1 Original Research

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3 **Chicken and turkey meat: consumer exposure to multidrug-resistant**

4 **Enterobacteriaceae including *mcr*-carriers, uropathogenic *E. coli* and high-risk**

5 **lineages such as ST131**

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Abstract

This study evaluates for first time the consumer exposure via poultry meat to Enterobacteriaceae with capacity to develop severe extraintestinal infections by either bacterial virulence and/or antibiotic resistance traits. The characterization of 256 isolates and the assessment of five parameters, showed that 96 of 100 poultry meat samples from supermarkets of northwest Spain posed ≥ one potential risk: i) 96% carried Enterobacteriaceae resistant to antimicrobials of categories A (64% to monobactams) or B (95% to cephalosporins 3rd and 4rd- generation, quinolones and/or polymixins) of the new categorization of EMA. ii) More than one extended-spectrum-β-lactamase (ESBL)-producing Enterobacteriaceae species were recovered from 28% of poultry meat. iii) High-risk lineages of *E. coli*, including multidrug-resistant ST131-*H*22, were present in 62% of samples. iv) *E. coli* recovered from 25% of samples conformed the ExPEC status. v) *E. coli* from 17% satisfied the UPEC status. Of note, the recovery from different samples of two *E. coli* CC10-A (CH11-54) carrying *mcr-1.1*-bearing IncX4 plasmids, and four *E. coli* CC10-A (*eae*-beta1) of the hybrid pathotype aEPEC/ExPEC. (ESBL)-producing *K. pneumoniae* were isolated from 27% of samples. In summary, poultry meat microbiota is a source of genetically diverse Enterobacteriaceae*,* resistant to relevant antimicrobials and potentially pathogenic for consumers.

Keywords: Food safety, *Escherichia coli*, *Klebsiella pneumoniae*, poultry meat, *mcr*, ESBL, ST131, hybrid pathotype

48 # 1. Introduction

49 The hypothesis that food, particularly poultry products, can act as a reservoir for human
50 extraintestinal Enterobacteriaceae pathogens is based on scientific evidence obtained
51 from different approaches (Davis et al., 2015; Hindermann et al., 2017; Jakobsen et al.,
52 2010; Jorgensen et al., 2019; Mellata et al. 2018; Mora et al., 2009; Mora et al., 2013;
53 Riley, 2020). In fact, extraintestinal pathogenic *Escherichia coli* (ExPEC) and
54 *Klebsiella penumoniae*, which are commonly implicated in nosocomial and community
55 extraintestinal infections (Mathers et al., 2015; Zowawi et al., 2015), have been newly
56 suggested as foodborne pathogens (Riley, 2020). Within a variety of pathologies,
57 ExPEC are the most important causative agents involved in the urinary tract infections
58 (UTIs), accounting for 75-85% of the cases (Foxman, 2010).

59 On the other hand, food production animals have been identified as the main cause of
60 the antimicrobial-resistance (AMR) increase, including resistance to colistin (Cyoia et
61 al., 2018; Garcia-Menino et al., 2018). A rapid spread of extended-spectrum beta-
62 lactamases (ESBL) has occurred in the last decades, mainly due to their presence in
63 plasmids and expansion through successful clonal groups, such as the pandemic ST131
64 of *E. coli* or the ST258 of *K. pneumoniae* (Dahbi et al., 2013; Mathers et al., 2015;
65 Mora et al., 2010). Of deep concern is the potential *in vivo* acquisition of *mcr*- and
66 bla_{ESBL}-bearing plasmids by human *E. coli* isolates following treatment with colistin, or
67 via animal transmission through direct contact or the food chain (Beyrouthy et al., 2017;
68 Gilrane et al., 2017). The emerging threat of multidrug-resistant (MDR) Gram-negative
69 bacteria in urology is also worrisome (Zowawi et al., 2015). Besides the clinical
70 problem, there is high economic impact derived from healthcare costs, estimated in a
71 mean of €70 for one episode of a suspected UTI and €58 million from the societal
72 perspective, according to a recent study conducted in France (Francois et al., 2016).

73 Due to the risk of resistance development associated with their use in animals and
74 potential impact (direct or indirect) on humans, the European Medicines Agency
75 (EMA) has recently proposed a new categorization of antimicrobials,
76 (EMA/CVMP/CHMP, 2019). This proposal establishes four categories, with Category
77 A ("Avoid") including those antimicrobials not currently authorized in veterinary
78 medicine in the European Union, such as fosfomycin or monobactams; and with
79 Category B ("Restrict") including those that should be restricted in animals to mitigate
80 the risk to public health, namely, quinolones, 3rd- and 4th-generation cephalosporins
81 and polymyxins.

82 In a recent review, Riley (2020) indicates that a surveillance system of ExPEC
83 genotypes causing extraintestinal infections, which does not currently exist, could
84 provide traceback investigation and elucidate the role of ExPEC as new extraintestinal
85 foodborne pathogens. On this bases, and using the concept "from the farm to the table",
86 the goals of this study were i) to gain knowledge regarding AMR on the current
87 situation in the Spanish poultry farming through the results obtained from the meat
88 samples, paying special attention to antimicrobial categories A and B; ii) to assess the
89 consumer exposure, via chicken and turkey meat, to high-risk Enterobacteriaceae clones
90 with potential to develop severe infections by either bacterial virulence and/or antibiotic
91 resistance traits; iii) to design a microbiological protocol for this risk assessment,
92 potentially applicable in routine laboratories.

93 ## 2. Material and methods

94 ### 2.1. Sample collection

95 Between September 2016 and September 2017, we randomly sampled 100 fresh poultry
96 meat products (50 chicken breasts and 50 turkey breasts) in six Spanish supermarket

chains located in Lugo (northwest Spain). Twenty-five g were aseptically cut from different points of each sample, homogenized in 225 ml of buffered peptone water and incubated for 6 hours at 37 °C. Afterwards, 1 ml of the pre-enriched sample was inoculated into 9 ml of MacConkey broth (18-24 h / 37 °C). From the positive tubes, 10 µl were plated on in each of Lactose MacConkey agar (LMA) (Oxoid), CHROMID® ESBL and CHROMID®CARBA SMART media (BioMérieux) (18-24 h / 37 °C). These media were used for the screening of *E. coli*, ESBL- and carbapenemase-producing Enterobacteriaceae, respectively. With this purpose, one representative colony of *E. coli* from LMA per sample, as well as the potentially ESBL- and carbapenemase-producing Enterobacteriaceae from CHROMID® ESBL and CHROMID®CARBA SMART according to manufacturer's instructions, were selected and stored at room temperature in nutrient broth (Difco™) with 0.75% nutrient agar (Difco™) for further characterization. In total, 256 different Enterobacteriaceae isolates recovered from the 100 meat samples were characterized (114 isolates from chicken and 142 from turkey). Bacterial identification was performed by MALDI-TOF MS (Bruker Daltonik, Bremen, Germany) in duplicated for each isolate. A reliable result (at the species level) was only considered if the score obtained was higher than 2.

