susceptible	%	No. non-susceptible	%	[2] Two-tailed *P* value
AMP	118	72.4	149	**93.1**	267	82.7	**0.000**
AMC	26	16.0	47	**29.4**	73	22.6	**0.005**
CXM	54	33.1	58	36.3	112	34.7	0.561
CAZ	55	33.7	73	**45.6**	128	39.6	**0.031**
CTX	69	42.3	82	**51.3**	151	46.7	**0.119**
FOX	3	1.8	5	3.1	8	2.5	0.499
ATM	57	35.0	77	**48.1**	134	41.5	**0.018**
IPM	0	0.0	0	0.0	0	0.0	-
GEN	48	**29.4**	16	10.0	64	19.8	**0.000**
TOB	21	12.9	13	8.1	34	10.5	0.205
AMK	0	0.0	0	0.0	0	0.0	-
FOF	0	0.0	0	0.0	0	0.0	-

CST*	1	0.6	5	3.1	6	1.9	0.119
DOX	96	58.9	117	**73.1**	213	65.9	**0.010**
CHL	35	21.5	69	**43.1**	104	32.2	**0.000**
NIT	11	6.7	11	6.9	22	6.8	1
SXT	47	28.8	74	**46.3**	121	37.5	**0.001**
CIP	115	70.6	117	73.1	232	71.8	0.623
NAL	123	75.5	116	72.5	239	74.0	0.612
TGC	0	0.0	1	0.6	1	0.3	0.495
MDR	121	74.2	137	**85.6**	258	79.9	**0.012**

Note: [1]Antimicrobial susceptibility tested by disc diffusion assay and interpreted according to the CLSI standard breakpoints (CLSI, 2020), where number of isolates and prevalence include intermediate values: ampicillin (AMP), amoxicillin-clavulanic acid (AMC), cefuroxime (CXM), ceftazidime (CAZ), cefotaxime (CTX), cefoxitin (FOX), aztreonam (ATM), imipenem (IMP), gentamicin (GEN), tobramycin (TOB), amikacin (AMK), fosfomycin (FOF), colistin (CST), doxycycline (DOX), chloramphenicol (CHL), nitrofurantoin (NIT), sulfamethoxazole-trimethoprim (SXT), ciprofloxacin (CIP), nalidixic acid (NAL), tigecycline (TGC) and multidrug-resistance (MDR) according to Magiorakos definition (Magiorakos et al., 2012). *Resistance to colistin was also performed by broth microdilution for the 13 *mcr*-positive isolates, which gave MIC values of 4 μg/mL (11 isolates), 2 and 1 μg/mL (1 isolate each).

[2] In bold, the statistically significant values ($P < 0.05$).

838 **Table 6**

839 *Foodborne risk assessment of the 100 meat poultry samples based on six parameters*

[1] Type of sample	No. sample	[2] ESBL/pAmpC/*mcr* types (No. of isolates)	[3] High-risk lineages of *E. coli*	[4]ExPEC	[5] UPEC	[6] Resistances to antimicrobials of categories A or B	[7] *E. coli* count	[8] TOTAL risk	[9] HP
Ch	1	SHV-12 (1 isolate)	**ST648-F (CH4-58);** ST117-G; ST162-B1	1	1	MB-CF3rd-FQ	10	4	
Ch	2	CTX-M-32 (1 isolate)	**O25b:H4-B2-ST131 (CH40-neg); ST115-E (CH26-270);** CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	30	4	*
Ch	3	SHV-12 (1 isolate)	CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	40	4	*
Ch	4	CTX-M-1 (1 isolate)	**ST117-G (CH45-97)**; **ST428-B2 (CH40-22)**; CC10-A (*eae*-beta1)	1	1	CF3rd-FQ	410	4	*
Ch	5	SHV-12, CTX-M-NT, CTX-M-9 **(1 isolate each)**	**O2:H9-E-ST115 (CH26-270)**; **ST69-D (CH35-27); ST1485-F (CH231-58)**; CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	20	5	*
Ch	6	TEM-52	**ST1485-F (CH231-58)**; CC10-A (*eae*-beta1)	1	1	CF3rd-FQ	10	4	*
Ch	7	SHV-12, CTX-M-32, CTX-M-1 (**1 isolate each)**	**ST23-C (CH4-35);** ST93-A**;** ST10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	100	5	*
Ch	8	SHV-12 **(2 isolates)**	**O25b:H4-B2-ST131 (CH40-neg)**; CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	70	5	*
Ch	9	CTX-M-NT, *mcr1.1* (**1 isolate each)**	**ST1485-F (CH231-58);** ST48-A	1	1	CF3rd-FQ-CST*	30	5	
Ch	10	CTX-M-1 **(2 isolates)**	**ST117-G (CH45-97)**; CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	40	5	*
Ch	11	-	-	0	0	-	<10	0	
Ch	12	-	-	0	0	-	<10	0	
Ch	13	SHV-12 **(2 isolates)**	**O25b:H4-B2-ST131 (CH40-22); ST1485-F (CH231-58)**	1	1	MB-CF3rd-FQ	50	5	
Ch	14	-	**O153:H10-A-ST10 (CH11-54) (*eae*-beta1)**	1	1	-	10	3	*

Ch	15	-	**ST93-A (CH11-41)**	1	0	FQ	<10	3	
Ch	16	SHV-12, CTX-M-1 **(2 isolates)**	ST10-A (*eae*-beta1)	1	1	MB-CF3rd-Q	20	5	*
Ch	17	SHV-12 **(2 isolates)**	**O153:HNM-A-ST10 (CH11-54) (*eae*-beta1);** ST428-B2	1	1	MB-CF3rd-FQ	20	5	*
Ch	18	SHV-12, CTX-M-1 (**1 isolate each)**	**O153:H10-A-ST10 (CH11-54) (*eae*-beta1)**	1	1	MB-CF3rd-FQ	440	5	*
Ch	19	CTX-M-1, TEM-52 (**1 isolate each)**	ST428-B2	1	1	MB-CF3rd-FQ	**510**	**6**	
Ch	20	SHV-12 (1 isolate)	ST10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	40	4	*
Ch	21	SHV-12, TEM-52 (**1 isolate each)**	**ST95-B2 (CH38-27)**	1	1	MB-CF3rd-FQ	10	5	
Ch	22	SHV-12 **(2 isolates)**	**ST93-A (CH11-41)**	1	0	MB-CF3rd-FQ	40	4	*
Ch	23	SHV-12 (1 isolate)	-	0	0	MB-CF3rd-FQ	310	1	
Ch	24	CTX-M-32 (1 isolate)	**ST117-G (CH45-97)**; **O153:H10-A-ST10 (CH11-54) (*eae*-beta1)**	1	1	MB-CF3rd-FQ	200	4	*
Ch	25	SHV-12, CTX-M-32, TEM-52 **(1, 2, and 1 isolates respectively)**	**ST10-A (CH11-54)**	0	0	MB-CF3rd-FQ	50	3	
Ch	26	-	**ST93-A (CH11-neg)**	1	0	FQ	<10	3	
Ch	27	SHV-12 (1 isolate)	**ST10-A (CH11-54)**	1	0	MB-CF3rd-FQ	10	3	
Ch	28	**SHV-12 (2 isolates)**	ST155-B1	1	0	MB-CF3rd-FQ	<10	4	
Ch	29	-	**O25b:H4-B2-ST131 (CH40-22)**	1	1	FQ	<10	4	
Ch	30	-	-	0	0	-	<10	0	
Ch	31	SHV-12 (1 isolate)	**ST93-A (CH11-neg)**	1	1	MB-CF3rd-FQ	10	4	
Ch	32	SHV-12 (1 isolate)	-	0	0	MB-CF3rd	<10	1	
Ch	33	SHV-12 (3 isolates)	ST162-B1	1	0	MB-CF3rd-FQ	20	3	
Ch	34	CTX-M-9 (1 isolate)	**ST93-A (CH11-neg)**	1	0	MB-CF3rd-FQ	80	3	
Ch	35	SHV-12, TEM-52, CTX-M-1 **(1, 1, and 2 isolates respectively)**	**ST117-G (CH45-97)**; **ST101-B1 (CH41-86)**	1	0	MB-CF3rd-FQ	50	4	

Ch	36	SHV-12 (1 isolate)	**O153:H10-A-ST10 (CH11-54) (*eae*-beta1); ST93-A (CH11-41)**	1	0	MB-CF3rd-FQ	20	3	*
Ch	37	-	**ST1485-F (CH231-58)**	1	1	FQ	<10	4	
Ch	38	-	**ST1485-F (CH231-58)**	1	1	FQ	<10	4	
Ch	39	SHV-12 (1 isolate)	**O25b:H4-B2-ST131 (CH40-22)**	1	1	MB-CF3rd-FQ	<10	4	
Ch	40	SHV-12 (1 isolate)	CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	<10	4	*
Ch	41	SHV-12 **(3 isolates)**	**ST69-D (CH35-27);** ST155-B1	1	0	MB-CF3rd-FQ	<10	4	
Ch	42	-	-	0	0	-	<10	0	
Ch	43	SHV-12 **(3 isolates)**	**ST117-G (CH45-97)**; **ST1485-F (CH231-58)**; ST57-E	1	1	MB-CF3rd-FQ	30	5	
Ch	44	CTX-M-9 (1 isolate)	**ST95-B2 (CH38-27)**	1	1	CF3rd-FQ	120	4	
Ch	45	SHV-12, CTX-M-14 (**1 isolate each)**	**O20:H9-C-ST410 (CH4-24); ST648-F (CH4-58)**	1	1	MB-CF3rd-FQ	480	5	
Ch	46	SHV-12 (1 isolate)	ST641-B1	0	0	MB-CF3rd-FQ	<10	2	
Ch	47	CTX-M-32 **(2 isolates)**	**ST93-A (CH11-neg)**	1	0	MB-CF3rd-FQ	<10	4	
Ch	48	SHV-12 **(2 isolates)**	**ST10-A (CH11-54);** ST48-A; ST744-A	1	0	MB-CF3rd-FQ	120	4	
Ch	49	SHV-12, CTX-M-15 **(2 and 1 isolate, respectively)**	**ST617-A (CH11-neg)**; ST155-B1	1	0	MB-CF3rd-FQ	30	4	
Ch	50	-	**O25b:H4-B2-ST131 (CH40-22)**	1	1	FQ	80	4	
T	1	SHV-12 and *mcr1.1* (**1**); SHV-12 (**2**); *mcr1.1* (**1**)	**ST744-A (CH11-54)**; ST155-B1	1	1	MB-CF3rd-FQ-CST*	**510**	**6**	
T	2	SHV-12 (1 isolate)	**O46:H31-B2-ST569 (CH38-5); ST10-A (CH11-54)**	1	1	MB-CF3rd-FQ	90	4	
T	3	CTX-M-1, CTX-M-15 (**1 isolate each)**	-	0	0	MB-CF3rd-FQ	100	2	
T	4	SHV-12 **(3 isolates)**	ST354-F	1	1	MB-CF3rd-FQ	40	5	
T	5	SHV-2, SHV-12 (**1 isolate each)**	**O51:H52-A-ST93 (CH11-neg)**	1	0	MB-CF3rd-FQ	70	4	
T	6	SHV-12 **(2 isolates**)	**ST1485-F (CH231-58)**	1	1	MB-CF3rd-FQ	180	5	

T	7	CTX-M-1, CTX-M-15 (**1 isolate each)**	ST48-A	0	0	MB-CF3rd-FQ	<10	3	
T	8	SHV-12, CTX-M-15 (**1 isolate each)**	**ST453-B1 (CH6-31)**	1	0	MB-CF3rd-FQ	20	4	
T	9	SHV-12 **(5 isolates)**	**ST117-G (CH45-97)**	1	1	MB-CF3rd-FQ	250	5	
T	10	CTX-M-32 (1 isolate)	**O51:H52-A-ST93 (CH11-neg)**	1	0	MB-CF3rd-FQ	300	3	
T	11	SHV-12 (1 isolate)	**ST115-E (CH26-270);** ST162-B1	1	0	MB-CF3rd-FQ	200	3	
T	12	SHV-12 (1 isolate)	-	0	0	MB-CF3rd-FQ	440	1	
T	13	SHV-12, CTX-M-1, *mcr1.1* **(1, 1, and 2 isolates, respectively)**	**ST10-A (CH11-23)**; **ST69-D (CH35-27); ST117-G (CH45-97)**	1	1	MB-CF3rd-FQ-CST*	210	5	
T	14	-	**O8:H4-C-ST88 (CH4-39); ST428-B2 (CH40-22)**	1	1	FQ	150	4	
T	15	CTX-M-15 (1 isolate)	**ST117-G (CH45-97)**	1	1	MB-CF3rd-FQ	70	4	
T	16	*mcr1.1* (2 isolates)	**ST93-A (CH11-41)**; **ST101-B1 (CH41-86)**; **ST1485-F (CH231-58)**	1	1	FQ-CST*	100	4	
T	17	SHV-12, TEM-52, mcr1.1 **(1, 1 and 3 isolates, respectively)**	**O153:H34-F-ST354 (CH88-58)**	1	1	MB-CF3rd-FQ-CST*	60	5	
T	18	SHV-12 (1 isolate)	**ST648-F (CH4-58)**; ST93-A	1	0	MB-CF3rd-FQ	40	3	*
T	19	-	-	0	0	-	<10	0	
T	20	SHV-12, CTX- 14 **(2 and 1 isolates, respectively)**	**ST1485-F (CH231-58)**	1	1	MB-CF3rd-FQ	60	5	
T	21	SHV-12 (1 isolate)	**ST1485-F (CH231-58)**	1	1	MB-CF3rd-FQ	130	4	
T	22	-	**O8:H4-C-ST88 (CH4-39);** ST95-B2	1	1	FQ	80	4	
T	23	-	-	0	0	-	<10	0	
T	24	SHV-12, *mcr1.1* **(5 and 2 isolates, respectively)**	**ONT:H9-A-ST744 (CH11-54); O153:H34-F-ST354 (CH88-58); ST141-B2 (CH52-14);** ST57-E; ST34-A	1	1	MB-CF3rd-FQ	150	5	
T	25	SHV-12 (1 isolate)	ST350-E	0	0	MB-CF3rd-FQ	420	2	
T	26	SHV-12 **(2 isolates)**	**O25b:H4-B2-ST131 (CH40-22); ST93-A (CH11-neg)**; **ST648-F (CH4-58)**	1	1	MB-CF3rd-FQ	20	5	

