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## Populations of extended-spectrum $\beta$ -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* are different in human-polluted environment and food items: a multicentre European study

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1 **Populations of extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli***  
2 **and *Klebsiella pneumoniae* are different in human-polluted environment**  
3 **and food items: A multicentre European study**

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29

30 **Running title:** Population structure of ESBL-*E. coli* and ESBL-*K. pneumoniae* retrieved in  
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32

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37 **Abstract**

38 **Objectives.** To assess the extent to which food items are a source of extended-spectrum  $\beta$ -  
39 lactamase (ESBL)-producing *Escherichia coli* (ESBL-Ec) and ESBL-producing *Klebsiella*  
40 *pneumoniae* (ESBL-Kp) for humans in five European cities.

41 **Methods.** We sampled 122 human polluted (hp)-environments (sewers and polluted rivers, as  
42 a proxy of human contamination) and 714 food items in Besançon (France), Geneva  
43 (Switzerland), Sevilla (Spain), Tübingen (Germany), and Utrecht (The Netherlands).  
44 254 ESBL-Ec and 39 ESBL-Kp isolates were cultured. All genomes were fully sequenced to  
45 compare their sequence types (ST) and core genomes, along the distribution of *bla*<sub>ESBL</sub> genes  
46 and their genetic supports (*i.e.* chromosome or plasmid).

47 **Results.** Sequence data revealed that ESBL-Ec and ESBL-Kp isolates from hp-environment  
48 were genetically different from those contaminating food items. ESBL-Ec ST131 was  
49 widespread in the hp-environment (21.5% of the isolates) but absent from the food items  
50 tested. ESBL-Ec ST10 was in similar proportions in hp-environment and food items (15 and  
51 10 isolates, respectively) but mostly carried reservoir-specific *bla*<sub>ESBL</sub>. *bla*<sub>CTX-M-1</sub> and *bla*<sub>SHV-12</sub>  
52 predominated in food-related *E. coli* isolates (32% and 34% of the isolates, respectively),  
53 while *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-27</sub> predominated in isolates from hp-environment (52% and  
54 15% of the isolates, respectively).

55 **Conclusions.** We found a very limited connection between ESBL-Ec and ESBL-Kp  
56 populations and *bla*<sub>ESBL</sub>, retrieved in food items and from hp-environment. This suggests that  
57 human-to-human contamination, rather than the food chain, is possibly the most frequent  
58 route of ESBL-Ec and ESBL-Kp transmission in high-income countries.

## 59 **Introduction**

60 Antimicrobial resistance is a global health issue and extended-spectrum  $\beta$ -lactamase  
61 producing *Enterobacterales* (ESBL-PE) are a major cause of AMR dissemination.  
62 *Escherichia coli* (ESBL-Ec) and *Klebsiella pneumoniae* (ESBL-Kp) are the two major ESBL-  
63 PE species causing infections in humans with third-generation cephalosporin resistant  
64 bacteria, limiting therapeutic options for patients [1]. ESBL-PE carriage by healthy people is  
65 frequent, with a prevalence of ~10% in Europe [2]. In addition, some populations, such as  
66 those found in long-term care facilities (LTCFs), are at higher risk of colonization and  
67 infections [3]. ESBL-PE have also been widely reported in slaughter animals [4], associated  
68 with a large proportion of the derived retail meat contaminated with these antibiotic-resistant  
69 pathogens. To a lesser extent, ESBL-PE can also be found in fresh vegetables [5,6]. Sewage  
70 systems bring ESBL-PE found in human faeces to the surface water [7]. There are many  
71 routes for ESBL-PE dissemination and the One Health concept supports the idea that bacterial  
72 populations found in animals, humans, and environment are heavily interconnected [8].  
73 However, the extent of transmission between these compartments remains uncertain,  
74 especially in high-income countries where hygiene standards probably limit the  
75 contaminations from non-human sources [9]. In particular, the role of the food chain is under  
76 debate [9–12].

77 Most studies neglected ESBL-Kp, also contaminating food items [13] and responsible for an  
78 important burden in human [14] with a rate of human-to-human transmission higher than of  
79 ESBL-Ec [15].

80 The exact identification of the route of contamination of an individual is nearly impossible to  
81 establish and requires the use of population-level proxies. In European countries, human-  
82 polluted (hp-) environments, and especially wastewaters, constitute a good proxy of human  
83 contamination [10,16,17].

84 To estimate the contribution of foodborne ESBL-Ec and ESBL-Kp to human colonization, we  
85 used whole genome sequences to assess the genetic relationships, and compared the identity  
86 of *bla*<sub>ESBL</sub> between the isolates from retail food and from hp-environments in five European  
87 cities.