2.2. Antimicrobial susceptibility and genetic characterization of β-lactamase and *mcr* genes

Antimicrobial susceptibility testing was conducted by disc difussion assay and/or by the Microscan system (Beckman Coulter, CA, USA) The antibiotics tested included ampicillin (AMP), amoxicillin-clavulanic acid (AMC), ceftazidime (CAZ), cefotaxime (CTX), cefoxitin (FOX), aztreonam (ATM), imipenem (IMP), gentamicin (GEN), tobramycin (TOB), amikacin (AMK), fosfomycin (FOF), doxycycline (DOX), chloramphenicol (CHL), nitrofurantoin (NIT), sulfamethoxazole-trimethoprim (SXT),

122 ciprofloxacin (CIP), nalidixic acid (NAL) and tigecycline (TGC). Furthermore, MICs
123 for colistin (CST) were manually obtained by broth microdilution for those suspected
124 colonies. All results were interpreted according to the CLSI guidelines (Clinical and
125 Laboratory Standards Institute, 2019). The collection was also investigated by PCR for
126 screening of specific *bla* genes using the TEM, CIT, SHV, CTX-M-1 and CTX-M-9
127 group-specific primers, and further sequencing (Mora et al., 2013), as well as for the
128 *mcr* genes (1 to 5) (Table S1).

129 **2.3. Multilocus sequence typing (MLST)**

130 MLST was performed for *E. coli* and *K. pneumoniae* isolates following the Achtman
131 (Wirth et al., 2006) and Institute Pasteur MLST (Diancourt et al., 2005) schemes,
132 respectively (Table S2).

133 **2.4. *E. coli* isolates**

134 **2.4.1. Phylogroups and clonotypes**

135 The phylogenetic relatedness of the *E. coli* isolates was further investigated using the
136 phylogroup assignment method of Clermont *et al.* (Clermont et al., 2013). Besides, the
137 clonotype (CH) of the *E. coli* isolates were established based on the internal 469-
138 nucleotide (nt) and 489-nt sequence of the *fumC* (allele obtained from MLST) and *fimH*
139 genes, respectively (Weissman et al., 2012) (Table S3).

140 **2.4.2. O and H typing**

141 O:H antigens of *E. coli* were determined following the method described by Guinée et
142 al. (1981) with O1 to O185 and H1 to H56 antisera, respectively. Isolates that did not
143 react with any O antisera were classified as non-typeable (ONT), and non-motile
144 isolates (HNM) were further analyzed by PCR for their flagellar genes (Mora et al.,
145 2018) (Table S4).

146 **2.4.3. Pathotypes**

147 All *E. coli* isolates were screened by PCR for the presence of specific virulence gene
148 factors (VF) associated with the diarrheagenic pathotypes: enteropathogenic EPEC (*eae*,
149 *bfpA*), verotoxigenic STEC (*eae*, stx_1, stx_2) and enteroaggregative EAEC (*aaiC*, *aggR*)
150 (Table S5). Likewise, all *E. coli* were tested for specific virulence markers defining the
151 status of extraintestinal pathogenic *E. coli* (ExPEC status) and the status of
152 uropathogenic *E. coli* (UPEC status): i) *papAH*, *sfa/focDE*, *afa/draBC*, *kpsM II* and *iutA*
153 (≥ 2 of these five markers conforms the ExPEC status) (Johnson et al., 2003); ii) *chuA*,
154 *fyuA*, *vat* and *yfcV* (≥ 3 of these four markers satisfies the uropathogenic status)
155 (Spurbeck et al., 2012). For those isolates exhibiting ExPEC and/or UPEC status, other
156 extraintestinal VF were analysed to complete their characterization (Table S5).

157 **2.4.4. Screening of ST131 and virotypes**

158 The O25b subtype associated with the clonal group ST131 was screened by PCR
159 (Clermont et al., 2008) within the *E. coli* collection (Table S4). The isolates confirmed
160 as O25b:H4-B2-ST31 were characterized for their virotypes according to the scheme
161 defined by Dahbi *et al.* (2014), based on the presence or absence of certain
162 extraintestinal VF (*afa/draBC*, *afa* operon FM955459, *iroN*, *sat*, *ibeA*, *papG II*, *papG*
163 *III*, *cnf1*, *hlyA*, *cdtB*, *kpsM II-K1*, *-K2 and -K5*).

164 **2.5. Pulsed field gel electrophoresis (PFGE)**

165 *XbaI*-PFGE profiles were performed following the PulseNet protocol
166 (https://www.cdc.gov/pulsenet/participants/international/index.html), and imported into
167 BioNumerics (Applied Maths, St-Martens-Latern Belgium) to obtain a dendrogram with
168 the UPGMA algorithm based on the Dice similarity coefficient and applying 1% of
169 tolerance in the band position.

170 **2.6. Whole-genome sequencing (WGS)**

171 Two isolates positive by PCR for the presence of *mcr* genes were futher characterized
172 by WGS. Genomic libraries were constructed using the Nextera XT DNA library
173 preparation kit (Illumina) following the manufactures´s instructions and sequenced on
174 an Illumina HiSeq X with 150 bp x 2 read length. Quality control checks on the
175 obtained raw sequence data was performed using FastQC version 0.11.3. Genome
176 assembly was performed *de novo* using SPAdes 3.11 (Bankevich et al., 2012) and were
177 *in silico* analysed using the bioinformatics tools of the Center for Genomic
178 Epidemiology (CGE) (https://cge.cbs.dtu.dk/services/) for the presence of antibiotic
179 resistance (ResFinder V2.1.), virulence genes (VirulenceFinder v1.5.), plasmid replicon
180 types (PlasmidFinder 1.3./PMLST 1.4.), and identification of clonotypes (CHTyper
181 1.0), sequence types (MLST 2.0) and serotypes (SerotypeFinder 2.0). All the CGE
182 predictions were called applying a select threshold for identification and a minimum
183 length of 95% and 80%, respectively. Phylogroups were predicted using the
184 ClermonTyping tool at the iame-research center web (http://clermontyping.iame-
185 research.center/).

186 **2.7. Statistical analysis**

187 Differences within groups were analyzed by a two-tailed Fisher's exact test. P values <
188 0.05 were considered statistically significant.

189 **2.8. Definition of food-borne risk**

190 In order to assess the level of microbiological risk exposure for consumers, each meat
191 sample was qualified between zero (lowest risk) to five (highest), on the basis of five
192 parameters which were individually analysed and considered as positive when
193 happened: i) the recovery of more than one ESBL-producing Enterobacteriaceae
194 species; ii) the identification of a high-risk clonal group of *E. coli*, according to recent
195 studies, due to their association within human extraintestinal and uropathogenic

196 pathologies (Mamani et al., 2019; Manges et al., 2019; Yamaji et al., 2018); iii) the
197 presence of *E. coli* conforming ExPEC status; iv) the presence of *E. coli* conforming
198 UPEC status; v) the recovery of Enterobacteriaceae resistant to antimicrobials of
199 categories A ("Avoid") or B ("Restrict") (EMA/CVMP/CHMP, 2019).