T	27	SHV-12 **(2 isolates)**	ST350-E; ST155-B1	0	0	MB-CF3rd-FQ	220	3	
T	28	SHV-12 (1 isolate)	**ST10-A (CH11-54); ST648-F (CH4-58)**	1	1	MB-CF3rd-FQ	70	4	
T	29	-	-	0	0	-	<10	0	
T	30	-	**ST115-E (CH26-270)**	1	0	FQ	<10	3	
T	31	SHV-12, *mcr1.1* (**1 isolate each**)	-	0	0	MB-CF3rd-FQ	350	2	
T	32	SHV-12, CTX-M-14 (**2 and 1 isolates, respectively**)	**ST10-A (CH11-54); ST617-A (CH11-neg)**	1	0	MB-CF3rd-FQ	100	4	
T	33	CTX-M-14 **(2 isolates)**	**O8:H4-C-ST88 (CH4-39);** ST1141-A	1	1	MB-CF3rd-FQ	30	5	
T	34	-	**ST453-B1 (CH6-31)**	1	0	FQ	<10	3	
T	35	SHV-12 (1 isolate)	-	0	0	MB-CF3rd-FQ	<10	1	
T	36	-	**O7:H6-E-ST362 (CH100-96)**	1	0	FQ	<10	3	
T	37	CTX-M-1 (1 isolate)	ST48-A	1	0	MB-CF3rd-FQ	20	3	
T	38	SHV-12 **(3 isolates)**	**ST155-B1 (CH4-neg)**	0	1	MB-CF3rd-FQ	30	4	
T	39	SHV-12 **(2 isolates)**	**O51:H52-A-ST93 (CH11-neg); ST10328-B1**	1	0	MB-CF3rd-FQ	210	4	
T	40	SHV-12, CTX-M-14 **(3 and 1 isolates, respectively)**	**O8:HNM-B1-ST58 (CH4-27); ST38-E (CH26-65)**	1	1	MB-CF3rd-FQ	**2320**	**6**	
T	41	SHV-12 (1 isolate)	**ST1485-F (CH231-58); ST95-B2 (CH38-30)**	1	1	MB-CF3rd-FQ	<10	4	
T	42	SHV-12 **(2 isolates)**	**ST10-A (CH11-54); ST602-B1 (CH19-86)**	0	0	MB-CF3rd-FQ	**680**	4	
T	43	SHV-12 **(5 isolates)**	**ST95-B2 (CH38-27)**; **ST155-B1 (CH4-neg); ST354-F (CH88-58);** ST34-A	1	1	MB-CF3rd-FQ	<10	5	
T	44	SHV-12 (1 isolate)	**O25b:H4-B2-ST131 (CH40-22); ST1485-F (CH231-58); ST359-B1 (CH41-35)**	1	1	MB-CF3rd-FQ	<10	4	
T	45	-	-	0	0	-	<10	0	
T	46	SHV-12 (1 isolate)	**O25b:H4-ST131-B2 (CH40-22)**; ST57-E	1	1	MB-CF3rd-FQ	**1130**	5	
T	47	CTX-M-1, CMY-2 **(1 and 2 isolates, respectively)**	**O101:HNM-A-ST167 (CH11-negative)**; ST10-A (*eae*-beta1)	0	0	MB-CF3rd-FQ	40	3	*

T	48	SHV-12 **(4 isolates)**	**ST95-B2 (CH38-27)**; **ST155-B1 (CH4-neg)**	1	1	MB-CF3rd-FQ	20	5
T	49	-	**ST1485-F (CH231-58)**	1	0	FQ	20	3
T	50	SHV-12, CTX-M-32 **(2 and 1 isolates, respectively)**	**ST115-E (CH26-270); ST1485-F (CH231-58); ST602-B1 (CH19-86)**; ST906-B1	1	0	MB-CF3rd-FQ	110	4

Note: [1] Type of sample: Ch (chicken meat), T (turkey meat).

[2] ESBL/pAmpC/*mcr* types determined by PCR and sequencing. Indicated in bold, the recovery of ≥ 2 different ESBL/pAmpC –producing or *mcr*-bearing isolates.

[3] High-risk lineages of *E. coli* associated with human extraintestinal and/or uropathogenic pathologies according to recent studies (Manges et al., 2019; Yamaji et al., 2018a,b). In bold, those clonal groups (phylogroup, ST and CH) found within our own collections of clinical human isolates (Mamani et al., 2019; Flament-Simon et al., 2020b,c).

[4] ExPEC status = 1: *E. coli* strains considered with higher capacity of developing extraintestinal pathologies when positive for two or more of five markers, including *papAH* and/or *papC*, *sfa/focDE*, *afa/draBC*, *kpsM II* and *iutA*; ExPEC status = 0: strains negative for those markers (Johnson et al. 2003).

[5] UPEC status = 1: strains considered with higher capacity of developing UTI pathologies when positive for three or more of four markers, including *chuA*, *fyuA*, *vat* and *yfcV*; UPEC status = 0: strains negative for those markers (Spurbeck et al., 2012).

[6] Detection of isolates resistant to antimicrobials categorized as A or B (European Medicines Agency, 2020); MB (monobactams); CF3rd (3^{rd}-generation cephalosporins); FQ (fluoroquinolones); Q (quinolones); CST (colistin) performed by broth microdilution , * MIC values = 4 µg/mL.

[7] Count of cfu (colony forming units) per g.

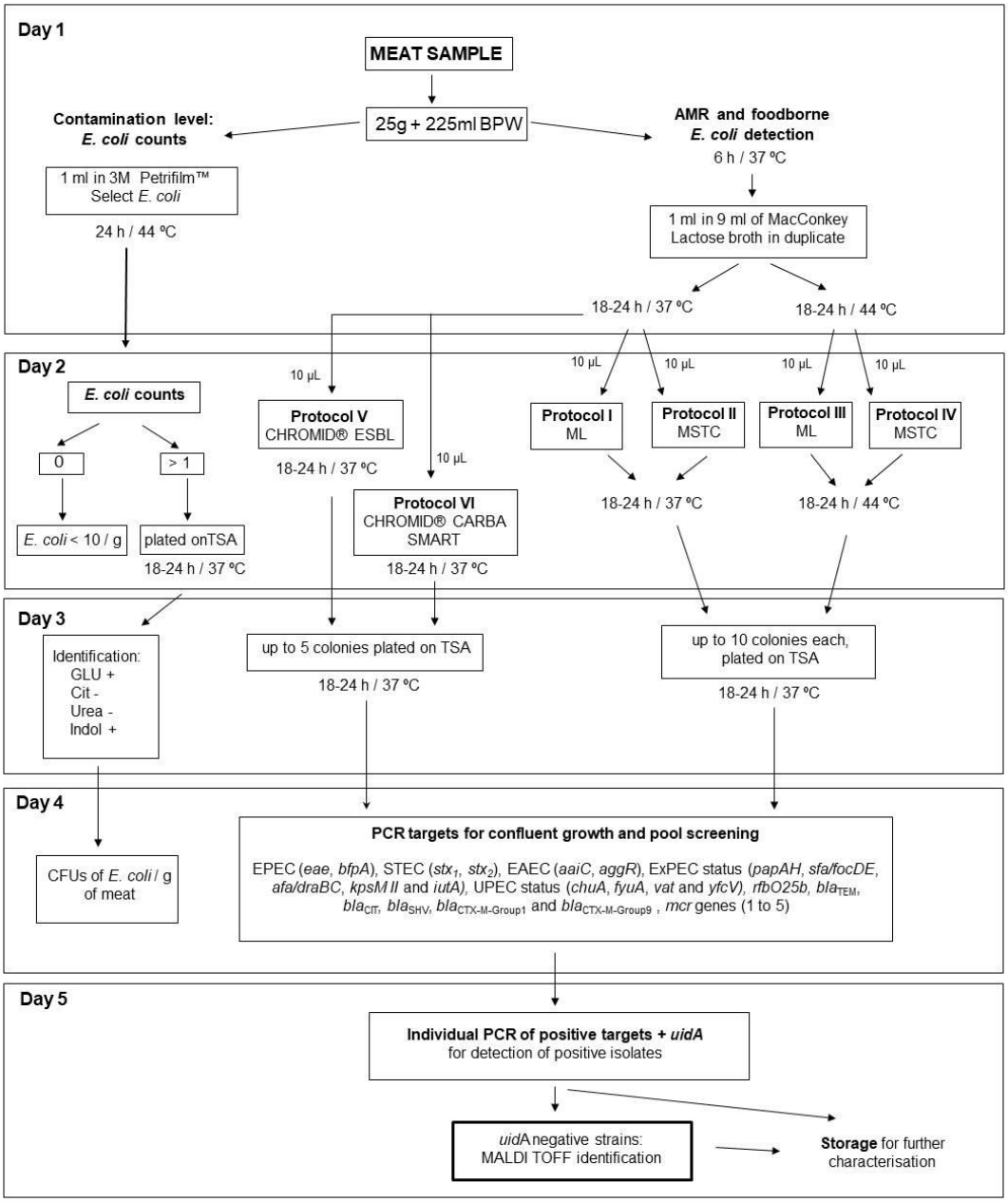
[8] Meat samples were qualified between zero (lowest) to six (highest) in association with the following microbiological parameters, considered as summative risks when happened: i) *E. coli* counts > 500 cfu / g of poultry meat. ii) The recovery of *E. coli* resistant to antimicrobials of categories A ("Avoid") or B ("Restrict"). iii) The recovery of

≥ 2 different ESBL/pAmpC –producing or *mcr*-bearing isolates. iv) The identification of high-risk clonal groups of *E. coli* associated with human extraintestinal pathologies. v) The isolation of *E. coli* conforming ExPEC status. vi) The isolation of *E. coli* conforming UPEC status.

[9] HP: the recovery of hybrid pathotypes aEPEC/ExPEC is indicated with asterisk (*)

Figure 1

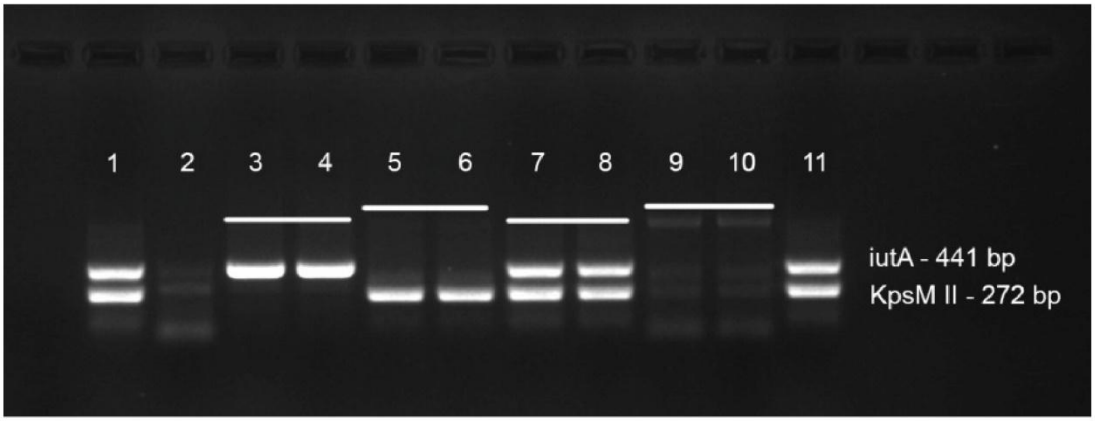
Lab workflow designed in this study to investigate the level of contamination, and the rates of AMR and foodborne pathogenic E. coli.



Note: AMR: antimicrobial resistance; ML: MacConkey Lactose agar; MSTC: MacConkey Sorbitol agar enriched with tellurite and cefixime; TSA: tryptone soy agar; CFU: colony forming units; EPEC: enteropathogenic *E. coli*; STEC: Shiga toxin-producing *E. coli*; EAEC: enteroaggregative *E. coli*; ExPEC: extraintestinal pathogenic *E. coli*.

Figure 2

Duplex PCR based on iutA (441 bp) and KpsM II (272 bp) targets and designed for the ExPEC screening on confluent growth, pooled and individual isolates.



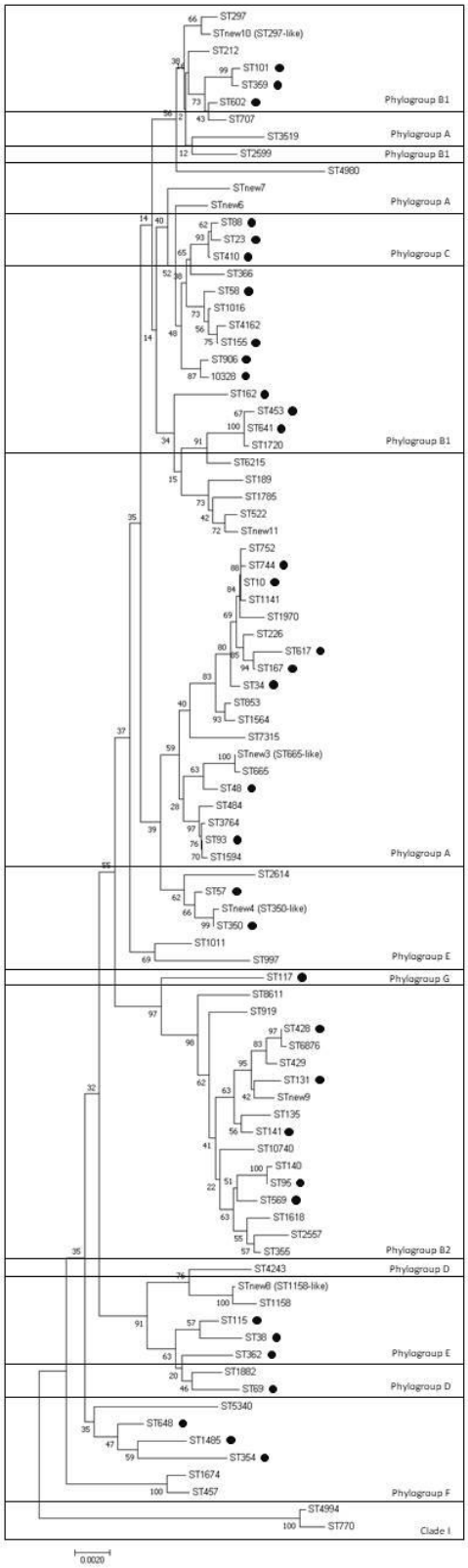
Target	Primers	Nucleotide sequence (5´- 3´)	Size (bp)	Reference
iutA	iutA-Al f	GCCGGAGCTGTCTCCGGCGG	441	This study
	aer-1152 r	CGTCGGGAACGGGTAGAATCG		Johnson *et al*. 1997
kpsM II	KpsII f	GCGCATTTGCTGATACTGTTG	272	Johnson & Stell 2000
	KpsII r	CATCCAGACGATAAGCATGAGCA		

Note: Lines 1 and 11, positive control (+ +); line 2, negative control (- -); lines 3 and 4, *iutA* carriers (+ -); lines 5 and 6, *KpsM II* carriers (- +); lines 7 and 8, *iutA* and *KpsM II* carriers (+ +) and lines 9 and 10, negative carriers (- -). PCR products were loaded on a 1.5% agararose gels with nzytech GreenSafe as stain. After electrophoresis, images were captured in an ultraviolet BioRad GelDoc. The thermal cycle included 35 cycles of amplification (denaturation 94°C, 1 min; annealing 60°C, 1 min; extension 72°C, 1.30 min).

877 **Figure 3**

878 *Representative E. coli collection. Phylogenetic tree based on concatenated sequences of the seven*

879 *housekeeping genes from the MLST Achtman scheme by the Neighbor-Joining method.*



880

Note: The optimal tree with the sum of branch length = 0.21789331 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 89 nucleotide sequences determined for 272 isolates. All positions containing gaps and missing data were eliminated. There were a total of 3423 positions in the final dataset. Evolutionary analyses were conducted in MEGA7. Marked with a black dot those STs of *E. coli* associated with human extraintestinal and/or uropathogenic pathologies.