## 88 **Methods**

89 **Study design.** We conducted a multicentre study involving five European cities: Besançon  
90 (France), Geneva (Switzerland), Sevilla (Spain), Tübingen (Germany), and Utrecht (The  
91 Netherlands). We collected samples of food products and from the hp-environment between  
92 January 2018 and August 2019, as part of the multicentre MODERN project. Samples from  
93 food products and hp-environment were collected within a prospective cohort study of ESBL-  
94 Ec and ESBL-Kp carriage in four LTCFs (manuscript in preparation) and during a four-month  
95 follow-up of ESBL-Ec and ESBL-Kp carriers after hospital discharge [15].

96

97 **Food and environmental samples.** The contamination by ESBL-Ec or ESBL-Kp was  
98 assessed in 714 food samples collected in LTCF kitchens before any processing (representing  
99 collective catering) and in 35 supermarkets located in or nearby each study site representing  
100 food bought by ESBL-PE carriers [18] (Supplementary Methods). The contamination of the  
101 hp-environment by ESBL-Ec or ESBL-Kp was assessed by collecting samples (n=122) eight  
102 times over a 32-week period in (i) the LTCF discharge sewer, (ii) the inflow of the  
103 downstream WWTP, and (iii) the river 200 m downstream of the WWTP outflow and >5 m  
104 from the riverbank (Supplementary Methods).

105

106 **Microbiological analysis.** For food samples, 25 g of meat or vegetables were incubated  
107 overnight at 35°C in 250 mL of tryptic soy broth supplemented by 8 mg/l vancomycin and  
108 0.25 mg/l cefotaxime. One hundred µL were then streaked on ESBL-specific plates  
109 (bioMérieux, Marcy-l'Etoile, France) and incubated overnight at 35°C.

110 Samples from WWTPs were diluted 1:10 in sterile water, 10 µL and 100 µL were streaked on  
111 ESBL-specific plates and incubated overnight at 35°C. One hundred mL of river and LTCF

112 sewage samples were filtrated on a 0.45- $\mu$ m filter deposited on ESBL-specific plates and  
113 incubated overnight at 35°C.  
114 Bacterial colonies suspected to be *E. coli* or *K. pneumoniae* were identified by MALDI-TOF  
115 mass spectrometry (Microflex LT, Bruker Daltonik GmbH, Bremen, Germany) with a log  
116 value  $\geq 2$  according to the manufacturer's recommendations. We kept a maximum of three  
117 *E. coli* or *K. pneumoniae* isolates with different morphotypes per sample. ESBL production  
118 was confirmed by double disk synergy tests (DDST20 and DDST30) as recommended by  
119 EUCAST (EUCAST guidelines for detection of resistance mechanisms and specific  
120 resistances of clinical and/or epidemiological importance, version 2.0). All isolates were  
121 stored in bead-containing cryotubes (Microbank, PRO-LAB Diagnostics, ON, Canada) at  
122 -80°C until further analysis.

123  
124 **Genome sequencing and analysis.** Bacterial DNA extraction and sequencing, read assembly,  
125 Sequence types (STs) determination with *in silico* multi-locus sequence typing (MLST),  
126 *bla*<sub>ESBL</sub> identification, and the identification of incompatibility (Inc) groups of plasmids are  
127 detailed in the Supplementary Methods.

128 Core genomes of ESBL-Ec and ESBL-Kp isolates were determined using existing schemes  
129 (<https://www.cgmlst.org/ncs>). The homemade pipeline pyMLST analysed the cgMLST  
130 (<https://github.com/bvalot/pyMLST>) by aligning genes present in >95% of the isolates and  
131 phylogenetic trees were then constructed (Supplementary Methods). A network was built with  
132 the package igraph on R 3.6.2 software to link genetically related ESBL-Ec, which genomes  
133 were distant by <10 genes.

134  
135 **Statistics.** The proportions of food samples contaminated by ESBL-Ec and ESBL-Kp were  
136 compared with non-parametric tests (Kruskal-Wallis test coupled with a post-hoc Dunn test).  
137 We then performed a test of equal proportions to determine in which cases the contamination

138 was higher or lower. The distribution and association of *bla*<sub>ESBL</sub> genes and plasmid  
139 incompatibility groups was also assessed. The  $\alpha$  value was set to 0.05. All analyses were  
140 performed with R 3.6.2 software (R Core Team Package 2019).