200 3. Results

201 3.1. Representative *E. coli* isolate per sample recovered from Lactose 202 MacConkey agar (LMA)

203 Of the 100 meat samples analyzed, 84% were positive for the presence of *E. coli* growth
204 in LMA (86% of the chicken and 82% of the turkey samples). From those positive, 84
205 representative *E. coli* (one colony per sample) were collected and characterized for their
206 phylogroups, STs, clonotypes, serotypes, bla_{ESBL} genes, resistance profiles,
207 ExPEC/UPEC status and VF associated with the enteropathogenic, verotoxigenic and
208 enteroaggregative pathotypes of *E. coli* (Table S6). As a result, most isolates belonged
209 to phylogroups B1 (25 isolates; 29.8%) and A (24; 28.6%); however, the other five
210 pylogroups of *E. coli sensu stricto* were also present, without differences regarding the
211 type of meat (chicken or turkey): C (15; 17.9%), F (8; 9.5%), E (6; 7.1%), B2 (5; 6%)
212 and D (1; 1.2%) (Figure S1). The analysis by MLST showed 41 different STs (Figure
213 1), including three new (STnew1, STnew2, and ST117-like with one single nucleotide
214 of difference in *fumC*45). Despite this diversity, eight STs accounted for 52.4% of the
215 isolates: ST10-A (eight isolates); ST23-C, ST58-B1 (seven isolates each); ST162-B1
216 (six isolates); ST410-C (five isolates); ST38-E, ST1485-F (four isolates each) and
217 ST744-A (three isolates) (Table S7). Clonotyping also showed high heterogeneity, with
218 23 different *fimH* alleles (seven isolates were negative for the amplification of the 489-
219 nt internal sequence) and 39 *fumC*-*fimH* combinations, but 51.2% of the isolates showed
220 any of the following seven clonotypes: CH11-54 (13 isolates of the CC10-A); CH4-35

221 (seven ST23-C); CH4-32 (six CC155-B1), CH65-32 (four ST162-B1 and two ST3580-
222 B1 isolates), CH26-65 (four ST38-E), CH231-58 (four ST1485-F) and CH4-24 (three
223 ST410-C) (Table S7). Regarding the serotyping, only three O:H combinations were
224 detected in more than two isolates belonging to the clonal groups O78:HNM-C-ST23
225 (three isolates), O83:H42-F-ST1485 (three isolates), ONT:H7-B1-ST3580 and
226 ONT:H7-A- ST5826 (two and one isolate, respectively) (Table S6).

227 Of the 84 representative *E. coli* isolates, 36 (42.9%) showed resistance to at least one
228 agent of ≥ three different antimicrobial categories and were defined as multidrug-
229 resistant (MDR) (Magiorakos et al., 2012). Moreover, only the isolates from four
230 samples showed susceptibility to the 19 antibiotics tested. The highest rates of
231 resistance were to AMP (78.6%), NAL (60.7%), CIP (39.3%), SXT (31.0%), DOX
232 (29.8%) and GEN (19.0%), being of note that the isolates recovered from turkey meat
233 showed higher values compared to those of chicken for AMP (90.2% *vs* 67.4%; P =
234 0.016), CIP (53.7% *vs* 25.6%; P = 0.013) and SXT (43.9% *vs* 18.6%; P = 0.018) (Table
235 S8). The screening and typing of bla_{ESBL} and *mcr* genes determined that two
236 ceftazidime-resistant isolates (one from chicken and one from turkey meat) were SHV-
237 12, and one colistin-resistant *E. coli* recovered from turkey was *mcr-1* (Table S6).

238 The screening of VF associated with diarrheagenic and extraintestinal pathotypes of *E.*
239 *coli* determined that 11 out of the 84 representative *E. coli* (13.1%) satisfied the status
240 ExPEC, and 12 isolates (14.3%) the status UPEC (the late comprised 10 of those
241 conforming also the ExPEC status). The 12 isolates positive for the UPEC status
242 belonged to the B2 and F phylogroups, and seven exhibited STs associated with high-
243 risk clonal groups (ST131-B2, ST95-B2, CC648-F) (Table S6; Table 1). None of the 84
244 isolates was positive for the presence of the diarrheagenic genes *bfpA,* stx_1, stx_2 *aaiC*, or
245 *aggR*. However, two *E. coli* isolates characterized as O153:H10-A-ST10 (CH11-54) and

246 O145:H40-A-ST752 (CH11-24) exhibited a hybrid atypical EPEC/ExPEC pathotype,
247 since both were carriers of the intimin-encoding gene *eae*-beta1 as well as of
248 extraintestinal VF (Table S9).

249 3.2. ESBL-producing Enterobacteriaceae recovered from CHROMID® ESBL

250 While none carbapenemase-producing colony was recovered from the
251 CHROMID®CARBA SMART medium, 82% of the 100 meat samples (40 of chicken
252 and 42 of turkey) were positive in CHROMID® ESBL. From those 82 samples, 172
253 different ESBL-producing isolates (71 of chicken and 101 of turkey) were recovered
254 and identified as *Escherichia coli* (137 isolates), *Klebsiella pneumoniae* (28 isolates),
255 *Serratia fonticola* (six isolates) and *Enterobacter cloacae* (one isolate). Significantly,
256 we found that 23 of the 50 turkey meat samples *versus* five of 50 chicken carried more
257 than one ESBL-producing species (46% *vs* 10%; P = 0.00) (Figures S2a and S2b). All
258 but one of the 172 ESBL-producing Enterobacteriaceae were MDR, showing the
259 highest rates of resistance to AMP (100%), NAL (72.7%), CTX (69.8%), ATM
260 (63.9%), CIP (63.4%), CAZ (55.2%), DOX (54.1%), CHL (41.3%) and SXT (36.6%).
261 ESBL-producing isolates obtained from turkey were significantly more resistant to
262 DOX (63.4% *vs* 40.8%; P = 0.005), SXT (49.5% *vs* 18.3%; P = 0.000), CIP (73.3% *vs*
263 49.3%; P = 0.002) and TGC (17.8% *vs* 2.8%; P = 0.003) (Table S8). Nineteen isolates
264 showed resistance to colistin: 11 *K. pneumoniae*, two *E. coli* (MICs >4 mg/L) (Table
265 S10), and the intrinsically resistant *S. fonticola* (six isolates).

266 ESBL-typing showed that 123 isolates out of 172 (71.5%) were carriers of SHV genes
267 (SHV-2, SHV-11, SHV-12, SHV-28); 50 isolates (29.1%) of CTX-M (CTX-M-1, CTX-
268 M-9, CTX-M-14, CTXM-15, CTX-M-32), six isolates (3.5%) of TEM-52 and four
269 *Serratia fonticola* (2.3%) of FONA (FONA-3/6 determined in three isolates and FONA-

270 5 in one) (Figure S3). Thirteen (7.5%) of the 172 ESBL-producing isolates were both
271 SHV and CTX-M carriers. By far, SHV-12 was the most prevalent ESBL type within
272 the 172 Enterobacteriaceae (94 of 172 isolates; 54.6%).