Table 1

Table 1

Association of E. coli counts with meat origin (chicken vs turkey) and packaging (modified atmosphere vs freshly butchered)

[1] CFU / g *E. coli*	Poultry meat n = 100	Chicken meat n = 50	Turkey meat n = 50	*P* two-tailed value	Modified atmosphere n = 49	Freshly butchered n = 51	*P* two-tailed value
< 10	27	16 (32%)	11 (22%)	0.367	10 (20.4%)	17 (33.3%)	0.179
10-49	30	19 (38%)	11 (22%)	0.125	15 (30.6%)	15 (29.4%)	1.000
50-500	38	14 (28%)	24 (48%)	0.063	22 (44.9%)	16 (31.4%)	0.216
> 500	5	1 (2%)	4 (8%)	0.322	2 (4.1%)	3 (5.9%)	1.000
≥ 50	43	15 (30%)	28 (56%)	**0.015**	24 (49%)	19 (37.3%)	0.419

Note: [1] CFU: colony forming units.

Statistically significant differences ($P < 0.05$) are highlighted in bold.

Table 2

Association of virulence traits with protocols and meat origin for the E. coli collection (N = 323)

Virulence trait	Protocol I ML 37 ºC	Protocol II MLST 37 ºC	Protocol III ML 44 ºC	Protocol IV MLST 44 ºC	Protocol V CHROMID® 37 ºC	Chicken origin	Turkey origin	[7]*P* two tail Chicken vs Turkey
[1] ExPEC status (%) N = 150	57 (38)	61 (40.7)	4 (2.7)	10 (6.6)	18 (12)	87 (58)	63 (42)	**0.014**
[2] UPEC status (%) N = 83	32 (38.6)	37 (44.6)	1 (1.2)	7 (8.4)	6 (7.2)	47 (56.6)	36 (43.4)	0.205
[3] ESBL/ pAmpC producer (%) N = 155	7 (4.5)	9 (5.8)	0	2 (1.3)	137 (88.4)	71 (45.8)	84 (54.2)	0.119
[4] *mcr-1* carrier (%) N = 13	4 (30.8)	6 (46.1)	0	2 (15.4)	1 (7.7)	1 (7.7)	12 (92.3)	**0.001**
[5] MDR (%) N = 253	48 (19.0)	52 (20.6)	3 (1.2)	13 (5.1)	137 (54.1)	118 (46.6)	135 (53.4)	**0.010**
[6] *rbfO25b* (%) N = 13	6 (46.1)	6 (46.1)	1 (7.7)	0	0	10 (76.9)	3 (23.1)	0.086
No. isolates per protocol and meat origin	79	86	5	16	137	163	160	-

Note: [1] No. of isolates conforming ExPEC status (Johnson *et al*., 2003).

[2] No. of isolates conforming status UPEC (Spurbeck *et al*., 2012).

[3] No. of ESBL/pAmpC-producing *E. coli*.

[4] No. of isolates carriers of the *mcr-1* gene.

[5] No. of MDR isolates according to Magiorakos *et al*. (2012) criteria.

[6] No. of *rbfO25b*-positive isolates: O25b subtype associated with the clonal group ST131 screened by PCR (Clermont *et al*., 2008).

[7] Statistically significant differences ($P < 0.05$) highlighted in bold.

Table 3

1 **Table 3**

2 *Association of virulence traits with phylogroup distribution for the E. coli collection (N = 323)*

Virulence trait	Phylogroup A	Phylogroup B1	Phylogroup B2	Phylogroup C	Phylogroup D	Phylogroup E	Phylogroup F	Phylogroup G	Clade I
[1]ExPEC status (%) N = 150	24 (**16**)	7 (4.7)	51 (**34**)	3 (2)	8 (5.3)	16 (**10.7**)	31 (**20.7**)	2 (1.3)	8 (5.3)
[2]UPEC status (%) N = 83	0	0	57 (**68.7**)	0	0	0	21 (**25.3**)	5 (**6**)	0
[3]ESBL/ pAmpC producer (%) N = 155	63 (**40.6**)	44 (**28.4**)	5 (3.2)	4 (2.6)	3 (1.9)	20 (**12.9**)	4 (2.6)	7 (4.5)	5 (3.2)
[4]*mcr-1* carrier (%) N = 13	6 (**46.2**)	4 (**30.8**)	2 (**15.4**)	0	1 (7.7)	0	0	0	0
[5]MDR (%) N = 253	91 (**36**)	55 (**21.7**)	20 (7.9)	8 (3.2)	6 (2.4)	33 (**13**)	25 (9.9)	9 (3.6)	6 (2.4)
[6]*rbfO25b* (%) N = 13	0	0	12 (**92.3**)	0	0	1 (7.7)	0	0	0
No. isolates per phylogroup (%) (N = 323)									
	105 (32.5)	56 (17.3)	57 (17.6)	9 (2.8)	11 (3.4)	35 (10.8)	33 (10.2)	9 (2.8)	8 (2.5)

Note: [1] No. of isolates conforming ExPEC status (Johnson *et al*., 2003).

[2] No. of isolates conforming UPEC status (Spurbeck *et al*., 2012).

[3] No. of ESBL/pAmpC-producing *E. coli*.

[4] No. of isolates carriers of the *mcr-1* gene.

[5] No. of MDR isolates according to Magiorakos *et al*. (2012) criteria.

[6] No. of *rbfO25b*-positive isolates: O25b subtype associated with the clonal group ST131 screened by PCR (Clermont *et al*., 2008).

[7] In bold, the most prevalent associations.

Table 4

1 **Table 4**

2 *Characterization of the 22 isolates exhibiting hybrid pathotype aEPEC/ExPEC recovered from 19 meat samples*

E. coli counts [1] cfu /g	[2]Sample code	Protocol	[3] Clones	*eae* type	Virulence profile	[4]Antibioresistance profile	ESBL /pAmpC typing
40	Ch2	IV	ONT:H40-A-ST752-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iutA traT iss*	AMP, GEN, DOX, CIP, NAL	-
40	Ch3	II	O123:H34-A-ST752-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iutA traT*	AMP, GEN, TOB*, DOX, SXT, CIP*, NAL	-
40	Ch4	II	O123:H34-A-ST752-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iutA traT*	AMP, NIT*, CIP*, NAL	-
20	Ch5	II	O11:H40-A-ST752-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iutA traT iss*	DOX, CIP*, NAL	-
20	Ch5	II	O80:H26-A-ST165-CC189 *	β1	*fimH fimAvMT78 traT fyuA*	AMP, GEN, TOB*, CIP, NAL	-
10	Ch6	I	O145:H40-A-ST752-CC10 (CH11-24)	β1	*fimH24 traT*	CIP*, NAL	-
100	Ch7	II	ONT:HNT-A-ST19-CC10 (CH11-122)	β1	*fimH122 hlyF iucD iroN cvaC traT tsh, ompT iss chuA yfcV*	AMP, CIP*, NAL	-
70	Ch8	II	O132:H37-ST10-CC10 (CH11-24)	β1	*fimH24 traT*	CIP*, NAL	-
40	Ch10	V	O123:H34-A-ST752-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iutA traT*	AMP, CXM, CTX, FOX*, ATM*, CHL*, NIT*, CIP, NAL	CTX-M-1
10	Ch14	I	O153:H10-A-ST10-CC10 (CH11-54)	β1	*fimH54 fimAvMT78 traT fyuA*	-	-

<10	Ch16	II	O68:H51-A-ST10-CC10 (CH11-24)	β1	*fimH24 traT*	AMP, GEN, TOB*, NAL	-
20	Ch17	II	O153:HNM-A-ST10-CC10 (CH11-54)	β1	*fimH54 fimAvMT78 traT fyuA*	AMP, DOX, CHL	-
440	Ch18	IV	O153:H10-A-ST10-CC10 (CH11-54)	β1	*fimH54 fimAvMT78 traT fyuA*	AMP, GEN, DOX, CHL	-
40	Ch20	II	O11:HNT-A-ST752-CC10 (CH11-54)	β1	*fimH54 traT fyuA*	AMP, CIP*, NAL	-
40	Ch20	II	O123:H34-A-ST10-CC10 (CH11-54)	β1	*fimH54*	AMP*, CIP*, NAL	-
40	Ch22	II	O154:H51-A-STnew11-CC10 (23-823)	β1	*fimH823 fimAvMT78 traT usp*	AMP, DOX*, SXT, CIP, NAL	-
200	Ch24	V	O153:H10-A-ST10-CC10 (CH11-54)	β1	*fimH54 fimAvMT78 traT fyuA*	AMP, CFZ, CXM, CAZ*, CTX, ATM, GEN, DOX, CHL	CTX-M-32
20	Ch36	I	O153:H10-A-ST10-CC10 (CH11-54)	β1	*fimH54 papEF papC papG II traT fyuA*	AMP, DOX, CHL, CIP, NAL	-
<10	Ch40	I	O145:H40-A-ST752-CC10 (CH11-24)	β1	*fimH24 traT*	AMP, NAL	-
40	T18	II	O57:HNT-A-ST165-CC189 *	β1	*fimH fimAvMT78 hlyF iucD iroN cvaCtraT usp iss fyuA*	AMP, GEN, TOB, DOX, SXT, CIP, NAL	-
40	T47	II	O2:H40-A-ST10-CC10 (CH11-24)	β1	*fimH24 hlyF traT*	AMP, AMC, CFZ, CXM, CAZ, CTX, FOX, SXT, CIP, NAL	CMY-2
40	T47	II	O2:H40-A-ST10-CC10 (CH11-24)	β1	*fimH24 hlyF iucD iroN cvaC traT iss*	AMP, AMC, CFZ, CXM, CAZ, CTX, FOX, DOX, SXT, CIP, NAL	CMY-2

3

4 *Note*: [1] cfu: colony forming units.

[2] Ch (chicken meat), T (turkey meat).

[3] Clone defined as combination of serotype-filogroup-Sequence Type-Clonal Complex (Clonotype); * CH not determined.

[4] Antimicrobial susceptibility tested by disc diffusion assay and interpreted according to the CLSI standard breakpoints (CLSI, 2020): ampicillin (AMP), amoxicillin-clavulanic acid (AMC), cefuroxime (CXM), ceftazidime (CAZ), cefotaxime (CTX), cefoxitin (FOX), aztreonam (ATM), gentamicin (GEN), tobramycin (TOB), doxycycline (DOX), chloramphenicol (CHL), sulfamethoxazole-trimethoprim (SXT), ciprofloxacin (CIP), nalidixic acid (NAL). Intermediate values are indicated with *.

Table 5

Study of susceptibility for the for the E. coli collection (N = 323)

[1] ATB	*E. coli* from chicken n = 163		*E. coli* from turkey n = 160		Total N = 323		Chicken *vs* turkey origin
	No. non-susceptible	%	No. non-susceptible	%	No. non-susceptible	%	[2] Two-tailed *P* value
AMP	118	72.4	149	**93.1**	267	82.7	**0.000**
AMC	26	16.0	47	**29.4**	73	22.6	**0.005**
CXM	54	33.1	58	36.3	112	34.7	0.561
CAZ	55	33.7	73	**45.6**	128	39.6	**0.031**
CTX	69	42.3	82	**51.3**	151	46.7	**0.119**
FOX	3	1.8	5	3.1	8	2.5	0.499
ATM	57	35.0	77	**48.1**	134	41.5	**0.018**
IPM	0	0.0	0	0.0	0	0.0	-
GEN	48	**29.4**	16	10.0	64	19.8	**0.000**
TOB	21	12.9	13	8.1	34	10.5	0.205
AMK	0	0.0	0	0.0	0	0.0	-
FOF	0	0.0	0	0.0	0	0.0	-
CST*	1	0.6	5	3.1	6	1.9	0.119
DOX	96	58.9	117	**73.1**	213	65.9	**0.010**
CHL	35	21.5	69	**43.1**	104	32.2	**0.000**
NIT	11	6.7	11	6.9	22	6.8	1
SXT	47	28.8	74	**46.3**	121	37.5	**0.001**
CIP	115	70.6	117	73.1	232	71.8	0.623
NAL	123	75.5	116	72.5	239	74.0	0.612
TGC	0	0.0	1	0.6	1	0.3	0.495
MDR	121	74.2	137	**85.6**	258	79.9	**0.012**

Note: [1]Antimicrobial susceptibility tested by disc diffusion assay and interpreted according to the CLSI standard breakpoints (CLSI, 2020), where number of isolates and prevalence include intermediate values:

ampicillin (AMP), amoxicillin-clavulanic acid (AMC), cefuroxime (CXM), ceftazidime (CAZ), cefotaxime (CTX), cefoxitin (FOX), aztreonam (ATM), imipenem (IMP), gentamicin (GEN), tobramycin (TOB), amikacin (AMK), fosfomycin (FOF), colistin (CST), doxycycline (DOX), chloramphenicol (CHL), nitrofurantoin (NIT), sulfamethoxazole-trimethoprim (SXT), ciprofloxacin (CIP), nalidixic acid (NAL), tigecycline (TGC) and multidrug-resistance (MDR) according to Magiorakos definition (Magiorakos et al., 2012). *Resistance to colistin was also performed by broth microdilution for the 13 *mcr*-positive isolates, which gave MIC values of 4 μg/mL (11 isolates), 2 and 1 μg/mL (1 isolate each).

[2] In bold, the statistically significant values ($P < 0.05$).

Table 6

Foodborne risk assessment of the 100 meat poultry samples based on six parameters

[1] Type of sample	No. sample	[2] ESBL/pAmpC/*mcr* types (No. of isolates)	[3] High-risk lineages of *E. coli*	[4]ExPEC	[5] UPEC	[6] Resistances to antimicrobials of categories A or B	[7] *E. coli* count	[8] TOTAL risk	[9] HP
Ch	1	SHV-12 (1 isolate)	**ST648-F (CH4-58);** ST117-G; ST162-B1	1	1	MB-CF3rd-FQ	10	4	
Ch	2	CTX-M-32 (1 isolate)	**O25b:H4-B2-ST131 (CH40-neg); ST115-E (CH26-270);** CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	30	4	*
Ch	3	SHV-12 (1 isolate)	CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	40	4	*
Ch	4	CTX-M-1 (1 isolate)	**ST117-G (CH45-97)**; **ST428-B2 (CH40-22)**; CC10-A (*eae*-beta1)	1	1	CF3rd-FQ	410	4	*
Ch	5	SHV-12, CTX-M-NT, CTX-M-9 **(1 isolate each)**	**O2:H9-E-ST115 (CH26-270)**; **ST69-D (CH35-27); ST1485-F (CH231-58)**; CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	20	5	*
Ch	6	TEM-52	**ST1485-F (CH231-58)**; CC10-A (*eae*-beta1)	1	1	CF3rd-FQ	10	4	*
Ch	7	SHV-12, CTX-M-32, CTX-M-1 (**1 isolate each)**	**ST23-C (CH4-35);** ST93-A**;** ST10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	100	5	*
Ch	8	SHV-12 **(2 isolates)**	**O25b:H4-B2-ST131 (CH40-neg)**; CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	70	5	*
Ch	9	CTX-M-NT, *mcr1.1* (**1 isolate each)**	**ST1485-F (CH231-58);** ST48-A	1	1	CF3rd-FQ-CST*	30	5	
Ch	10	CTX-M-1 **(2 isolates)**	**ST117-G (CH45-97)**; CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	40	5	*
Ch	11	-	-	0	0	-	<10	0	
Ch	12	-	-	0	0	-	<10	0	
Ch	13	SHV-12 **(2 isolates)**	**O25b:H4-B2-ST131 (CH40-22); ST1485-F (CH231-58)**	1	1	MB-CF3rd-FQ	50	5	
Ch	14	-	**O153:H10-A-ST10 (CH11-54) (*eae*-beta1)**	1	1	-	10	3	*