141 **Results**

142 **ESBL-Ec and ESBL-Kp isolated from food and hp-environment.** Overall, we obtained  
143 265 food samples from the LTCF kitchens and 449 food samples from 35 supermarkets  
144 (Table 1). Overall, ESBL-Ec or ESBL-Kp contaminated 26.7% (93/349) of the meat samples  
145 and 1.9% (7/365) of the vegetable samples. Among these samples, chicken (33.2%  
146 positive; n=63) and turkey (75.0% positive; n=20) were more frequently contaminated than  
147 other types of food products ( $p = 2e-16$ ; Table 1). We also collected 122 samples from hp-  
148 environment (Table 1). We retrieved 293 isolates: 254 ESBL-Ec and 39 ESBL-Kp. The  
149 contamination of food samples by ESBL-Kp was infrequent with only 12 isolates retrieved in  
150 the 714 samples analysed (Table 1). Except for two chicken samples, all other food samples  
151 positive to ESBL-Kp came from Tübingen. The country-specific distribution of positive  
152 samples is shown in the Table S1.

153

154 **Genomic comparison of ESBL-producing *E. coli* cultured from food and hp-**  
155 **environment.**

156 The 254 ESBL-Ec isolated from food (n=96) and hp-environment (n=158) were distributed  
157 into 77 different STs (Table 2). Twenty-two and 45 STs were exclusively represented by  
158 isolates found in food and in hp-environment, respectively. In contrast, 10 STs (99/254  
159 isolates) were found in both food and hp-environment samples (Table 2). ST131 was  
160 specifically found in samples of the hp-environment where it accounted for 21.5% of total  
161 ESBL-Ec.

162 We assessed the genetic relatedness of ESBL-Ec isolates retrieved from food and hp-  
163 environment by constructing a Bio Neighbor Joining (BioNJ) tree from the 254 genomes. It  
164 showed that although the genomes of isolates cultured from food and hp-environment were  
165 intermixed in the tree, the cgMLST distances were generally high between the two reservoirs

166 (Fig. 1). We identified only five clusters that contained ESBL-Ec isolates from both hp-  
167 environment and food, having a cgMLST distance <40 genes. They were represented by ten  
168 genomes of ESBL-Ec from the hp-environment and seven genomes of ESBL-Ec from food  
169 (see the green sectors in Fig. 1).

170 To evaluate the interconnections between the ESBL-Ec populations contaminating food and  
171 hp-environment, we built a network linking reservoirs with ESBL-Ec isolates whose genomes  
172 had a cgMLST distance <10 genes (Fig. 2). To define the cut-off value under which genomes  
173 were considered as genetically related, we created epidemiological groups based on the cities  
174 of isolation of the ESBL-Ec, considering that links between cities were epidemiologically  
175 unlikely. We then built networks that link the different sources: two sources were linked if  
176 each of them included at least one genome having less than 10, 15, 20, 30, or 40 differences in  
177 core genes. We then counted the number of unlikely links for each cut-off value. The cut-off  
178 value of 10, confirming previous findings [20], was chosen since it minimized the number of  
179 mistakenly connected compartments (Fig. S1).

180 Most of the links connected sources of the same type (food or hp-environment) and from the  
181 same city. Indeed, we found 15 clusters of hp-environment isolates, one cluster of nine  
182 isolates, one of five isolates, and 13 clusters of two isolates. Besides, there were 20 clusters of  
183 food isolates, one cluster of seven isolates, two of five isolates, four of three isolates, and 13  
184 of two isolates). The only link between the two compartments involved genomes of three  
185 meat-contaminating isolates and that of an isolate found in the receiving river in Tübingen  
186 (Fig. 2).

187

188 ***bla*<sub>ESBL</sub> distribution in *E. coli* cultured from food and hp-environment.** Thirteen different  
189 *bla*<sub>ESBL</sub> were identified. *bla*<sub>SHV-2</sub> was specific to food-related isolates while *bla*<sub>CTX-M-3</sub>, *bla*<sub>CTX-</sub>  
190 <sub>M-8</sub>, *bla*<sub>CTX-M-24</sub>, and *bla*<sub>CTX-M-65</sub> were specific to isolates from hp-environments (Fig. S2).