273 **3.2.1. ESBL-producing *E. coli* recovered from CHROMID® ESBL**

274 *E. coli* represented 80% of the 172 ESBL-producing Enterobacteriaceae recovered by
275 means of the CHROMID® ESBL (Figure S2b). The 137 ESBL-producing *E. coli* (64
276 isolates of chicken and 73 of turkey) from 76 meat samples (38 chicken and 38 turkey)
277 showed a similar phylogroup distribution to that found within the representative *E. coli*
278 collection obtained from LMA. Thus, most isolates belonged to the phylogroups A (55
279 isolates; 40.1%) and B1 (40; 29.2%), but there was also presence of phylogroups E (18;
280 13.1%), F (10; 7.3%), C (4; 2.9%), B2 (3; 2.2%) and D (2; 1.4%). Interestingly, five
281 isolates (5; 3.6%) belonged to Clade I (Figure 2). By MLST, the ESBL-producing *E.*
282 *coli* showed high heterogeneity with 51different STs, including five new: STnew3
283 (related with the ST665-A), STnew4 (related with the ST350-E), STnew5 (related with
284 the ST906-B1), STnew6 and STnew7 (Table S11). In fact, only seven STs (ST10-A,
285 ST93-A, ST117-F, ST155-B1, ST354-F, ST602-B1 and ST770-Clade I) were found in
286 ≥ 3 isolates. Sixteen of the 51 STs were also present within the representative *E. coli*
287 collection (Figure 2). Accordingly, clonotyping also showed high diversity with 46
288 *fumC-fimH* different combinations, of which only six were determined in ≥ 3 isolates:
289 CH4-32 (seven isolates of the CC155-B1), CH11-54 (ten CC10-A), CH19-86 (three
290 ST602-B1), CH31-54 (three CC350-E), CH45-97 (six ST117-F) and CH116-552 (five
291 ST770-Clade I) (Table S11). ESBL-typing determined that 68.6% of the 137 *E. coli*
292 produced SHV (93 isolates SHV-12 and one SHV-2), 27% CTX-M (14 isolates CTX-
293 M-1, 9 CTX-M-32, 6 CTX-M-14, 5 CTX-M-15 and 3 CTX-M-9), and 4.4% showed
294 type TEM-52. All but one chicken isolate were MDR, showing the highest rates of

295 resistance to AMP (100%), NAL and ATM (73.0%), CAZ (63.5%), CTX (62.0%), CIP
296 (59.8%), DOX (51.8%), CHL (45.3%). ESBL-producing *E. coli* from turkey were
297 significantly more resistant to DOX (61.6% *vs* 40.6%; P = 0.023), CHL (54.8% *vs*
298 34.4%; P = 0.025); SXT (38.4% *vs* 17.2%; P = 0.008) and CIP (68.5% *vs* 50.0%; P =
299 0.036) (Table S8). Of note that one SHV-12 colistin-resistant *E. coli* recovered from
300 turkey was positive for the *mcr-1* gene. The screening of VF determined that 18 out of
301 the 137 ESBL-*E. coli* (13.1%) satisfied the status ExPEC, including isolates of
302 phylogroups A, B1, B2, D, E, F and the five isolates of Clade I. Besides, six isolates
303 (4.4%) conformed the UPEC status and included risk clonal groups such as ST141-B2
304 and ST117-F (Table 1). Interestingly, two *E. coli* isolates from the ESBL-producing
305 collection, recovered from different samples, exhibited also a hybrid atypical
306 EPEC/ExPEC pathotype, being carriers of *eae*-beta1 and extraintestinal VF: O153:H10-
307 A-ST10 (CH11-54), CTX-M-32, and O123/186:H34-A-ST752 (CH11-24), CTX-M-1
308 (Table S9).

309 **3.2.2. ESBL-producing *K. pneumoniae* recovered from CHROMID® ESBL**

310 Twenty-eight *K. pneumoniae* were recovered from 27 different samples, representing
311 16% of the ESBL-producing isolates (Figure S2b). Significantly, only two isolates were
312 from chicken meat compared to 26 isolates from turkey. All isolates were MDR, with
313 the highest rates of resistances to AMP and CTX (100%), CIP (89.3%), SXT and NAL
314 (82.1%), DOX (67.9%), TGC (62.3%), TOB (42.9%), CST (39.3%), ATM (35.7%),
315 CAZ (28.6%). The ESBL typing revealed that 13 isolates were CTX-M-15, and eight of
316 those also positive for SHV-28. All isolates were negative by PCR for the presence of
317 *mcr-1* to *mcr-5* genes, including eleven phenotypically resistant to colistin (Table S10).
318 The 28 *K. pneumoniae* were further characterized by MLST and PFGE as shown in
319 Figure 3. Eleven different STs were established, being the most prevalent: ST307

320 (seven isolates), ST147 (four isolates), the new assignation ST4028 (four isolates) and
321 ST15 (three isolates). The 28 *K. pneumoniae* isolates exhibited 25 macrorestriction
322 profiles which grouped in the *XbaI*-PFGE dendrogram according to their ST, with five
323 clusters of similarity ≥85% (the two ST111 isolates, the four ST147, six of the seven
324 ST307, two of the three ST15 and the four ST4028) (Figure 3).

325 **3.3. WGS of *mcr-E. coli* isolates**

326 Within the collection analyzed here (84 representative *E. coli* and 172 ESBL-producing
327 Enterobacteriaceae), two colistin-resistant *E. coli* recovered from two meat turkey
328 samples were *mcr*-carriers (2/50; 4% turkey and 0/50 chiken meat). They showed MIC
329 values of >4 mg/L (ESBL-producing *E. coli*) and >32 mg/L (representative *E. coli*)
330 (Table S10). Table 2 summarizes their phenotypic traits and *in silico* characterization
331 using CGE tools. The two isolates belonged to the clonal group CC10-A (CH11-54).
332 The resistome analysis revealed that both genomes encoded mechanisms of antibiotic
333 resistance for ≥ three different antimicrobial categories, and both were carriers of the
334 *mcr-1.1* variant located in an IncX4 plasmid type. Furthermore, PlasmidFinder showed
335 a high plasmid diversity based on the identified replicons, with five to seven different
336 plasmid types per genome (Table 2). To highlight the fact that LREC-204 carried
337 double-serine mutations in *gyrA* S83L and *parC* S80I, with additional substitutions in
338 *gyrA* D87N and *parC* A56T, which corresponded to the fluoroquinolone resistance
339 determined *in vitro* for its isolate.

340 **3.4. Food-borne risk assessment**

341 The results of the present study determined that the majority (97%) of meat samples
342 were positive for the presence of Enterobacteriaceae. Besides, a comprehensive analysis
343 based on the assessment of five parameters showed that 96% of the samples meant a

344 consumer exposure to ≥ one risk, and 82% to ≥ two risks (Table S12). In detail, the
345 result for each parameters was: i) 96 out of the 100 samples with positive recovery of
346 isolates resistant to antimicrobials of the EMA categories A ("Avoid") or B ("Restrict"),
347 including 64 meat (31 chicken and 33 turkey) carriers of isolates resistant to
348 monobactams (ATM), and one of those also to FOF (category A). ii) Sixty-two samples
349 (31 chicken and 31 turkey) with presence of high-risk clonal groups of *E. coli*
350 associated with human extraintestinal and/or uropathogenic pathologies (ST10, ST23,
351 ST38, ST48, ST58, ST69, ST88, ST93, ST95, ST101, ST115, ST117, ST131, ST141,
352 ST167, ST350, ST345, ST354, ST359, ST410, ST602, ST617, ST641, ST906, ST1485).
353 iii) The ExPEC and iv) UPEC status, based on the presence of certain virulence markers
354 associated with a higher capacity of developing extraintestinal or UTI pathologies, was
355 determined in *E. coli* isolates recovered from 25 and 17 samples, respectively. v) More
356 than one ESBL-producing bacterial species were recovered from 28 samples (23 turkey
357 meat and five chicken; P = 0.000). Although it was not taken into account as an
358 additional risk, it is of note that 37 samples carried more than one type of ESBL-
359 producing *E. coli*.