Ch	15	-	**ST93-A (CH11-41)**	1	0	FQ	<10	3	
Ch	16	SHV-12, CTX-M-1 **(2 isolates)**	ST10-A (*eae*-beta1)	1	1	MB-CF3rd-Q	20	5	*
Ch	17	SHV-12 **(2 isolates)**	**O153:HNM-A-ST10 (CH11-54) (*eae*-beta1);** ST428-B2	1	1	MB-CF3rd-FQ	20	5	*
Ch	18	SHV-12, CTX-M-1 (**1 isolate each)**	**O153:H10-A-ST10 (CH11-54) (*eae*-beta1)**	1	1	MB-CF3rd-FQ	440	5	*
Ch	19	CTX-M-1, TEM-52 (**1 isolate each)**	ST428-B2	1	1	MB-CF3rd-FQ	**510**	**6**	
Ch	20	SHV-12 (1 isolate)	ST10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	40	4	*
Ch	21	SHV-12, TEM-52 (**1 isolate each)**	**ST95-B2 (CH38-27)**	1	1	MB-CF3rd-FQ	10	5	
Ch	22	SHV-12 **(2 isolates)**	**ST93-A (CH11-41)**	1	0	MB-CF3rd-FQ	40	4	*
Ch	23	SHV-12 (1 isolate)	-	0	0	MB-CF3rd-FQ	310	1	
Ch	24	CTX-M-32 (1 isolate)	**ST117-G (CH45-97)**; **O153:H10-A-ST10 (CH11-54) (*eae*-beta1)**	1	1	MB-CF3rd-FQ	200	4	*
Ch	25	SHV-12, CTX-M-32, TEM-52 **(1, 2, and 1 isolates respectively)**	**ST10-A (CH11-54)**	0	0	MB-CF3rd-FQ	50	3	
Ch	26	-	**ST93-A (CH11-neg)**	1	0	FQ	<10	3	
Ch	27	SHV-12 (1 isolate)	**ST10-A (CH11-54)**	1	0	MB-CF3rd-FQ	10	3	
Ch	28	**SHV-12 (2 isolates)**	ST155-B1	1	0	MB-CF3rd-FQ	<10	4	
Ch	29	-	**O25b:H4-B2-ST131 (CH40-22)**	1	1	FQ	<10	4	
Ch	30	-	-	0	0	-	<10	0	
Ch	31	SHV-12 (1 isolate)	**ST93-A (CH11-neg)**	1	1	MB-CF3rd-FQ	10	4	
Ch	32	SHV-12 (1 isolate)	-	0	0	MB-CF3rd	<10	1	
Ch	33	SHV-12 (3 isolates)	ST162-B1	1	0	MB-CF3rd-FQ	20	3	
Ch	34	CTX-M-9 (1 isolate)	**ST93-A (CH11-neg)**	1	0	MB-CF3rd-FQ	80	3	
Ch	35	SHV-12, TEM-52, CTX-M-1 **(1, 1, and 2 isolates respectively)**	**ST117-G (CH45-97)**; **ST101-B1 (CH41-86)**	1	0	MB-CF3rd-FQ	50	4	

Ch	36	SHV-12 (1 isolate)	**O153:H10-A-ST10 (CH11-54) (*eae*-beta1); ST93-A (CH11-41)**	1	0	MB-CF3rd-FQ	20	3	*
Ch	37	-	**ST1485-F (CH231-58)**	1	1	FQ	<10	4	
Ch	38	-	**ST1485-F (CH231-58)**	1	1	FQ	<10	4	
Ch	39	SHV-12 (1 isolate)	**O25b:H4-B2-ST131 (CH40-22)**	1	1	MB-CF3rd-FQ	<10	4	
Ch	40	SHV-12 (1 isolate)	CC10-A (*eae*-beta1)	1	1	MB-CF3rd-FQ	<10	4	*
Ch	41	SHV-12 **(3 isolates)**	**ST69-D (CH35-27);** ST155-B1	1	0	MB-CF3rd-FQ	<10	4	
Ch	42	-	-	0	0	-	<10	0	
Ch	43	SHV-12 **(3 isolates)**	**ST117-G (CH45-97)**; **ST1485-F (CH231-58)**; ST57-E	1	1	MB-CF3rd-FQ	30	5	
Ch	44	CTX-M-9 (1 isolate)	**ST95-B2 (CH38-27)**	1	1	CF3rd-FQ	120	4	
Ch	45	SHV-12, CTX-M-14 (**1 isolate each)**	**O20:H9-C-ST410 (CH4-24); ST648-F (CH4-58)**	1	1	MB-CF3rd-FQ	480	5	
Ch	46	SHV-12 (1 isolate)	ST641-B1	0	0	MB-CF3rd-FQ	<10	2	
Ch	47	CTX-M-32 **(2 isolates)**	**ST93-A (CH11-neg)**	1	0	MB-CF3rd-FQ	<10	4	
Ch	48	SHV-12 **(2 isolates)**	**ST10-A (CH11-54);** ST48-A; ST744-A	1	0	MB-CF3rd-FQ	120	4	
Ch	49	SHV-12, CTX-M-15 **(2 and 1 isolate, respectively)**	**ST617-A (CH11-neg)**; ST155-B1	1	0	MB-CF3rd-FQ	30	4	
Ch	50	-	**O25b:H4-B2-ST131 (CH40-22)**	1	1	FQ	80	4	
T	1	SHV-12 and *mcr1.1* (**1**); SHV-12 (**2**); *mcr1.1* (**1**)	**ST744-A (CH11-54)**; ST155-B1	1	1	MB-CF3rd-FQ-CST*	**510**	**6**	
T	2	SHV-12 (1 isolate)	**O46:H31-B2-ST569 (CH38-5); ST10-A (CH11-54)**	1	1	MB-CF3rd-FQ	90	4	
T	3	CTX-M-1, CTX-M-15 (**1 isolate each)**	-	0	0	MB-CF3rd-FQ	100	2	
T	4	SHV-12 **(3 isolates)**	ST354-F	1	1	MB-CF3rd-FQ	40	5	
T	5	SHV-2, SHV-12 (**1 isolate each)**	**O51:H52-A-ST93 (CH11-neg)**	1	0	MB-CF3rd-FQ	70	4	
T	6	SHV-12 **(2 isolates**)	**ST1485-F (CH231-58)**	1	1	MB-CF3rd-FQ	180	5	

T	7	CTX-M-1, CTX-M-15 (**1 isolate each)**	ST48-A	0	0	MB-CF3rd-FQ	<10	3	
T	8	SHV-12, CTX-M-15 (**1 isolate each)**	**ST453-B1 (CH6-31)**	1	0	MB-CF3rd-FQ	20	4	
T	9	SHV-12 **(5 isolates)**	**ST117-G (CH45-97)**	1	1	MB-CF3rd-FQ	250	5	
T	10	CTX-M-32 (1 isolate)	**O51:H52-A-ST93 (CH11-neg)**	1	0	MB-CF3rd-FQ	300	3	
T	11	SHV-12 (1 isolate)	**ST115-E (CH26-270);** ST162-B1	1	0	MB-CF3rd-FQ	200	3	
T	12	SHV-12 (1 isolate)	-	0	0	MB-CF3rd-FQ	440	1	
T	13	SHV-12, CTX-M-1, *mcr1.1* **(1, 1, and 2 isolates, respectively)**	**ST10-A (CH11-23)**; **ST69-D (CH35-27); ST117-G (CH45-97)**	1	1	MB-CF3rd-FQ-CST*	210	5	
T	14	-	**O8:H4-C-ST88 (CH4-39); ST428-B2 (CH40-22)**	1	1	FQ	150	4	
T	15	CTX-M-15 (1 isolate)	**ST117-G (CH45-97)**	1	1	MB-CF3rd-FQ	70	4	
T	16	*mcr1.1* (2 isolates)	**ST93-A (CH11-41)**; **ST101-B1 (CH41-86)**; **ST1485-F (CH231-58)**	1	1	FQ-CST*	100	4	
T	17	SHV-12, TEM-52, mcr1.1 **(1, 1 and 3 isolates, respectively)**	**O153:H34-F-ST354 (CH88-58)**	1	1	MB-CF3rd-FQ-CST*	60	5	
T	18	SHV-12 (1 isolate)	**ST648-F (CH4-58)**; ST93-A	1	0	MB-CF3rd-FQ	40	3	*
T	19	-	-	0	0	-	<10	0	
T	20	SHV-12, CTX- 14 **(2 and 1 isolates, respectively)**	**ST1485-F (CH231-58)**	1	1	MB-CF3rd-FQ	60	5	
T	21	SHV-12 (1 isolate)	**ST1485-F (CH231-58)**	1	1	MB-CF3rd-FQ	130	4	
T	22	-	**O8:H4-C-ST88 (CH4-39);** ST95-B2	1	1	FQ	80	4	
T	23	-	-	0	0	-	<10	0	
T	24	SHV-12, *mcr1.1* **(5 and 2 isolates, respectively)**	**ONT:H9-A-ST744 (CH11-54); O153:H34-F-ST354 (CH88-58); ST141-B2 (CH52-14);** ST57-E; ST34-A	1	1	MB-CF3rd-FQ	150	5	
T	25	SHV-12 (1 isolate)	ST350-E	0	0	MB-CF3rd-FQ	420	2	
T	26	SHV-12 **(2 isolates)**	**O25b:H4-B2-ST131 (CH40-22); ST93-A (CH11-neg)**; **ST648-F (CH4-58)**	1	1	MB-CF3rd-FQ	20	5	

T	27	SHV-12 **(2 isolates)**	ST350-E; ST155-B1	0	0	MB-CF3rd-FQ	220	3	
T	28	SHV-12 (1 isolate)	**ST10-A (CH11-54); ST648-F (CH4-58)**	1	1	MB-CF3rd-FQ	70	4	
T	29	-	-	0	0	-	<10	0	
T	30	-	**ST115-E (CH26-270)**	1	0	FQ	<10	3	
T	31	SHV-12, *mcr1.1* (**1 isolate each**)	-	0	0	MB-CF3rd-FQ	350	2	
T	32	SHV-12, CTX-M-14 (**2 and 1 isolates, respectively**)	**ST10-A (CH11-54); ST617-A (CH11-neg)**	1	0	MB-CF3rd-FQ	100	4	
T	33	CTX-M-14 **(2 isolates)**	**O8:H4-C-ST88 (CH4-39);** ST1141-A	1	1	MB-CF3rd-FQ	30	5	
T	34	-	**ST453-B1 (CH6-31)**	1	0	FQ	<10	3	
T	35	SHV-12 (1 isolate)	-	0	0	MB-CF3rd-FQ	<10	1	
T	36	-	**O7:H6-E-ST362 (CH100-96)**	1	0	FQ	<10	3	
T	37	CTX-M-1 (1 isolate)	ST48-A	1	0	MB-CF3rd-FQ	20	3	
T	38	SHV-12 **(3 isolates)**	**ST155-B1 (CH4-neg)**	0	1	MB-CF3rd-FQ	30	4	
T	39	SHV-12 **(2 isolates)**	**O51:H52-A-ST93 (CH11-neg); ST10328-B1**	1	0	MB-CF3rd-FQ	210	4	
T	40	SHV-12, CTX-M-14 **(3 and 1 isolates, respectively)**	**O8:HNM-B1-ST58 (CH4-27); ST38-E (CH26-65)**	1	1	MB-CF3rd-FQ	**2320**	**6**	
T	41	SHV-12 (1 isolate)	**ST1485-F (CH231-58); ST95-B2 (CH38-30)**	1	1	MB-CF3rd-FQ	<10	4	
T	42	SHV-12 **(2 isolates)**	**ST10-A (CH11-54); ST602-B1 (CH19-86)**	0	0	MB-CF3rd-FQ	**680**	4	
T	43	SHV-12 **(5 isolates)**	**ST95-B2 (CH38-27)**; **ST155-B1 (CH4-neg); ST354-F (CH88-58);** ST34-A	1	1	MB-CF3rd-FQ	<10	5	
T	44	SHV-12 (1 isolate)	**O25b:H4-B2-ST131 (CH40-22); ST1485-F (CH231-58); ST359-B1 (CH41-35)**	1	1	MB-CF3rd-FQ	<10	4	
T	45	-	-	0	0	-	<10	0	
T	46	SHV-12 (1 isolate)	**O25b:H4-ST131-B2 (CH40-22)**; ST57-E	1	1	MB-CF3rd-FQ	**1130**	5	
T	47	CTX-M-1, CMY-2 **(1 and 2 isolates, respectively)**	**O101:HNM-A-ST167 (CH11-negative)**; ST10-A (*eae*-beta1)	0	0	MB-CF3rd-FQ	40	3	*

T	48	SHV-12 **(4 isolates)**	**ST95-B2 (CH38-27)**; **ST155-B1 (CH4-neg)**	1	1	MB-CF3rd-FQ	20	5
T	49	-	**ST1485-F (CH231-58)**	1	0	FQ	20	3
T	50	SHV-12, CTX-M-32 **(2 and 1 isolates, respectively)**	**ST115-E (CH26-270); ST1485-F (CH231-58); ST602-B1 (CH19-86)**; ST906-B1	1	0	MB-CF3rd-FQ	110	4

Note: [1] Type of sample: Ch (chicken meat), T (turkey meat).

[2] ESBL/pAmpC/*mcr* types determined by PCR and sequencing. Indicated in bold, the recovery of ≥ 2 different ESBL/pAmpC –producing or *mcr*-bearing isolates.

[3] High-risk lineages of *E. coli* associated with human extraintestinal and/or uropathogenic pathologies according to recent studies (Manges et al., 2019; Yamaji et al., 2018a,b). In bold, those clonal groups (phylogroup, ST and CH) found within our own collections of clinical human isolates (Mamani et al., 2019; Flament-Simon et al., 2020b,c).

[4] ExPEC status = 1: *E. coli* strains considered with higher capacity of developing extraintestinal pathologies when positive for two or more of five markers, including *papAH* and/or *papC*, *sfa/focDE*, *afa/draBC*, *kpsM II* and *iutA*; ExPEC status = 0: strains negative for those markers (Johnson et al. 2003).

[5] UPEC status = 1: strains considered with higher capacity of developing UTI pathologies when positive for three or more of four markers, including *chuA*, *fyuA*, *vat* and *yfcV*; UPEC status = 0: strains negative for those markers (Spurbeck et al., 2012).

[6] Detection of isolates resistant to antimicrobials categorized as A or B (European Medicines Agency, 2020); MB (monobactams); CF3rd (3^{rd}-generation cephalosporins); FQ (fluoroquinolones); Q (quinolones); CST (colistin) performed by broth microdilution , * MIC values = 4 µg/mL.

[7] Count of cfu (colony forming units) per g.

[8] Meat samples were qualified between zero (lowest) to six (highest) in association with the following microbiological parameters, considered as summative risks when happened: i) *E. coli* counts > 500 cfu / g of poultry meat. ii) The recovery of *E. coli* resistant to antimicrobials of categories A (“Avoid”) or B (“Restrict”). iii) The recovery of

≥ 2 different ESBL/pAmpC –producing or *mcr*-bearing isolates. iv) The identification of high-risk clonal groups of *E. coli* associated with human extraintestinal pathologies. v) The isolation of *E. coli* conforming ExPEC status. vi) The isolation of *E. coli* conforming UPEC status.