191 *bla*<sub>SHV-12</sub> and *bla*<sub>CTX-M-1</sub> were more often associated with food-related isolates (34% and 32%  
192 of the isolates, respectively) compared to isolates from hp-environments (3% [p = 1e-11] and  
193 12% [p = 8e-5] of the isolates, respectively). *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-27</sub> dominated in ESBL-  
194 Ec from hp-environments (52% and 15% of the isolates, respectively) in contrast with food-  
195 related ESBL-Ec (11% [p = 1e-13] and 6% [p = 0.043] of the isolates, respectively). Although  
196 we found the ST10 clone in similar proportions in the ESBL-Ec populations in food and in the  
197 hp-environment (11.5% and 9.5% of the isolates, respectively; p = 0.62), ST10 isolates  
198 generally harboured different *bla*<sub>ESBL</sub> genes (11/15 hp-environment samples harboured *bla*<sub>CTX-</sub>  
199 <sub>M-15</sub> while 1/10 food-related isolates harboured this gene). Moreover, we found only one hp-  
200 environment isolate belonging to ST10 that had <40 different core genes with two food  
201 isolates, with these three isolates being collected in distinct cities (Geneva, Besancon, and  
202 Tübingen).

203 We then analysed the distribution of plasmid incompatibility groups and we found that it was  
204 homogeneous among the ESBL-Ec isolates of the two reservoirs except for IncB/O/K/Z -  
205 more often associated with food-related isolates (p = 3e-7), and IncFIA groups - more  
206 frequent in isolates from the hp-environment (p = 0.019) (Table S2).

207 .

208

209 **Comparison of genomes and *bla*<sub>ESBL</sub> of ESBL-producing *K. pneumoniae* isolates cultured**  
210 **from food and hp-environment.** Among the 39 ESBL-Kp isolates retrieved in this study, 12  
211 were found in food and 27 in the hp-environment. We identified 22 different STs among  
212 which 16 were singletons. Eight STs were specific to the food, 13 were specific to hp-  
213 environment, and one ST was shared between the two reservoirs. Indeed, ST219 isolates were  
214 found in one river sample of Geneva and Sevilla and in two meat samples (one chicken and  
215 one turkey) in Tübingen. We built a maximum-likelihood tree to investigate the relatedness of

216 all ESBL-Kp genomes (Fig. 3). Only the ST219 was found in both food and the hp-  
217 environment, but of different cities: two isolates collected from food products in Tübingen  
218 had <40 different core genes with one isolate collected in the river in Sevilla. We identified  
219 six different ESBL-encoding genes with an overrepresentation of *bla*<sub>CTX-M-15</sub>, carried by  
220 87.2% of the ESBL-Kp isolates (Fig. 3).

## 221 **Discussion**

222 We compared the genomes of ESBL-Ec and ESBL-Kp isolates cultured from food items (96  
223 ESBL-Ec and 12 ESBL-Kp) with those from hp-environment (158 ESBL-Ec and 27 ESBL-  
224 Kp) in five European cities. The main finding of this international study was that the  
225 population of these ESBL-Ec and ESBL-Kp cultured from these two reservoirs were  
226 genetically different, and that ESBL-Ec had generally *bla*<sub>ESBL</sub> genes of different nature.

227 Samples were collected in five European cities distributed from North to South of Western  
228 Europe to reflect the diversity of climate, food production, and veterinary antibiotic  
229 consumption that could affect the contamination level of the different reservoirs by ESBL-PE.  
230 Our collection of ESBL-Ec found in hp-environment, dominated by ST131 and ST38 mostly  
231 carrying *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-27</sub>, mirrored that of ESBL-Ec found in humans  
232 [10,16,17,21–23]. Similarly, we identified pandemic clones of ESBL-Kp (*i.e.* ST37, ST307)  
233 in the hp-environments. Food samples were gathered from LTCF kitchens and supermarkets  
234 to enable the collection being representative for most consumed food, both in collective  
235 catering and by ESBL-PE carriers [18] (Table 1 and Table S1). The contamination frequency  
236 of meat products and vegetables found in this study was consistent with those observed in the  
237 literature, with infrequent contamination of vegetables and more frequent contamination of  
238 poultry products [5,6,10,24].

239 The cgMLST analysis revealed that the populations of ESBL-Ec cultured from food and hp-  
240 environment are generally dissociated, with only six isolates out of 254 (four from food  
241 products and two from the hp-environment) that could be linked (Fig. 1 and Fig. 2). We found  
242 a majority of *bla*<sub>CTX-M-15</sub> carriers among ESBL-Kp. The dominance of *bla*<sub>CTX-M-15</sub> in  
243 wastewater and retail meat has been previously described [16,25]. The population of ESBL-  
244 Kp was mostly represented by distinct clones, with 22 different STs and with no linked  
245 isolates (*i.e.* which genomes had <10 different core genes between the reservoirs). Overall,

246 this suggests that transmission of ESBL-Ec and ESBL-Kp strains from food items to humans  
247 is a rare event. This supports the idea that *E. coli* ST131 and other B2 clonal groups are not  
248 food-related and are mostly transmitted from human to human [10,15,24,26]. ST10 has been  
249 previously detected in humans, animals, and in the environment [27]. Here, ESBL-Ec ST10  
250 was equally distributed between food and the hp-environment but the isolates were  
251 genetically distinct and carried reservoir-specific *bla*<sub>ESBL</sub> genes, which limits the chance of a  
252 common origin [12].