360 # 4. Discussion

361 Common human extraintestinal diseases, namely, UTIs or blood stream infections, may
362 be caused by bacteria not traditionally defined as foodborne pathogens. Currently, there
363 is not a surveillance system of ExPEC genotypes, or other Enterobacteriace causing
364 extratintestinal infections, to elucidate their real role (Riley, 2020). For first time, and
365 based on a comprehensive characterization of 256 isolates, this study evaluates the
366 consumer exposure via poultry meat to Enterobacteriaceae with capacity to develop, not
367 only intestinal, but also severe extraintestinal infections by either bacterial virulence
368 and/or antibiotic resistance traits. For this purpose, we aimed to develop a suitable

369 protocol potentially applicable in the routine of food microbiological laboratories. This
370 protocol comprises a meat sample enrichment, followed by the characterization of one
371 representative *E. coli* colony grown on Lactose MacConkey agar (LMA), and those
372 species suspected of being ESBL/Carbapenemase-producing Enterobacteriaceae grown
373 on CHROMID® ESBL or CHROMID®CARBA SMART. The combination of these
374 selective media effectively provided complementary information on the presence and
375 prevalence of specific high-risk clonal groups of *E. coli*, as well as other ESBL-
376 producing Enterobacteriacea.
377 A high-risk clone, such as the ST131 of *E. coli*, is that defined as globally distributed,
378 associated with multiple antimicrobial resistance determinants, able to colonize and
379 persist in hosts for more than six months, capable of effective transmission between
380 hosts, enhanced pathogenicity and fitness, and able to cause severe and/or recurrent
381 infections (Mathers et al., 2015). It is within the group of ExPEC where the successful
382 risk clones of *E. coli* emerge. According to a recent meta-analysis, 20 major ExPEC STs
383 accounted for 85% of the studies included, being considered global extraintestinal
384 pathogenic lineages (Manges et al., 2019). In this study, 13 of those top 20 ExPEC
385 lineages were detected in 50% of our meat samples (ST10, ST23, ST38, ST58, ST69,
386 ST88, ST95, ST117, ST131, ST167, ST354, ST410, ST617). Seven of the 13 STs were
387 determined within both the representative 84 *E. coli* and the 137 ESBL-*E. coli*;
388 however, certain isolates could be recovered only via LMA (those belonging to ST23,
389 ST95 and the pandemic ST131) while others (ST69, ST167, ST354, and ST617) of the
390 ESBL-producing isolates, were detected mostly via CHROMID® ESBL.
391 The increasing evidence that retail food may serve as a source of *E. coli* implicated in
392 UTIs was recently analysed by Yamaji *et al*. (2018) through the characterizacion of 233
393 *E. coli* isolates from human urine samples and 177 *E. coli* from retail meat (poultry,

394 pork and beef) collected in the same geographic region. Within their collection, 21% of
395 *E. coli* isolates from suspected cases of UTIs belonged to STs found in poultry, stating
396 that poultry may serve as possible reservoir of UPEC. In our study, 40% of the meat
397 poultry samples carried *E. coli* belonging to STs (ST10, ST38, ST69, ST88, ST95,
398 ST101, ST117, ST131, ST141, ST354, ST906) identified by Yamaji *et al*. (2018) within
399 the UTI human cases, corresponding to 20.8% of our 221 *E. coli* isolates. Significantly,
400 we found a higher prevalence of turkey isolates belonging to STs associated to UPEC in
401 comparison with those of chicken origin (32 of 114; 28.1% *vs* 16 of 107; 14.9%) (P =
402 0.022).
403 We further investigated, within the 221 *E. coli*, the presence of four genes (*yfcV*, *vat*,
404 *fyuA*, and *chuA*) that predicts whether isolates can colonize the bladder more efficiently
405 than *E. coli* isolates without these genes (Spurbeck et al., 2012). We found that 18 *E.*
406 *coli* from 17 different meat samples conformed the UPEC status (Table 2). The 18
407 isolates belonged to B2 and F phylogroups, exhibited a high number of extraintestinal
408 VF and included reported ST/CC linked to UTIs, such as ST95, ST117, ST131, ST141
409 or CC648. Importantly, the isolates of the following seven clones carried the four genes
410 *yfcV*, *vat*, *fyuA*, and *chuA*: O1:H7-B2-ST95 (CH38-30); O50/O2:H6-B2-ST141 (CH52-
411 14); O115:HNM-B2-ST187 (CH24-187); O120:H4-B2-ST428 (CH40-22); O120:H4-
412 B2-ST428 (CH40-neg); O11:H25-F-ST457 (CH88-145); O113:H5-B2-ST8611 (CH24-
413 26).
414 *E. coli* ST131 has clearly become the major cause of MDR UTIs worldwide within
415 healthcare and community settings. WGS-analysis of the population structure of *E. coli*
416 ST131 identified three genetically distinct Clades (A, B, C), and numerous subclades
417 from the dominant fluoroquinolone-resistant Clade C (Johnson et al., 2010; Price et al.,
418 2013; Stoesser et al., 2016). Clade C carries a type 1 fimbrial adhesin gene *H30* variant

419 (*fimH*30; clonotype CH40-30), and compensatory mutations at regulatory regions which
420 seems to confer adaptive advantages for the fitness cost of AMR, plasmid acquisition
421 and maintenance, differently from the fluoroquinolone-susceptible Clades A (*fimH*41;
422 clonotype CH40-41) and B (*fimH*22; clonotype CH40-22) (Decano and Downing, 2019;
423 Stoesser et al., 2016). While ST131-*H*30 is the most prevalent, Clades A and B are also
424 important agents of community and hospital-acquired UTIs (de Toro et al., 2017; Liu et
425 al., 2018; Mora et al., 2014). ST131 isolates can be further classified into 12 virotypes
426 (A to F), regarding the presence/absence of certain virulence genes, which show
427 different host distribution, prevalence, and *in vivo* virulence in the mouse model
428 (Blanco et al., 2013; Dahbi et al., 2014; Mora et al., 2014). In this study, ST131-*H*22
429 (CH40-22) was determined in two *E. coli* isolates recovered from two chicken samples
430 in the LMA medium. Both ST131 isolates conformed virotype D4 (carriers of *ibe*A
431 gene and K1 variant of group II capsule) and showed MDR to aminoglycoxides,
432 tetracyclines and quinolones. In previous studies, we proved that the ST131 poultry
433 linage typically conforms virotype D4 (Cortes et al., 2010; Mora et al., 2010; Sola-
434 Gines et al., 2015). Importantly, we also found virotype D4 within clinical human
435 ST131, with a prevalence of 3.8% among 157 isolates (unpublished data), and some of
436 them showing a high genetic similarity compared to avian isolates (Mora et al., 2010).
437 Recently, we also proved by WGS, that porcine (meat and animal origin) and clinical
438 human ST131-H22 isolates of new subclades B6 and B7, were strongly related (average
439 distance of 20 and 15 SNP/Mb, respectively) (Flament-Simon et al., 2020). Liu *et al.*
440 (2018), combining detection of poultry-associated ColV plasmids with high-resolution
441 phylogenetics, quantified the proportion of human infections (from urine and blood
442 cultures). From their results, the authors stated that sublineage ST131-H22 has become
443 established in poultry populations around the world and that meat may serve as a

444 vehicle for human exposure and infection. According to the authors, ST131-*H22* would
445 be just one of many *E. coli* lineages that may be transmitted from food animals to
446 humans.