[9] HP: the recovery of hybrid pathotypes aEPEC/ExPEC is indicated with asterisk (*)

Figure 1

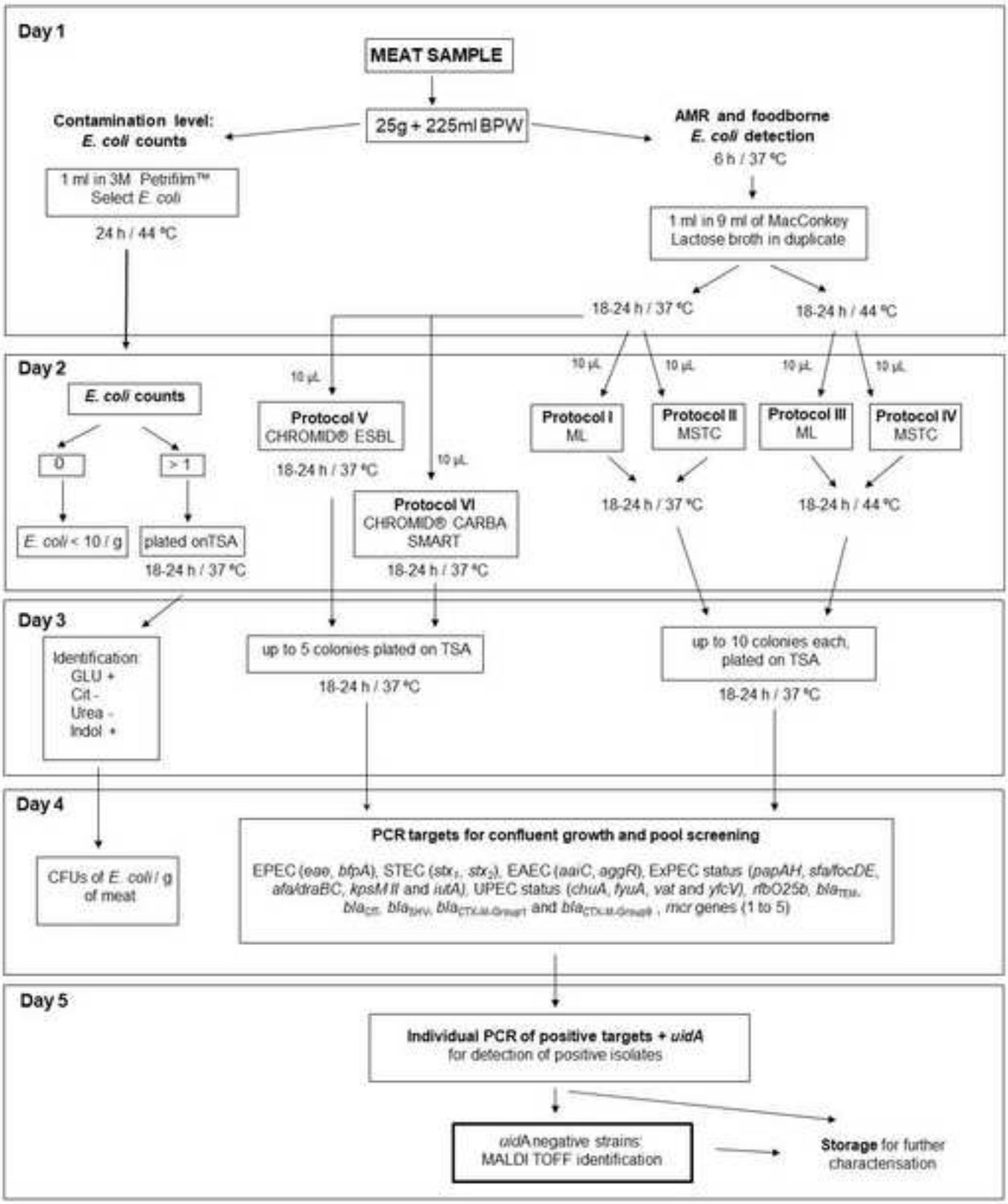
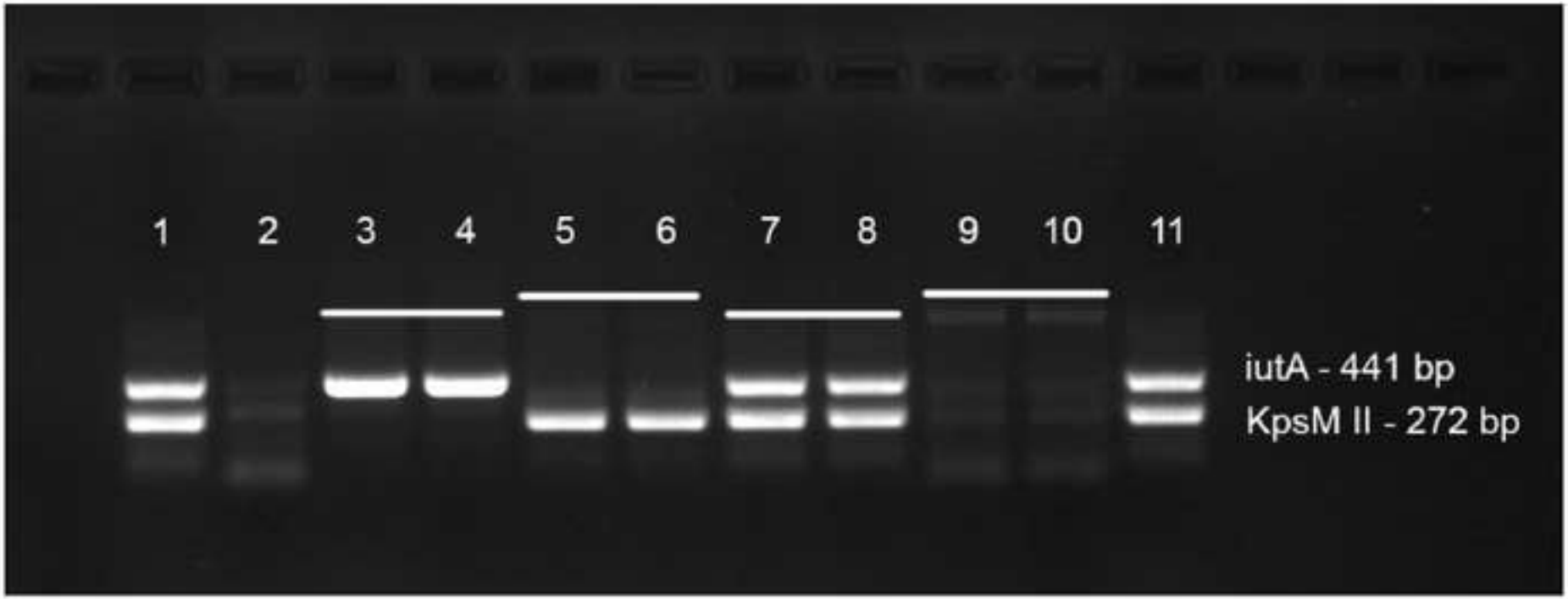
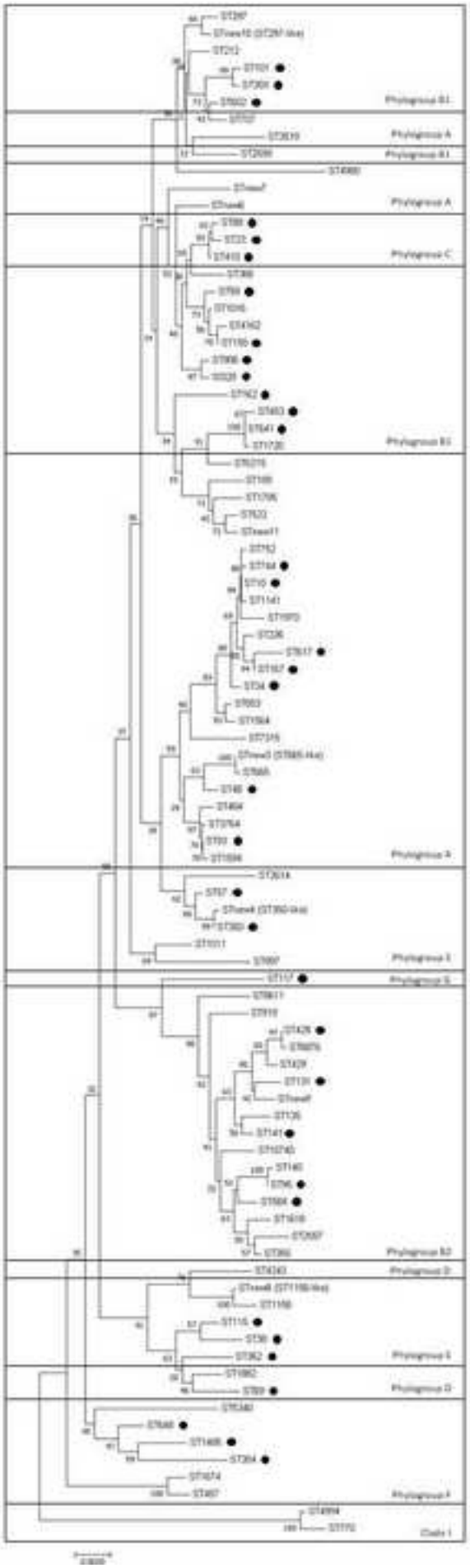

Day 1
MEAT SAMPLE
25g + 225ml BPW
Contamination level: E. coli counts
1 ml in 3M Petrifilm™ Select E. coli
24 h / 44 °C
AMR and foodborne E. coli detection
6 h / 37 °C
1 ml in 9 ml of MacConkey Lactose broth in duplicate
18-24 h / 37 °C
18-24 h / 44 °C
Day 2
E. coli counts
0
> 1
E. coli < 10 / g
plated onTSA
18-24 h / 37 °C
10 µL
Protocol V CHROMID® ESBL
18-24 h / 37 °C
10 µL
Protocol VI CHROMID® CARBA SMART
18-24 h / 37 °C
10 µL
Protocol I ML
Protocol II MSTC
Protocol III ML
Protocol IV MSTC
18-24 h / 37 °C
18-24 h / 44 °C
Day 3
Identification GLU + Cit - Urea - Indol +
up to 5 colonies plated on TSA
18-24 h / 37 °C
up to 10 colonies each, plated on TSA
18-24 h / 37 °C
Day 4
CFUs of E. coli / g of meat
PCR targets for confluent growth and pool screening
EPEC (eae, bfpA), STEC (stx1, stx2), EAEC (aaiC, aggR), ExPEC status (papAH, sfa/focDE, afa/draBC, kpsM II and iutA), UPEC status (chuA, fyuA, vat and yfcV), rfbO25b, blaTEM, blaCTX-M, blaSHV, blaCTX-M Group1 and blaCTX-M Group9 , mcr genes (1 to 5)
Day 5
Individual PCR of positive targets + uidA for detection of positive isolates
uidA negative strains: MALDI TOFF identification
Storage for further characterisation

Figure 2



Target	Primers	Nucleotide sequence (5´- 3´)	Size (bp)	Reference
iutA	iutA-Al f	GCCGGAGCTGTCTCCGGCGG	441	This study
	aer-1152 r	CGTCGGGAACGGGTAGAATCG		Johnson *et al.* 1997
kpsM II	KpsII f	GCGCATTTGCTGATACTGTTG	272	Johnson & Stell 2000
	KpsII r	CATCCAGACGATAAGCATGAGCA		

Figure 3


Phylogroup B1
Phylogroup A
Phylogroup B1
Phylogroup A
Phylogroup C
Phylogroup B1
Phylogroup A
Phylogroup E
Phylogroup B2
Phylogroup D
Phylogroup E
Phylogroup D
Phylogroup F
Clade I

Supplementary Material

Microbiological risk assessment of turkey and chicken meat for consumers: significant differences regarding multidrug resistance, *mcr* or presence of hybrid aEPEC/ExPEC pathotypes of *E. coli*

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Table S1. Sampling date, type and packaging system of the 100 meat products and points of sale

Sampling batch	Sampling date	Meat type (no. of samples)	Packaging system (C=chicken; T=turkey)	[a] Points of sale
1	05/09/2016	Chicken (10)	5 modified atmosphere 5 freshly cut	A, B, C, D, E
2	20/09/2016	Turkey (8)	4 modified atmosphere 4 freshly cut	B, C, E, F, G
3	19/10/2016	Chicken (2)	2 freshly cut	H
4	02/11/2016	Chicken (4) Turkey (4)	4 modified atmosphere (2 C + 2 T) 4 freshly cut (2 C + 2 T)	C, D, E
5	22/11/2016	Chicken (4) Turkey (4)	4 modified atmosphere (2 C + 2 T) 4 freshly cut (2 C + 2 T)	A, B, C, G
6	13/03/2017	Chicken (4) Turkey (4)	4 modified atmosphere (2 C + 2 T) 4 freshly cut (2 C + 2 T)	B, C, E, G
7	03/04/2017	Chicken (4) Turkey (4)	4 modified atmosphere (2 C + 2 T) 4 freshly cut (2 C + 2 T)	B, C D, E, G
8	24/04/2017	Chicken (4) Turkey (4)	4 modified atmosphere (2 C + 2 T) 4 freshly cut (2 C + 2 T)	A, B, D, E
9	22/05/2017	Chicken (4) Turkey (4)	4 modified atmosphere (2 C + 2 T) 4 freshly cut (2 C + 2 T)	B, C, E, G
10	08/06/2017	Chicken (4) Turkey (4)	4 modified atmosphere (2 C + 2 T) 4 freshly cut (2 C + 2 T)	B, D, E, G
11	27/06/2017	Chicken (4) Turkey (4)	4 modified atmosphere (2 C + 2 T) 4 freshly cut (2 C + 2 T)	B, C, E, G
12	04/09/2017	Chicken (6) Turkey (4)	5 modified atmosphere (3 C + 2 T) 5 freshly cut (3 C + 2 T)	C, D, E
13	18/09/2017	Turkey (6)	3 modified atmosphere 3 freshly cut	D, E, B, C

[a] Points of sale: A to F (supermarket chains); G, H (local retailers).

Table S2. Primers and targets associated with diarrheagenic and extraintestinal pathotypes of *E. coli*

Pathotype	Target	Primers	Nucleotide sequence (5´- 3´)	Size (bp)	Reference
STEC	*stx*$_1$	VT1-F	TCGCTGAATGTCATTCGCTCTGC	539	Mora et al., 2011
		VT1-R	TCAGCAGTCATTACATAAGAAC		
	stx$_2$	VT2-F1	TTTCTTCGGTATCCTATTCCC	358	Mora et al., 2011
		VT2-F2	TGTCTTCAGCATCTTATGCAG		
		VT2-R	CTGCTGTCCGTTGTCATGGAA		
STEC EPEC	*eae*	EAE-V3F	CATTGATCAGGATTTTTCTGGT	510	Mora et al., 2011
		EAE-MBR	TCCAGAATAATATTGTTATTACG		
	eae	[c]EAE-R11	TCTTCGGAGGGTTTTTTATT	1125	Alonso et al., 2017
		[c]EAE-FBN	CAGGTCGTCGTGTCTGCTAAAAC		
	eae	[c]EAE-R12	CCAGACGAATATATACATATTC	1181	Alonso et al., 2017
		[c]EAE-FBN	CAGGTCGTCGTGTCTGCTAAAAC		
tEPEC	*bfpA*	BFP-NF1	ATGGTTTCTAAAATCATGAATAAG	262	Bennett, 2003
		BFP-NR1	ATTATTCCGGAATTGCAGATGTGT		García-Meniño et al. 2018
EAEC	*aaiC*	aaiC-F	TGGTGACTACTTTGATGGACATTGT	313	Boisen et al. 2012
		aaiC-F	GACACTCTCTTCTGGGGTAAACGA		
	aggR	aggR-F	GCAATCAGATTAARCAGCGATACA	426	Boisen et al. 2012
		aggR-R	CATTCTTGATTGCATAAGGATCTGG		
ExPEC	*fimH*	FimH-f	TGCAGAACGGATAAGCCGTGG	508	Johnson and Stell, 2000
		FimH-r	GCAGTCACCTGCCCTCCGGTA		
	fimAv$_{MT78}$	fimA201	TCTGGCTGATACTACACC	266	Marc and Dho-Moulin, 1996
		fimA215	ACTTTAGGATGAGTACTG		
	papC	Forward	GTGGCAGTATGAGTAATGACCGTTA	205	Johnson et al., 2015
		Reverse	ATATCCTTTCTGCAGGGATGCAATA		
	[a]*papAH*	papA-F	ATGGCAGTGGTGTCTTTTGGTG	720	Johnson and Stell, 2000
		papA-R	CGTCCCACCATACGTGCTCTTC		
	papEF	PapEF-F	GCAACAGCAACGCTGGTTGCATCAT	336	Yamamoto et al., 1995
		PapEF-R	AGAGAGAGCCACTCTTATACGGACA		
	papG I	pap-I F	TTAGCTGGATGGCACAATG	335	Mora et al., 2013
		pap-I R	TTGTCCATGTATCCCATTCAT		
	papG II	pap-II F	GGGCATTGCTACGGTAACCTG	545	Mora et al., 2013
		pap-II R	CGCTATTAATAGACAGATCACC		
	papG III	pap-III F	CGGCAACTTTAAGCTATGTG	720	Mora et al., 2013
		pap-III R	TGTACCATCTCATCGTTGTCTC		
	[a]*sfa/focDE*	sfa1	CTCCGGAGAACTGGGTGCATCTTAC	410	Le Bouguenec et al., 1992
		sfa2	CGGAGGAGTAATTACAAACCTGGCA		
	[a]*afa/draBC*	afa1	GCTGGGCAGCAAACTGATAACTCTC	750	Le Bouguenec et al., 1992
		afa2	CATCAAGCTGTTTGTTCGTCCGCCG		
	afaFM955459	AFA-O25F	GAGTCACGGCAGTCGCGGCGG	207	Blanco et al., 2009
		AFA-O25R	TTCACCGGCGCACAGCCATCTCC		
	cnf1	cnf1-f2	CAGGAGGTACTTAGCAGCGT	468	Mora et al., 2013
		cnf1-rc	TAATTTTGGGTTTGTATC		
	cdtB	cdt-s1	GAAAGTAAATGGAATATAAATGTCCG	466	Tóth et al., 2003
		cdt-as1	AAATCACCAAGAATCATCCAGTTA		
		cdt-s2	GAAAATAAATGGAACACACATGTCCG		
		cdt-as2	AAATCTCCTGCAATCATCCAGTTA		
	sat	SatF	GCAGCTACCGCAATAGGAGGT	937	Johnson et al., 2003a
		SatR	CATTCAGAGTACCGGGGCCTA		
	hlyA	hly F	AACAAGGATAAGCACTGTTCTGGCT	1177	Yamamoto et al., 1995
		hly R	ACCATATAAGCGGTCATTCCCGTCA		
	hlyF	hlyF f	TCGTTTAGGGTGCTTACCTTCAAC	444	Morales et al., 2004
		hlyF r	TTTGGCGGTTTAGGCATTCC		
	iucD	Aer F	TACCGGATTGTCATATGCAGACCGT	602	Yamamoto et al., 1995
		Aer R	AATATCTTCCTCCAGTCCGGAGAAG		
	iroN	Ironec-F	AAGTCAAAGCAGGGGTTGCCCG	665	Johnson et al., 2000
		Ironec-R	GACGCCGACATTAAGACGCAG		
	[a]*iutA*	iutA-Ale-R	GCCGGAGCTGTCTCCGGCGG	441	Johnson et al., 1997
		aer-1152R	CGTCGGGAACGGGTAGAATCG		
	[a]*kpsM II*	KpsII f	GCGCATTTGCTGATACTGTTG	272	Johnson and Stell, 2000
		KpsII r	CATCCAGACGATAAGCATGAGCA		
	kpsM II-K2	KpsII f	GCGCATTTGCTGATACTGTTG	570	Johnson and O'Bryan, 2004
		KpsII-K2r	AGGTAGTTCAGACTCACACCT		

Pathotype	Target	Primers	Nucleotide sequence (5´- 3´)	Size (bp)	Reference
	kpsM II-K5	K5-f	CAGTATCAGCAATCGTTCTGTA	159	Johnson and Stell, 2000
		KpsII r	CATCCAGACGATAAGCATGAGCA		
	neuC (K1)	neu1	AGGTGAAAAGCCTGGTAGTGTG	676	Moulin-Schouleur et al., 2006
		neu2	GGTGGTACATCCCGGGATGTC		
	kpsM III	KpsIII f	TCCTCTTGCTACTATTCCCCCT	392	Johnson and Stell, 2000
		KpsIII r	AGGCGTATCCATCCCTCCTAAC		
	cvaC	ColV-CF	CACACACAAACGGGAGCTGTT	680	Johnson and Stell, 2000
		ColV-CR	CTTCCCGCAGCATAGTTCCAT		
	traT	TraT f	GGTGTGGTGCGATGAGCACAG	290	Johnson and Stell, 2000
		TraT r	CACGGTTCAGCCATCCCTGAG		
	ibeA	ibe10 f	AGGCAGGTGTGCGCCGCGTAC	170	Johnson and Stell, 2000
		ibe10 r	TGGTGCTCCGGCAAACCATGC		
	malX	MALX-F	GCATGAGCAGTGCGATACATCGC	828	Mora et al., 2013
		MALX-R	AGGGCTGGGAAGTGGTTTAGCC		
	usp	usp-F	ACATTCACGGCAAGCCTCAG	440	Bauer et al., 2002
		usp-R	AGCGAGTTCCTGGTGAAAGC		
	ompT	ompT-F	ATCTAGCCGAAGAAGGAGGC	559	Johnson, et al. 2015
		ompT-R	CCCGGGTCATAGTGTTCATC		
	tsh	tsh03	GGTGGTGCACTGGAGTGG	640	Dozois et al., 2000
		tsh15	AGTCCAGCGTGATAGTGG		
	[b]*vat*	vat-F	TCAGGACACGTTCAGGCATTCAGT	1100	Spurbeck et al., 2012
		vat-R	GGCCAGAACATTTGCTCCCTTGTT		
	[b]*fyuA*	fyuA-F	GTAAACAATCTTCCCGCTCGGCAT	850	Spurbeck et al., 2012
		fyuA-R	TGACGATTAACGAACCGGAAGGGA		
	[b]*yfcV*	yfcV-F	ACATGGAGACCACGTTCACC	292	Spurbeck et al., 2012
		yfcV-R	GTAATCTGGAATGTGGTCAGG		
	[b]*chuA*	ChuA-F	CTGAAACCATGACCGTTACG	652	Spurbeck et al., 2012
		ChuA-R	TTGTAGTAACGCACTAAACC		
	rfbO25b	rfb. 1bis.f	ATACCGACGACGCCGATCTG	300	Clermont et al., 2008
		rfbO25b.r	TGCTATTCATTATGCGCAGC		
E. coli ID	*uid*A	uidA-F	GCGTCTGTTGACTGGCAGGTGGTGG	503	Gómez-Duarte et al., 2010
		uidA-R	GTTGCCCGCTTCGAAACCAATGCCT		

[a] Virulence factors (VF) screened to assess the extraintestinal pathogenic *E. coli* status (ExPEC status).

[b] VF screened to assess the uropathogenic *E. coli* status (UPEC status).

[c] Primers used for the *eae* typing (sequencing). Those isolates exhibiting ExPEC and /or UPEC status, were further characterized for other extraintestinal VF: *fimAv*$_{MT78}$, *papEF*, *papC*, *papG I*, *papG II* and *papG III*, *cnf1*, *cdtB*, *sat*, *hlyA*, *hlyF*, *iucD*, *iroN*, *kpsM II* (establishing neuC-K1, K2 and K5 variants), *kpsM III*, *cvaC*, *iss*, *traT*, *ibeA*, *malX*, *usp*, *tsh* and *ompT*.

Table S3. Targets and primers to determine sequence types and clonotypes of *E. coli*

Target	Primers	Nucleotide sequence (5´- 3´)	Locus size (bp)	Reference
adk	adkF	ATTCTGCTTGGCGCTCCGGG	536	Wirth et al., 2006
	adkR	CCGTCAACTTTCGCGTATTT		
fumC	fumCF	TCACAGGTCGCCAGCGCTTC	469	
	fumCR	GTACGCAGCGAAAAAGATTC		
gyrB	gyrBF	TCGGCGACACGGATGACGGC	460	
	gyrBR	ATCAGGCCTTCACGCGCATC		
icd	icdF	ATGGAAAGTAAAGTAGTTGTTCCGGCACA	518	
	icdR	GGACGCAGCAGGATCTGTT		
mdh	mdhF	ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG	452	
	mdhR	TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT		
purA	purAF	CGCGCTGATGAAAGAGATGA	478	
	purAR	CATACGGTAAGCCACGCAGA		
recA	recAR1	AGCGTGAAGGTAAAACCTGTG	510	
	recAF1	ACCTTTGTAGCTGTACCACG		
Clonotyping				
fimH	fimH-F	CACTCAGGGAACCATTCAGGCA	locus size 469	Weissman et al., 2012
	fimH-R	CTTATTGATAAACAAAAGTCAC		

The STs were assigned through the Enterobase website for *E. coli* (http://enterobase.warwick.ac.uk/species/ecoli/allele_st_search). Allele assignments for *fimH* were determined using the FimTyper database available at the Center for Genomic Epidemiology (CGE) website https://bitbucket.org/genomicepidemiology/fimtyper_db/downloads/, and the combination of *fumC* and *fimH* allele designations determined the CH "type".

Table S4. Primers used for the detection and typing of *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{CIT}$ and *mcr* genes

Target	Primers	Nucleotide sequence (5´- 3´)	Size (bp)	Reference
bla$_{CTX-M}$	CTX-C3	ATGTGCAGCACCAGTAAAGTGATG	542	Mora et al., 2013
	CTX-C4	ACCGCGATATCGTTGGTGGTGCC		
bla$_{CTX-M}$ group1	M13U	GGTTAAAAAATCACTGCGTC	863	Saladin et al., 2002
	M13L	TTGGTGACGATTTTAGCCGC		
bla$_{CTX-M}$ group9	[a]CTX-M9-F	GTGACAAAGAGAGTGCAACGG	856	Simarro et al., 2000
	[a]CTX-M9-R	ATGATTCTCGCCGCTGAAGCC		
bla$_{CTX-M}$ group9	[b]CTX-M9-14-14B-24F	GAATACTGATGTAACACGGA	998	García-Meniño et al. 2018
	[b]CTX-M9-R	AGCTGAAGATGTATATCAAG		
bla$_{CTX-M}$ group9	[b]CTX-M9-14-14B-24F	GAATACTGATGTAACACGGA	989	García-Meniño et al. 2018
	[b]CTX-M14-24-R	CTGCGTTGTCGGGAAGATACG		
bla$_{CTX-M}$ group9	[b]CTX-M9-14B-F	CCTATACCCGAGGCGCGACAG	1059	García-Meniño et al. 2018
	[b]CTX-M9-R	AGCTGAAGATGTATATCAAG		
bla$_{CTX-M}$ group9	[b]CTX-M14-24-F	CTAAATTCTTCGTGAAATAGTG	1049	García-Meniño et al. 2018
	[b]CTX-M14-24-R	CTGCGTTGTCGGGAAGATACG		
bla$_{SHV}$	SHV-F2	TTGTCGCTTCTTTACTCGCC	879	Mora et al., 2013
	SHV-R2	CCCGGCGATTTGCTGATTTCGC		
bla$_{SHV}$	[b]SHV-1	GGGTTATTCTTATTTGTCGC	930	Rasheed et al., 1997
	[b]SHV-2	TTAGCGTTGCCAGTGCTC		
bla$_{TEM}$	[a]TEM-1-F	ATGAGTATTCAACATTTCCG	868	Rasheed et al., 1997
	[a]TEM-1-R	CTGACAGTTACCAATGCTTA		
LAT-1 a LAT-4, CMY-2 a CMY-7, BIL-1	CITMF	TGGCCAGAACTGACAGGCAAA	462	Pérez-Pérez FJ et al. 2002
	CITMR	TTTCTCCTGAACGTGGCTGGC		
CMY-2	[b]CMY-2F	AACACACTGATTGCGTCTGAC	1226	Pérez-Pérez FJ et al. 2002
	[b]CMY-2R	CTGGGCCTCATCGTCAGTTA		
mcr-1	CLR5-F	CGGTCAGTCCGTTTGTTC	309	Liu et al., 2016
	CLR5-R	CTTGGTCGGTCTGTAGGG		
mcr-2	mcr-2 IF	TGTTGCTTGTGCCGATTGGA	567	Xavier et al., 2016
	mcr-2 IR	AGATGGTATTGTTGGTTGCTG		
mcr-3	MCR3-F	TTG GCACTGTATTTTGCATTT	542	Yin et al., 2017
	MCR3-R	TTAACGAAATTGGCTGGAACA		
mcr-4	mcr-4 FW	ATTGGGATAGTCGCCTTTTT	487	Carattoli et al., 2017
	mcr-4 RV	TTACAGCCAGAATCATTATCA		
mcr-5	MCR5_FW	ATGCGGTTGTCTGCATTTATC	1644	Borowiak et al., 2017
	MCR5_RV	TCATTGTGGTTGTCCTTTTCTG		
mcr-1	[b]mcrS1-F	GGGATTGCGCAATGATTGC	548	García-Meniño et al., 2018
	[b]mcrS1-R	CACCCAAACCAATGATACG		

[a] Primers used for amplification and sequencing. [b] Primers used for sequencing.

Table S5. No. of positive isolates and positive samples regarding virulence traits and protocols

Virulence traits	**Protocol I + II** (ML + MSTC 37 ºC) No. positive /total: [a]isolates (%); [b]samples (%)	**Protocol V (**CHROMID® 37 ºC) No. positive /total: [a]isolates (%); [b]samples (%)
[1]**ExPEC status** N = 150 from 78 meat samples	[a] 118/150 (78.7); [b] 71/78 (91)	
[2]**UPEC status** N = 83 from 53 meat samples	[a] 69/83 (83.1); [b] 47/53 (88.7)	
[3]**ESBL/AmpC producer** N = 155 from 78 meat samples		[a] 137/155 (88.4); [b] 76/78 (97.4)
[4]***mcr-1* carrier** N = 13 from 7 meat samples	[a] 10/13 (76.9); [b] 6/7 (85.7)	
[5]**MDR** N = 253 from 88 meat samples	[a] 100/253 (39.5); [b] 59/88 (67)	[a] 137/253 (54.1); [b] 76/88 (87.5)
[6]***rbfO25b*** N = 13 from 10 meat samples	[a] 12/13 (92.3); [b] 9/10 (90)	

This table shows only the results for the protocol(s) of election in relation to each virulence trait. [1] No. of isolates conforming ExPEC status (Johnson *et al*., 2003b). [2] No. of isolates conforming status UPEC (Spurbeck *et al*., 2012). [3] No. of extended-spectrum beta-lactamase (ESBL) or AmpC-beta-lactamase (pAmpC)-producing *E. coli*. [4] No. of isolates carriers of the *mcr-1* gene. [5] No. of MDR isolates according to Magiorakos *et al*. (2012) criteria. [6] No. of *rbfO25b*-positive isolates: O25b subtype associated with the clonal group ST131 screened by PCR (Clermont *et al*., 2008).