447 We also studied within meat isolates the presence of diarrheagenic *E. coli*. While none
448 of the 221 *E. coli* was positive for the specific VF associated with the verotoxigenic
449 (stx_1, stx_2) or enteroaggregative (*aaiC*, *aggR*) pathotypes, four CC10-A isolates obtained
450 from four different meat samples carried the *eae*-beta1 intimin gene, together with
451 extraintestinal pathogenic genes, and conforming an atypical EPEC/ExPEC hybrid
452 pathotype: two isolates O153:H10-A-ST10 (CH11-54); one O145:H40-A-ST752
453 (CH11-24) and one O123/186:H34-A-ST752 (CH11-24). In our geographical region
454 (NW Spain), we have been periodically detecting a hybrid MDR aEPEC/ExPEC of
455 clonal group O153:H10-A-ST10 (CH11-54) recovered from different sources (food-
456 producing animals; chicken, beef and pork meat; wildlife and human clinical samples).
457 Importantly, we proved genomic evidence of the close relatedness of the isolates that
458 may be playing a successful role in spreading ESBLs (CTX-M-32) in our region within
459 different hosts, including wildlife. Besides, it would be potentially implicated in human
460 diarrhea via food (meat) transmission (Díaz-Jiménez, 2020). Since 2011, when a novel
461 verotoxigenic/enteroaggregative (STEC/EAEC) *E. coli* O104:H4 emerged in Germany
462 and neighboring countries (Mora et al., 2011), other hybrid virulent *E. coli* have been
463 reported. The most outstanding is the recently emerged STEC/ExPEC O80:H2 hybrid
464 reported to cause HUS and bacteremia (Mariani-Kurkdjian et al., 2014), but there are
465 also STEC/UPEC hybrids which have been identified from hospitalized patients (Toval
466 et al., 2014), or some STEC/ETEC strains associated with diarrheal disease and HUS in
467 humans (Nyholm et al., 2015). Given the public health importance of hybrid pathotypes,
468 it seems necessary the surveillance of potentially emerging types.

469 According to the diversity of STs found within the 221 *E. coli* isolates of our study, and
470 despite more than 50% of the 84 representative *E. coli* as well as the ESBL-producing
471 *E. coli* belonged to the phylogroups A + B1, the other five (B2, C, D, E, F) of *E. coli*
472 *sensu stricto* were represented in the collection. The most anciently diverged
473 phylogroups B2, F and D comprises the majority of ExPEC isolates, whereas the
474 intestinal pathologies are linked to the most recently diverged phylogroups (E, C, B1
475 and A) (Clermont et al., 2019). Interestingly, five isolates from different samples
476 belonged to *Escherichia* clade I, which is also considered a phylogroup of *E. coli* based
477 on the extent of recombination detected between strains belonging to clade I and *E. coli*
478 (Clermont et al., 2013). The five isolates, recovered from CHROMID® ESBL,
479 belonged to the clonal group ST770 (CH116-552), conformed the ExPEC status, were
480 CTX-M-9 (two) or SHV-12 (three isolates) and MDR (the five FQ-resistant).
481 Besides the virulence traits associated to intestinal and extraintestinal *E. coli* pathotypes,
482 we investigated here the consumers´ exposure to antibiotic-resistant bacteria. Based on
483 the complementary analysis of Enterobacteriaceae recovered from the two selective
484 media, we found that 90% of the meat samples were carriers of MDR isolates.
485 Specifically, 96% samples carried resistant isolates to antimicrobials of categories A or
486 B, including 18% of the meat samples with colistin-resistant isolates, 64% with
487 resistance to monobactams, and one of those also to FOF (category A). Resistance
488 prevalence was significantly higher among turkey isolates (in both representative *E. coli*
489 and ESBL-producing Enterobacteria) for SXT and CIP. In a study conducted in USA on
490 poultry meat, the authors found higher resistance prevalence among *E. coli* isolates from
491 conventionally-raised turkey for most of the antibiotics tested compared to chicken meat
492 (Davis et al., 2018). We also found in our study that turkey meat was significantly more
493 contaminated with other ESBL-producing species than chicken. The differences found

494 for turkey meat can be probably associated with a longer exposition to antibiotics due to
495 the much longer fattening period.
496 On the other hand, the marked variation of prevalence and type of ATB resistances
497 reported by the countries would be linked to the current and past usage of antibiotics in
498 the respective animal species. The European Union summary report on antimicrobial
499 resistance in indicator *E. coli* (EFSA, 2018) shows comparable results to ours from the
500 84 representative *E. coli*, however, this *E. coli* collection alone would not reflect the real
501 figures of MDR occurrence in the poultry samples.
502 The ESBL types determined in our study within the *E. coli* isolates are mostly the same
503 as those reported in other studies for poultry meat (Egea et al., 2012; Kaesbohrer et al.,
504 2019; Nüesch-Inderbinen et al., 2019), but with an outstanding prevalence of SHV
505 (SHV-12, mainly) (71.5% of the 172 ESBL-producing Enterobacteriacea and 68.6% of
506 the 137 ESBL-producing *E. coli*). In the south of Spain, Egea *et al*. also found this
507 predominance, but with a decrease in favor of CTX-M ESBLs in comparison with a
508 previous study (Doi et al., 2010; Egea et al., 2012). Interestingly, our studies on poultry,
509 suggest an increase of the SHV isolates. Thus, of the 84 avian ESBL-producing *E. coli*
510 recovered from fecal avian samples in 52 farms located in the same geographical area
511 (2010-2012), 70.2% were of CTX-M type and 29.8% of SHV (García et al., 2018).
512 Likewise, 62.8% and 37.2% of 98 ESBL-producing *E. coli* from chicken meat sampled
513 in our city (2010-2011) were CTX-M and SHV, respectively (Herrera, 2015).
514 We also investigated here the colistin resistance linked to *mcr* genes within the meat
515 isolates. Since the *mcr-1* plasmid gene was first described (Liu et al., 2016), different
516 authors corroborate that large conjugative plasmids of types IncHI2, IncX4 and IncI2
517 would be the maximum responsible for the dissemination of the *mcr-1* gene among *E.*
518 *coli* isolates from different sources and geographical locations (Dominguez et al., 2019;

519 Doumith et al., 2016; Hasman et al., 2015). We report in this study two CC10-A
520 (CH11-54) carriers of the *mcr-1.1* variant located in an IncX4 plasmid type. Based on
521 the different replicons identified by PlasmidFinder, it is of note the high plasmid
522 diversity found within these isolates. In a recent study, we investigated the
523 characteristics of colistin-resistant *E. coli* clones successfully spread in swine in Spain.
524 We found high variability in the location of *mcr-1.1* genes, although they were located
525 mainly on plasmids of the IncHI2 and IncX4 types (six and four of the 12 *mcr-1.1*
526 plasmid-located genes, respectively); however, *mcr-1.1* also appeared integrated in the
527 chromosome of four genomes (Garcia-Meniño et al., 2019).
528 We also recovered 28 ESBL-producing *K. pneumoniae* from 27 meat samples (mainly
529 from turkey). *K. pneumoniae* is a major cause of nosocomial infections worldwide,
530 capable to persist in a wide range of reservoirs including health care settings, retail
531 meat, livestock and wastewater (Holt et al., 2015; Ludden et al., 2019). A recent study
532 explored the genetic relatedness of *K. pneumoniae* isolated from the same and different
533 reservoirs within a defined geographic region of England. The authors found few STs
534 shared between the different sources, and the WGS-based analysis showed no evidence
535 for livestock as a source of *K. pneumoniae* infecting humans (Ludden et al., 2019). In
536 our collection, at least eight of the 11 STs identified were previously reported within
537 human clinic isolates: ST15, ST45, ST111, ST147, ST307, ST627, ST966 and ST1086
538 (Esposito et al., 2018; Holt et al., 2015; Hu et al., 2013; Moradigaravand et al., 2017;
539 Uz Zaman et al., 2014). Since *K. pneumoniae* is an opportunistic pathogen, the main
540 concern here would be the high rates of resistance to CTX, CIP, SXT, DOX and TGC
541 (more than 60% of isolates), together with the high prevalence of *bla*$_{\text{CTX-M-15}}$ (13
542 isolates from 12 meat samples). In contrast, CTX-M-15 producing *E. coli* was recovered

only from five samples, and two of them with co-occurrence of *K. pneumoniae* SHV-28, CTX-M-15 isolates.