Table S6. STs and clonotypes of 272 *E. coli* isolates

adk	*fumC*	*gyrB*	*icd*	*mdh*	*purA*	*recA*	[1]CC	[2]ST	[3]Clonotype	No. isolates and origin (C=chicken; T=turkey)
10	11	4	8	8	8	2	10	10	11-23	1T
10	11	4	8	8	8	2	10	10	11-24	3 (1C + 2T)
10	11	4	8	8	8	2	10	**10**	**11-54**	**12 (8C + 4T)**
10	11	4	8	8	8	2	10	10	11-122	1C
10	11	4	8	8	8	2	10	10	11-neg	2C
10	11	4	1	8	8	2	10	34	11-neg	2T
6	11	4	8	8	8	2	10	48	11-23	1C
6	11	4	8	8	8	2	10	48	11-41	2 (1C + 1T)
6	11	4	8	8	8	2	10	48	11-400	1T
10	11	4	8	8	13	2	10	167	11-neg	1T
10	11	4	8	8	13	73	10	617	11-neg	2 (1C + 1T)
10	11	135	8	8	8	2	10	744	11-54	2T
10	11	135	8	8	8	2	10	744	11-58	1C
10	11	4	8	8	8	49	10	**752**	**11-24**	**9C**
10	11	4	8	20	8	2	10	853	11-54	3T
10	11	183	8	8	8	2	10	1141	11-32	1T
10	11	57	8	8	8	185	10	1970	11-54	1C
6	4	12	1	20	13	7	23	23	4-35	1C
6	4	12	1	20	12	7	23	**88**	**4-39**	**4T**
6	4	12	1	20	18	7	23	410	4-24	1C
18	3	17	6	5	5	4	31	**1158**	**3-47**	**6C**
18	3	32	6	5	5	4	31	STnew8 (ST1158-like)	3-47	1C
4	26	2	25	5	5	19	38	38	26-65	1T
4	26	39	25	5	31	19	38	**115**	**26-270**	**7 (4C + 3T)**
21	35	27	6	5	5	4	69	**69**	**35-27**	**4 (2C + 2T)**
36	24	10	13	17	10	25	73	**355**	**24-154**	**7 (3C + 4T)**
36	24	9	13	17	11	159	73	1618	24-9	2C
99	6	33	33	24	8	7	86	453	6-31	2T
9	6	33	131	24	8	7	86	641	6-25	2C
9	270	33	131	24	8	7	86	1720	270-54	1T
37	38	19	37	17	11	26	95	**95**	**38-27**	**6 (4C + 2T)**
37	38	19	37	17	11	26	95	95	38-30	2T
55	38	19	37	17	11	26	95	140	38-15	3T
43	41	15	18	11	7	6	101	101	41-86	3 (1C + 2T)
43	41	15	90	11	8	6	101	359	41-35	1T
6	4	14	16	11	8	6	115	10328	4-32	1C
53	40	47	13	36	28	29	131	**131**	**40-22**	**8 (5C + 3T)**
53	40	47	13	36	28	29	131	**131**	**40-neg**	**4C**
6	4	4	16	24	8	14	155	58	4-27	1T
6	4	14	16	24	8	14	155	**155**	**4-32**	**7 (3C + 4T)**
6	4	14	16	24	8	14	155	155	4-121	1C

6	4	14	16	24	8	14	155	155	4-neg	3T
6	4	14	16	7	8	14	155	1016	4-32	1C
6	4	14	16	24	2	14	155	4162	4-38	1C
10	27	5	10	12	8	49	165	189	27-neg	2 (1C + 1T)
6	11	4	10	7	8	6	168	**93**	**11-41**	**4 (3C + 1T)**
6	11	4	10	7	8	6	168	93	11-47	1T
6	11	4	10	7	8	6	168	93	11-58	1C
6	11	4	10	7	8	6	168	**93**	**11-neg**	**11 (6C + 5T)**
6	11	4	10	7	84	6	168	484	11-neg	1T
6	11	4	234	7	8	6	168	1594	11-31	1C
6	482	4	10	7	8	6	168	3764	482-41	1T
10	27	5	8	8	7	2	226	226	27-41	2C
6	31	5	28	1	1	2	350	57	31-27	2 (1C + 1T)
6	31	5	28	1	1	2	350	57	31-31	1T
6	31	83	28	1	1	2	350	350	31-54	2T
6	31	83	28	1	1	new	350	STnew4 (ST350-like)	31-54	1T
85	88	78	29	59	58	62	354	354	88-58	3T
85	88	78	29	59	58	62	354	354	88-neg	1T
6	19	33	26	11	8	6	446	602	19-86	3T
9	65	5	1	9	13	6	469	**162**	**65-32**	**4 (2C + 2T)**
10	23	109	8	8	8	2	522	522	23-neg	1T
92	4	87	96	70	58	2	648	**648**	**4-58**	**6 (2C + 4T)**
92	231	87	96	70	58	2	648	**1485**	**231-58**	**19 (10C + 9T)**
6	new	4	16	7	13	2	None	STnew6	new-1319	2C
13	new	19	13	23	28	109	None	STnew9	new-664	1T
6	7	57	1	new	8	2	None	STnew7	7-54	1T
20	45	41	43	5	32	2	None	**117**	**45-97**	**8 (5C + 3T)**
20	45	41	43	5	32	2	None	117	45-151	1C
13	39	50	13	16	37	25	None	135	39-2	2C
13	52	10	14	17	25	17	None	141	52-14	1T
6	29	4	18	11	8	6	None	212	29-38	1C
6	65	32	26	9	8	2	None	297	65-38	1C
6	65	32	26	5	8	2	None	STnew10 (ST297-like)	65-276	1T
62	100	17	31	5	5	4	None	362	100-96	1T
6	4	15	1	22	8	7	None	366	4-30	2T
96	40	13	100	23	28	66	None	428	40-22	2 (1C + 1T)
96	40	13	100	23	28	66	None	**428**	**40-neg**	**4C**
96	40	93	13	23	28	66	None	429	40-20	2C
101	88	97	108	26	79	2	None	457	88-145	1T
13	38	84	13	17	64	34	None	569	38-5	1T
122	11	125	12	96	8	2	None	665	11-30	1C
122	11	125	12	8	8	2	None	STnew3 (ST665-like)	11-30	1C
6	41	33	18	9	8	6	None	707	41-60	1C
52	116	55	101	113	40	38	None	**770**	**116-552**	**7 (5C + 2T)**

6	4	3	16	11	8	6	None	906	4-61	1T
38	24	84	13	17	30	34	None	919	24-187	2T
83	23	155	170	133	1	2	None	997	23-31	1T
6	4	159	44	112	1	17	None	1011	4-31	2 (1C + 1T)
10	252	5	8	7	8	2	None	1564	252-neg	1T
101	88	97	108	7	13	2	None	1674	88-138	1T
10	168	4	8	12	35	2	None	1785	168-54	1T
18	22	67	31	5	5	4	None	1882	22-123	1T
36	43	19	13	16	10	25	None	2557	43-225	2C
267	6	5	26	9	13	98	None	2599	6-32	1T
31	276	83	140	1	187	19	None	2614	276-108	1C
6	8	32	159	9	23	7	None	3519	8-31	1C
6	8	32	159	9	23	7	None	3519	8-39	1C
79	3	206	451	5	16	182	None	**4243**	**3-1002**	**5 (4C + 1T)**
410	153	118	83	7	8	6	None	4980	153-39	2C
52	116	55	101	113	31	38	None	4994	116-270	1C
443	271	24	198	7	214	359	None	5340	271-58	1T
9	7	1	8	24	8	7	None	6215	7-34	1T
96	925	13	100	23	28	66	None	6876	925-neg	1T
136	11	4	1	9	18	7	None	7315	11-398	1T
88	24	19	36	17	11	91	None	8611	24-26	2T
76	1544	19	89	17	1	10	None	**10740**	**1544-9**	**5 (4C + 1T)**
101	88	97	108	26	79	2	None	457	88-145	1C
10	23	4	8	571	1	2	None	STnew11	23-823	1C

[1] Clonal complexes (CC) and [2] Sequence types (ST) according to the Achtman scheme (Wirth et al., 2006): STnew was assigned to allelic combinations not found in Enterobase, or to those including a new allele within the 7 gene; ST-like indicates one nucleotide of difference with the original ST. [2] Clonotype (CH) based on the internal 469-nucleotide (nt) and 489-nt sequence of the *fumC* (allele obtained from MLST) and *fimH* genes, respectively (Weissman et al., 2012); neg: negative result for the amplification of the 489-nt internal sequence.

Table S7. Characterization of the 13 *mcr-1* isolates recovered from seven meat samples

E. coli **counts** [1]**cfu /g**	[2]**Sample code**	**Protocol (code of isolate)**	[3] **Clones**	**ESBL /*mcr* typing**	**MIC value for colistin**	[4] **Resistance profile**	**Virulence profile**
30	Ch9	II (a)	O18ac:H49-B1-ST212 (CH29-38)	***mcr1.1***	4 µg/mL	AMP, AMC*, GEN, TOB*, CST, DOX, CHL, NIT*, SXT, CIP, NAL	*fimH38 traT*
510	T1	IV (a)	O109:H51-B1-ST155-CC155 (CH4-32)	***mcr1.1***	4 µg/mL	AMP, AMC*, GEN, DOX, CHL, CIP, NAL	*fimH32 hlyF iroN traT iss*
	T1	V (b)	O162/O89:H9-A-ST744-CC10 (CH11-54)	**SHV-12** / ***mcr1.1***	4 µg/mL	AMP, CXM*, CAZ, CTX, ATM, CST, DOX, CHL, SXT, CIP, NAL	*fimH54 fimAvMT78 hlyF iucD iutA iroN cvaC traT tsh iss*
210	T13	II (a)	O105:H32-A-ST10-CC10 (CH11-23)	***mcr1.1***	4 µg/mL	AMP, AMC*, DOX, CHL, CIP*, NAL	*fimH23 hlyF iroN ompT iss*
	T13	II (b)	O15:H6-D-ST69-CC69 (CH35-27)	***mcr1.1***	4 µg/mL	AMP, DOX, CHL, NAL	*fimH27 hlyF iroN traT ompT iss chuA fyuA*
100	T16	II (a)	O21:HAA-B1-ST101-CC101 (CH41-86)	***mcr1.1***	4 µg/mL	AMP, DOX, CHL, SXT, CIP*	*fimH86 traT ompT*
	T16	IV (b)	O21:H21-B1-ST101-CC101 (CH41-86)	***mcr1.1***	4 µg/mL	AMP, CST, DOX, CHL, SXT, CIP*	*fimH86 traT ompT*
60	T17	I (a)	O162/O89:H37-A-ST853-CC10 (CH11-54)	***mcr1.1***	4 µg/mL	AMP, CST, DOX*, SXT	*fimH54 fimAvMT78 hlyF iucD iutA iroN traT ompT iss*
	T17	I (b)	O101:HNM-A-ST853-CC10 (CH11-54)	***mcr1.1***	4 µg/mL	AMP, CST, DOX*, SXT	*fimH54 fimAvMT78 hlyF iucD iutA iroN traT ompT iss*
	T17	I (c)	O101:HNM-A-ST853-CC10 (CH11-54)	***mcr1.1***	4 µg/mL	AMP, CST, DOX*, SXT	*fimH54 fimAvMT78 hlyF iucD iutA iroN traT ompT iss*
150	T24	II (a)	O50/O2:H5-B2-ST140-CC95 (CH38-15)	***mcr1.1***	4 µg/mL	AMP, GEN, TOB*, CHL, CIP*	*fimH15 fimAvMT78 hlyF iucD iutA kpsM II-K1 traT ibeA malX usp ompT chuA vat fyuA yfcV*
	T24	II (b)	O50/O2:H5-B2-ST140-CC95 (CH38-15)	***mcr1.1***	2 µg/mL	AMP, GEN, TOB, CHL, CIP*	*fimH15 fimAvMT78 hlyF iucD iutA kpsM II-K1 traT ibeA malX usp ompT chuA vat fyuA yfcV*
350	T31	I (a)	O148:H30-A-ST522-CC522 (CH23-neg)	***mcr1.1***	1 µg/mL	AMP, AMC*, CFZ, DOX, NIT, CIP*, NAL*	*hlyF iroN traT*

[1] CFU: colony forming units. [2] Ch (chicken meat), T (turkey meat).[3] Clone defined as combination of serotype-filogroup-Sequence Type-Clonal Complex (Clonotype); "neg" when PCR was negative for the 489-nt internal sequence amplification of the *fimH* gene (Weissman et al., 2012). [4] Antimicrobial susceptibility tested by disc diffusion assay and interpreted according to the CLSI standard breakpoints (Clinical and Laboratory Standards Institute, 2020): ampicillin (AMP), amoxicillin-clavulanic acid (AMC), cefuroxime (CXM), ceftazidime (CAZ), cefotaxime (CTX), cefoxitin (FOX), aztreonam (ATM), gentamicin (GEN), tobramycin (TOB), colistin (CST), doxycycline (DOX), chloramphenicol (CHL), nitrofurantoin (NIT), sulfamethoxazole-trimethoprim (SXT), ciprofloxacin (CIP), nalidixic acid (NAL). Intermediate values are indicated with *.