5. Conclusions

Our results show that poultry meat microbiota is a source of genetically diverse *Enterobacteriaceae,* resistant to relevant antimicrobials and potentially pathogenic for humans, including hybrid pathotypes of *E. coli*, high-risk clonal groups of *E. coli* associated with human extraintestinal and/or uropathogenic pathologies, as well as *K. pneumoniae* clonal groups of clinical interest. Given this scenario, antibiotic pressure reduction in poultry as well as surveillance of bacterial evolution is a public health priority. It would be highly recommended the implementation of a systematic antimicrobial resistance and ExPEC monitoring of food at retail as a follow-up tool "from the farm to the table" under the "One-Health" strategy.

5. Nucleotide sequence accession numbers

The nucleotide sequence of the *mcr*-positive isolates have been deposited in the NCBI sequence databases with accession codes SAMN12430141 (isolate T-1-V-e; genome LREC-204) and SAMN12430147 (isolate T-17-R; genome LREC-210) and these sequences are part of BioProject ID PRJNA558228.

Declarations

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TABLES

Table 1. Phenotypic and genotypic characterization of the 18 *E. coli* conforming UPEC status: 12 from the representative collection ("R" code) plus six recovered from CHROMID® ("ESBL" code)

[1]Isolate code	[2]Serotype	[3]PG	[4]ST	[5]CH	[6]ESBL	[7]Resistances	[8]Virulence-gene profile
T-24-ESBL	O50/O2:H6	B2	141	52-14	SHV-12	AMP, CAZ, ATM, DOX, CHL	*fimH14 hlyF iucD iutA iroN kpsM II-K1 cvaC traT ibeA malX usp tsh ompT iss chuA vat fyuA yfcV*
T-40-ESBL	O113:H5	B2	8611	24-26	SHV-12	AMP, CAZ, ATM, DOX, CHL, NAL	*fimH26 iroN traT malX usp ompT chuA vat fyuA yfcV*
T-48-ESBL	O115:HNM	B2	919	24-187	SHV-12	AMP, CAZ, CTX, ATM, SXT, NAL	*fimH187 iucD iutA iroN traT ibeA malX hlyF ompT iss chuA vat fyuA yfcV*
Ch-2-R	O25:H4	B2	131	40-22	-	GEN, DOX, NAL	*fimH22 iucD iutA iroN kpsM II-K1 traT ibeA malX usp tsh ompT iss chuA fyuA yfcV*
Ch-13-R	O25:H4	B2	131	40-22	-	GEN, DOX, NAL	*fimH22 hlyF iucD iutA iroN kpsM II-K1traT ibeA malX usp tsh ompT iss chuA fyuA yfcV*
Ch-19-R	O120:H4	B2	428	40-neg	-	AMP, SXT	*fimH fimAvMT78 hlyF iucD iutA iroN kpsM II-K1 cvaC traT ibeA malX usp ompT iss chuA vat fyuA yfcV*
T-14-R	O120:H4	B2	428	40-22	-	AMP, SXT	*fimH22 hlyF iucD iutA iroN kpsM II-K1 cvaC traT ibeA malX usp ompT iss chuA vat fyuA yfcV*
T-22-R	O1:H7	B2	95	38-30	-	AMP, NAL	*fimH30 hlyF papAH papaEF papC papG II cdtB iucD iutA iroN kpsM II-K1 cvaC traT malX usp ompT iss chuA vat fyuA yfcV*
Ch-1-ESBL	O24:H18	F	117	45-151	SHV-12	AMP, CAZ, ATM, DOX, CHL, CIP, NAL	*fimH151 cdtB hlyF iucD iutA traT malX ompT chuA vat fyuA*
Ch-43-ESBL	O57:HNM	F	117	45-97	SHV-12	AMP, CAZ, ATM, DOX, CHL	*fimH97 cdtB iucD iutA iroN traT malX tsh ompT iss chuA vat fyuA*
T-9-ESBL	O118:H4	F	117	45-97	SHV-12	AMP, CAZ, CTX, ATM, DOX, CHL	*fimH97 cdtB iucD iutA iroN traT malX tsh ompT iss chuA vat fyuA*
Ch-6-R	O15:H42	F	1485	231-58	-	AMP, AMC, GEN, TOB, SXT, CIP, NAL	*fimH58 hlyF iucD iutA iroN kpsM II-K5 cvaC traT malX tsh ompT iss chuA vat yfcV*
Ch-9-R	O83:H42	F	1485	231-58	-	AMP, GEN, SXT, NAL	*fimH58 hlyF iucD iutA iroN kpsM II-K5 cvaC traT malX tsh ompT iss chuA vat yfcV*
Ch-16-R	O11:H25	F	457	88-145	-	GEN, DOX	*fimH145 hlyF iucD iutA iroN kpsM II-K2 cvaC traT malX tsh ompT iss chuA vat fyuA yfcV*
Ch-38-R	O83:H42	F	1485	231-58	-	AMP, SXT, CIP, NAL	*fimH58 hlyF iucD iutA iroN kpsM II-K5 cvaC traT malX tsh ompT iss chuA vat yfcV*
Ch-47-R	O53:HNM	F	117-like	new - 97	-	AMP, GEN, DOX, NAL	*fimH97 hlyF iucD iutA iroN traT malX tsh ompT iss chuA vat fyuA*
T-20-R	O83:H42	F	1485	231-58	-	AMP, SXT, CIP, NAL	*fimH58 hlyF iucD iutA iroN kpsM II-K5 cvaC traT malX tsh ompT iss chuA vat yfcV*
T-40-R	O8:HNM	F	5340	271-58	-	AMP, SXT	*fimH58 hlyF iucD iutA iroN cvaC traT malX tsh iss chuA vat yfcV*

[1] Origin of isolation-sample number-type of isolate: Ch (chicken meat), T (turkey meat), R (representative *E. coli*), ESBL (ESBL-producing *E. coli*). [2] H antigen: HNM for nonmotile isolates. [3] Phylogroup (PG) was designated by PCR according to Clermont scheme (Clermont et al., 2013). [4] Sequence type (ST) was performed following the Achtman scheme (Wirth et al., 2006). [5] Clonotype 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 when PCR was negative for the 489-nt internal sequence amplification. [6] *bla*$_{ESBL}$ typing (Garcia-Meniño et al., 2018). [7]Phenotypic resistance interpreted according to the CLSI guidelines (Clinical and Laboratory Standards Institute, 2019): ampicillin (AMP), ceftazidime (CAZ), cefotaxime (CTX), aztreonam

(ATM), gentamicin (GEN), tobramycin (TOB), doxycycline (DOX), chloramphenicol (CHL), sulfamethoxazole-trimethoprim (SXT), ciprofloxacin (CIP), nalidixic acid (NAL). [8]Specific extraintestinal VF (Johnson et al., 2003; Spurbeck et al., 2012).

[10] bla_{ESBL} and *mcr* typing. The collection was also investigated by PCR for specific *mcr* (1 to 8) and *bla* genes using the TEM, CIT, SHV, CTX-M-1 and CTX-M-9 group-specific primers, followed by amplicon sequencing for those positive (Mora et al., 2013).

Table 2. *In silico* characterization and phenotypic traits of the *mcr* positive isolates recovered from two turkey meat samples

Code[1]	O:H antigens[2]	Phylo Group[3]	ST[4]	CHType[5]	Acquired resistances (in black) and point mutations (in blue)[6]	Plasmid content Inc group (pMLST)[7]	*mcr* type / location[8]	Virulence genes[9]	Phenotypic resistances[10]	Virulence profile[11]
LREC-204 / T1-ESBL	O162/O89:H9	A	744	11-54	***bla_{SHV-12}***; *aadA1, aadA2, aadA5, aph(3'')-Ib, aph(6)-Id; catA1, cmlA1; dfrA17; mdf(A); sul1, sul2, sul3; tet(A), tet(B);* ***mcr-1*** *gyrA S83L, gyrA D87N, parC A56T, parC S80I*	IncF (F18:A-:B20*) IncI1 (ST26) IncQ1 IncX1 IncX4 Col(MG828)-like ColpVC	*mcr-1.1* / IncX4	*mchF, iroN, iss, tsh, cba, cma*	AMP, CAZ, CTX, ATM, CST, DOX, CHL, SXT, CIP, NAL	*fimH54, $fimAv_{MT78}$, hlyF, iutA, iucD, iroN, cvaC, iss, traT, tsh*
LREC-210 / T17-R	O162/O89:H37	A	853	11-54	*bla_{TEM-1B}; aadA1, aph(3'')-Ib, aph(6)-Id; dfrA1; mdf(A); mph(B); sul1, sul2; tet(A);* ***mcr-1***	IncF (F18:A-:B1) IncQ1 IncX4 Col(MG828)-like Col156-like	*mcr-1.1* / IncX4	*iroN, cma, iss, celb*	AMP, CST, SXT	*fimH54, $fimAv_{MT78}$, hlyF, iutA, iucD, iroN, iss, traT, ompT*

[1]Genomic and isolate reference, respectively; [2]serotypes, [4]sequence types, [5]clonotypes, [6]acquired antimicrobial resistance genes and/or chromosomal mutations, [7]replicon/plasmid STs, and [9]virulence genes were determined using SerotypeFinder 2.0, MLST 2.0, CHtyper 1.0, ResFinder 3.1, PlasmidFinder 2.0, pMLST 2.0, and VirulenceFinder 2.0 online tools at the Center of Genomic Epidemiology (https://cge.cbs.dtu.dk/services/), respectively; [3]phylogroups were predicted using the ClermonTyping tool at the Iame-research Center web (http://clermontyping.iame-research.center/)

886 [6]Resistome. Acquired resistance genes: beta-lactam: *bla*$_{TEM-1B}$, *bla*$_{SHV-12}$, aminoglycosides: *aadA, aph(3'')-Ib, aph(6)-Id*; phenicols: *catA1, cmlA1*;

887 macrolides: *mdf(A)*; sulphonamides: *sul1, sul2, sul3*; tetracycline: *tet(A), tet(B)*; trimethoprim: *dfrA1, dfrA17*; colistin: *mcr*. Point mutations:

888 quinolones and fluoroquinolones: *gyrA* S83L: TCG-TTG, *gyrA* D87N: GAC-AAT, *parC* S80I: AGC-ATC, *parC* A56T: GCC-ACC.

889 [7]Plasmid STs: "*" indicates the nearest ST allele (with less than 100% but >95% identity and 100% coverage).

890 [8]*mcr* gene location determined by PlasmidFinder/ResFinder predictions.

891 [9]Virulence genes: *cba*: colicin B, celb: endonuclease colicin E2, *cma*: colicin M, *iroN*: enterobactin siderophore receptor protein, *iss*: increased

892 serum survival, *mchF*: ABC transporter protein MchF, *tsh*: temperature-sensitive hemagglutinin.

893 [10]Phenotypic resistance interpreted according to the CLSI standard breakpoints (Clinical and Laboratory Standards Institute, 2019): ampicillin

894 (AMP), ceftazidime (CAZ), cefotaxime (CTX), aztreonam (ATM), colistin (CST), doxycycline (DOX), chloramphenicol (CHL),

895 sulfamethoxazole-trimethoprim (SXT), ciprofloxacin (CIP), nalidixic acid (NAL).

896 [11]Specific extraintestinal VF determined by PCR (Johnson et al., 2003; Spurbeck et al., 2012).

897 **FIGURES**

898 **Figure 1. Representative *E. coli* collection.** Phylogenetic tree based on concatenated
899 sequences of the seven housekeeping genes from the MLST Achtman scheme by the
900 Neighbor-Joining method using MEGA6. The optimal tree with the sum of branch
901 length = 0.13442852 is shown. The percentage of replicate trees in which the associated
902 taxa clustered together in the bootstrap test (1000 replicates) are shown next to the
903 branches [2]. The tree is drawn to scale, with branch lengths in the same units as those
904 of the evolutionary distances used to infer the phylogenetic tree. The evolutionary
905 distances were computed using the p-distance method [3] and are in the units of the
906 number of base differences per site. The analysis involved 41 nucleotide sequences
907 determined within the 84 representative *E. coli*. All positions containing gaps and
908 missing data were eliminated. Highlighted in red those STs of *E. coli* associated with
909 human extraintestinal and/or uropathogenic pathologies.

910

911 **Figure 2. ESBL-producing *E. coli* collection.** Phylogenetic tree based on concatenated
912 sequences of the seven housekeeping genes from the MLST Achtman scheme by the
913 Neighbor-Joining method using MEGA6. The optimal tree with the sum of branch
914 length = 0.15093159 is shown. The percentage of replicate trees in which the associated
915 taxa clustered together in the bootstrap test (1000 replicates) are shown next to the
916 branches [2]. The tree is drawn to scale, with branch lengths in the same units as those
917 of the evolutionary distances used to infer the phylogenetic tree. The evolutionary
918 distances were computed using the p-distance method [3] and are in the units of the
919 number of base differences per site. The analysis involved 51 nucleotide sequences
920 determined within the 137 ESBL-producing *E. coli* collection. All positions containing
921 gaps and missing data were eliminated. There were a total of 3414 positions in the final

922 dataset. Highlighted in red those STs of *E. coli* associated with human extraintestinal

923 and/or uropathogenic pathologies.

924

925 **Figure 3.** Dendrogram of the *Xba*I macrorestriction profiles of the 28 *Klebsiella*

926 *pneumoniae* isolates. The dendrogram was obtained with the UPGMA algorithm based

927 on the Dice similarity coefficient and applying 1% of tolerance in the band position

928 using the BioNumerics software (Applied Maths, St-Martens-Latern Belgium).

929 Association between isolation code, ST, ESBL type (NT: not typable) and resistance

930 profile is indicated on the right**.** Clusters of ≥85% identity are highlighted in red.