Table S8. Characterization of the 13 *rbfO25b* isolates recovered from 10 meat samples

***E. coli* counts [1]CFU /g**	**[2]Sample code**	**Protocol code of isolate**	**[3]Clones**	**[4]Virotype ST131**	**ExPEC/UPEC status**	**[5]Resistance profile**	**Virulence profile**
30	Ch2	I (a)	O25b:H4-B2-ST131-CC131 (CH40-neg)	D4	+ / +	GEN, DOX, CIP*, NAL	*fimH hlyF iucD iutA iroN kpsM II-K1 traT ibeA malX usp tsh ompT iss chuA fyuA yfcV*
	Ch2	I (b)	O25b:H4-B2-ST131-CC131 (CH40-neg)	D4	+ / +	GEN, DOX*, CIP*, NAL	*fimH hlyF iucD iutA iroN kpsM II-K1 traT ibeA malX usp tsh ompT iss chuA fyuA yfcV*
	Ch2	I (c)	O25b:H4-B2-ST131-CC131 (CH40-neg)	D4	+ / +	CIP*, NAL	*fimH hlyF iucD iutA iroN kpsM II-K1 traT ibeA malX usp tsh ompT iss chuA fyuA yfcV*
70	Ch8	II	O25b:H4-B2-ST131-CC131 (CH40-neg)	D4	+ / +	NAL*	*fimH hlyF iucD iutA iroN kpsM II-K1 cvaC traT ibeA malX usp tsh ompT iss chuA vat fyuA yfcV*
50	Ch13	I	O25b:H4-B2-ST131-CC131 (CH40-22)	D4	+ / +	GEN*, DOX, CIP*, NAL	*fimH22 hlyF iucD iutA iroN kpsM II-K1 traT ibeA malX usp tsh ompT iss chuA fyuA yfcV*
	Ch13	II	O25b:H4-B2-ST131-CC131 (CH40-22)	D4	+ / +	GEN, DOX, CIP*, NAL	*fimH22 hlyF iucD iutA iroN kpsM II-K1 traT ibeA malX usp tsh ompT iss chuA vat fyuA yfcV*
10	Ch27	III	O25b:H45-E-ST1011 (CH4-31)	-	- / -	AMP, DOX*, SXT, CIP,	*fimH31 hlyF iroN traT tsh ompT iss chuA fyuA*
<10	Ch29	II	O25b:H4-B2-ST131-CC131 (CH40-22)	D4	+ / +	CIP*, NAL	*fimH22 hlyF iucD iutA iroN kpsM II-K1 traT ibeA malX tsh ompT iss chuA vat fyuA yfcV*
<10	Ch39	I	O25b:H4-B2-ST131-CC131 (CH40-22)	D4	+ / +	CIP*, NAL	*fimH22 hlyF iucD iutA iroN kpsM II-K1 cvaC traT ibeA malX usp tsh ompT iss chuA vat fyuA yfcV*
80	Ch50	II	O25b:H4-B2-ST131-CC131 (CH40-22)	D4	+ / +	GEN, DOX*, NAL	*fimH22 hlyF iucD iutA iroN kpsM II-K1 traT ibeA malX usp tsh ompT iss chuA vat fyuA yfcV*
20	T26	I	O25b:H4-B2-ST131-CC131 (CH40-22)	D-nt	+ / +	-	*fimH22 hlyF iucD iutA iroN kpsM II-K5 cvaC traT ibeA malX usp tsh ompT iss chuA vat fyuA yfcV*
<10	T44	II	O25b:H4-B2-ST131-CC131 (CH40-22)	D1	- / +	AMP, AMC*, DOX*,	*fimH22 cdtB kpsM II-K5 traT ibeA malX usp ompT chuA fyuA yfcV*
1130	T46	II	O25b:H4-B2-ST131-CC131 (CH40-neg)	D4	+ / +	AMP, DOX*, NAL	*fimH22 hlyF iucD iutA iroN kpsM II-K1 cvaC traT ibeA malX usp tsh ompT iss chuA fyuA yfcV*

[1] CFU: colony forming units. [2] Ch (chicken meat), T (turkey meat).[3] Clone defined as combination of serotype-filogroup-Sequence Type-Clonal Complex (Clonotype); "neg" when PCR was negative for the 489-nt internal sequence amplification of the *fimH* gene (Weissman et al., 2012). [4] Virotypes according to Dahbi et al. (2014). [5] Antimicrobial susceptibility tested by disc diffusion assay and interpreted according to the CLSI standard breakpoints (Clinical and Laboratory Standards Institute, 2020): ampicillin (AMP),

amoxicillin-clavulanic acid (AMC), gentamicin (GEN), doxycycline (DOX), sulfamethoxazole-trimethoprim (SXT), ciprofloxacin (CIP), nalidixic acid (NAL). Intermediate values are indicated with *.

Figure S1. No. of *E. coli* isolates recovered per protocol in correlation with the six virulence traits

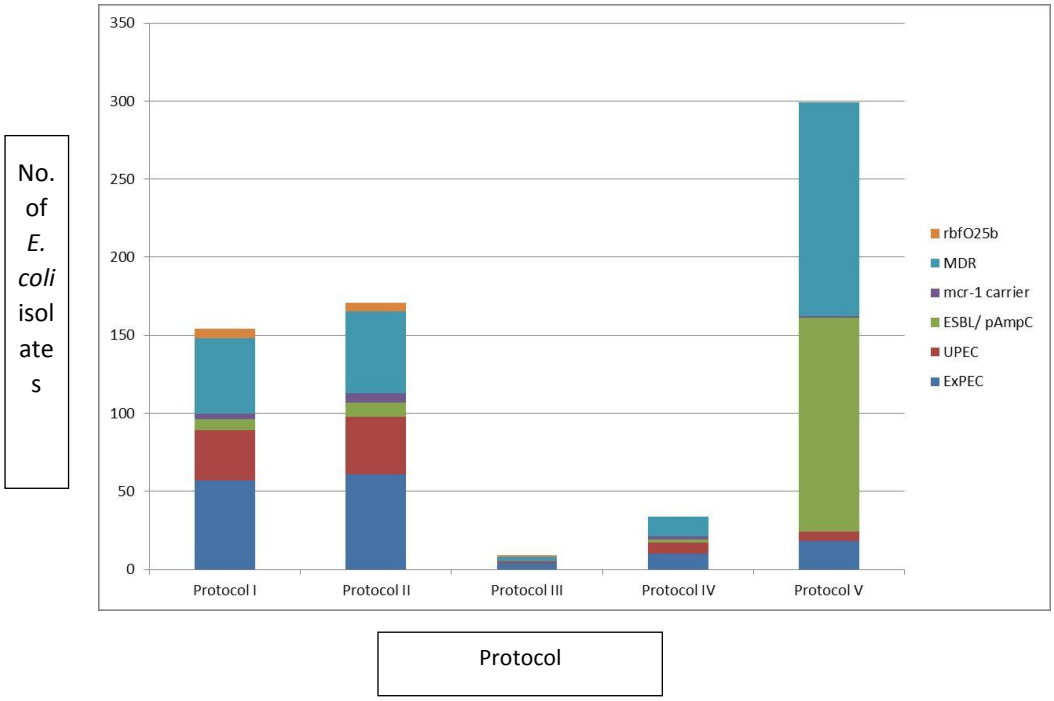


Figure S2. No. of *E. coli* isolates recovered per virulence trait in correlation with the meat origin

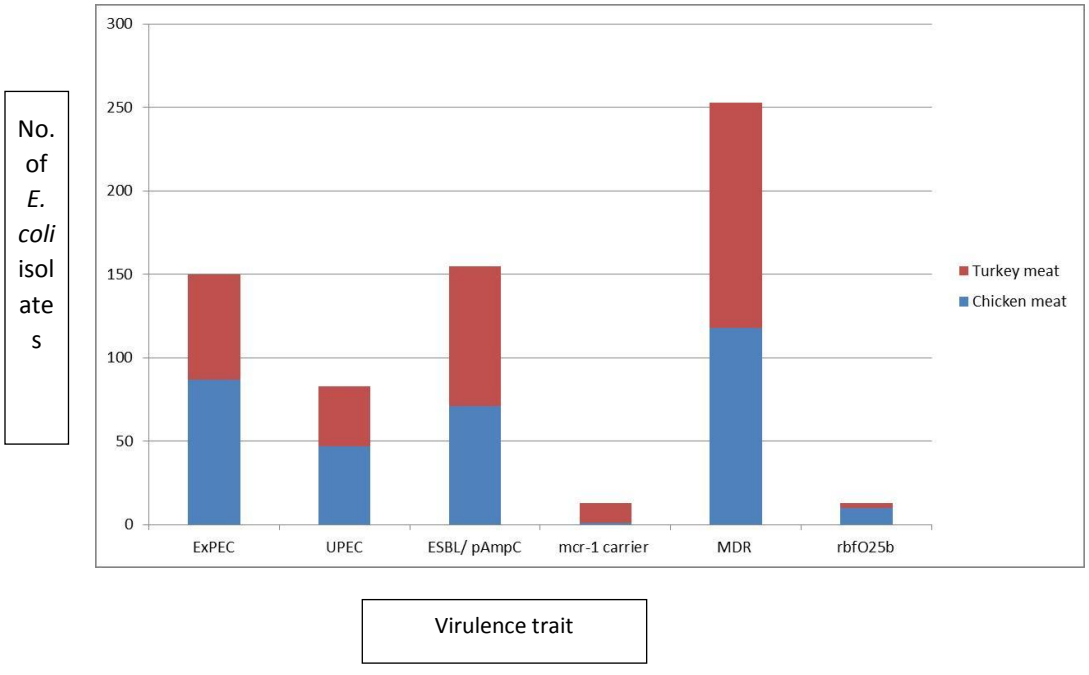


Figure S3. Proposal of optimized workflow to investigate the level of contamination, and the rates of AMR and foodborne pathogenic *E. coli*. AMR: antimicrobial resistance; ML: MacConkey Lactose agar; MSTC: MacConkey Sorbitol agar enriched with tellurite and cefixime; TSA: tryptone soy agar; CFU: colony forming units; EPEC: enteropathogenic *E. coli*; STEC: Shiga toxin-producing *E. coli*; EAEC: enteroaggregative *E. coli*; ExPEC: extraintestinal pathogenic *E. coli*.

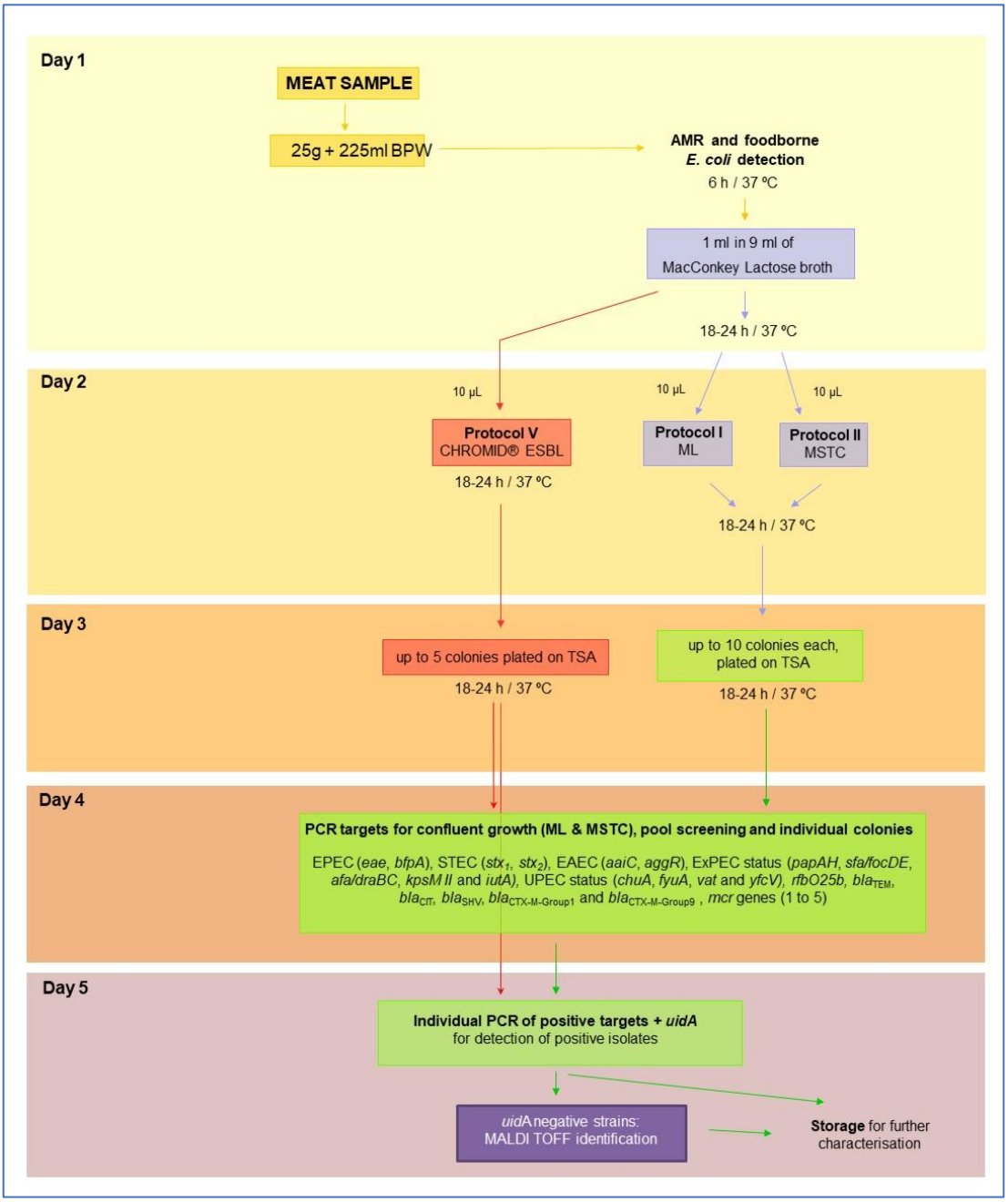
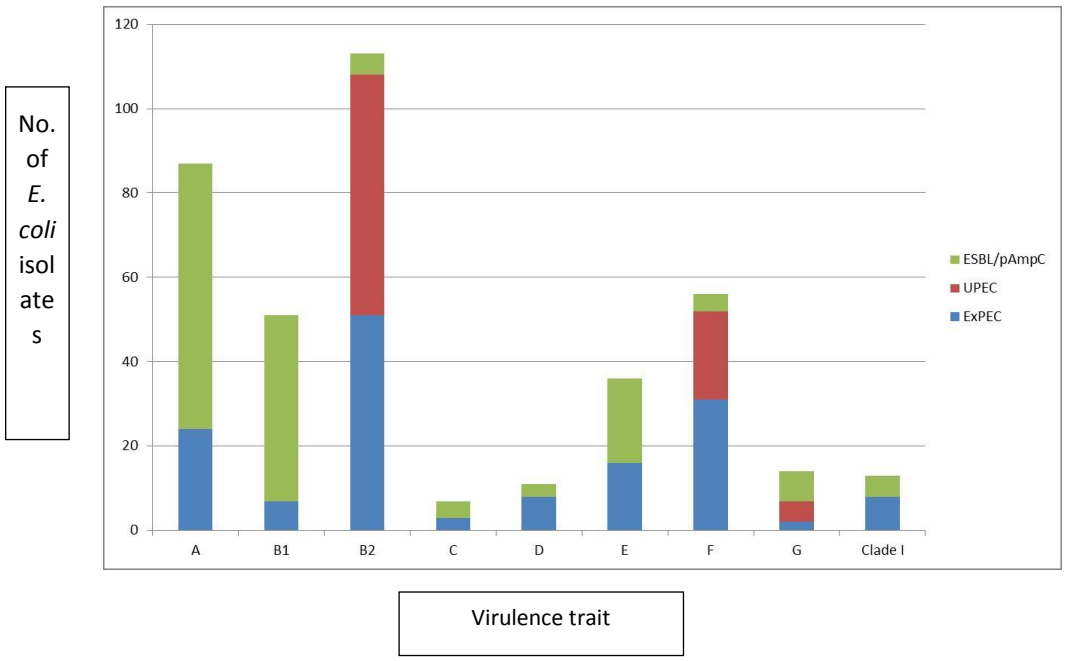


Figure S4. Phylogroup distribution within the isolates positive for the traits ESBL/pAmpC production, UPEC and ExPEC status



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Highlights

- Lab workflow for microbiological risk assessment and PCR for the screening of ExPEC
- Poultry meat is a rich source of *E. coli* (phylogroups A to G) and *Escherichia* clade I
- 73% of the poultry meat samples carried ExPEC clones identified in human isolates
- Turkey meat showed significant higher rates of *mcr*-carriage and multidrug resistance
- In a “Farm to Fork Strategy”, ExPEC should be clearly included in food surveillance

CRediT author statement

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