253 We further compared the distribution of *bla*<sub>ESBL</sub> genes in isolates from food and hp-  
254 environment (Fig. S2) and found that 5 out of 13 genes were specific to a compartment.  
255 Among the other genes, *bla*<sub>SHV-12</sub> and *bla*<sub>CTX-M-1</sub> predominated in food isolates, as reported by  
256 others [22,26,28]. Overall, distinct *bla*<sub>ESBL</sub> genes predominated in the two reservoirs. Plasmids  
257 carrying *bla*<sub>ESBL</sub> can spread between phylogenetically distinct *E. coli* populations possibly  
258 contributing to the human contamination with ESBL through the food chain [29]. It has been  
259 hypothesized that other food-related clones might serve as the source for plasmids or mobile  
260 genetic elements with resistance determinants to supply the major clone ST131, which is wide  
261 open for plasmid exchange [30]. Even if distinct genes predominated in the two reservoirs,  
262 plasmid reconstruction with long-read sequencing could help to identify transfer between the  
263 two reservoirs for the other genes.

264 We found here a limited concordance between the food and hp-environment reservoirs. This  
265 is in line with previous studies [10,12] but might appear as contrasting with a monocentric  
266 modelling study of the source distribution in a high-income country [9] where food products  
267 accounted for 18.9% of human contamination. However, this contribution could have been  
268 overestimated since using a model based on identity of *bla* genes, while we also considered  
269 their bacterial vehicle. Although we cannot exclude the possibility of human colonization with  
270 food-borne ESBL-Ec and ESBL-Kp, our data suggest that, in Western Europe, human-to-

271 human transmission plays a more important role in colonization of humans rather than a  
272 contamination by food items. However, the situation may be radically different in low- and  
273 middle-income countries where interconnections between human, animals, and the  
274 environment are stronger [8].

275 Our study has several limitations. Although we isolated the two most relevant ESBL  
276 producers in the community and in hospitals (ESBL-Ec and ESBL-Kp, respectively), we have  
277 neglected *bla*<sub>ESBL</sub> borne by other species [22,31]. The ultimate identification of HGT events  
278 requires plasmid reconstruction from long-read sequencing data and the comparison of  
279 plasmids carrying identical *bla*<sub>ESBL</sub> gene (manuscript in preparation). However, long-read  
280 sequencing would have not changed the main conclusions because there is a limited similarity  
281 between *bla*<sub>ESBL</sub> genes carried by the isolates of the two reservoirs.

282 Overall, we found a very limited connection between ESBL-Ec and ESBL-Kp populations  
283 and *bla*<sub>ESBL</sub> genes retrieved in retail food and that retrieved from hp-environment. This  
284 suggests that the human-to-human contamination, rather than the food chain, is likely the  
285 most frequent route of transmission for ESBL-Ec and ESBL-Kp in high-income countries.

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294

295 **Transparency declaration**

296 SP reports consulting fees from Illumina and IDbyDNA and honoraria for presentation at  
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299

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316

### 317 **Author contributions**

318 All authors contributed to the study design or conduct. DH, DM, AM, TV, SH, ET, JAJWK,  
319 and JRB wrote the study protocol. DH, JAJWK, SH, JRB, ET, and BSC obtained funding.  
320 DM, TV, NC, SB, AM, MER, ES, and CB collected the samples. DM, AM, AC, SP, ACF,  
321 JG, AP, MD-V, and DH performed or supervised the microbiological analyses in their local  
322 centres. JAJWK, ACF, TV, SP and JG provided a standardized procedure for whole-genome-  
323 sequencing. JG and SP performed and supervised sequencing. CPH and BV performed  
324 statistical analyses. DM, BV, and JG performed genetic analyses. DH, JAJWK, SH, JRB, and  
325 ET supervised the study in their local institution as principal investigators. DM, XB, and DH  
326 drafted the manuscript and all authors reviewed and contributed to the manuscript. DH  
327 coordinated the project.

328

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- 457

458 **Figure and table legends**

459

460 **Figure 1. BioNJ tree based on the core genome of the 254 ESBL-Ec isolates cultured**  
461 **from food items and hp-environment (Europe, 2018-2019).** The tree was based on HKY85  
462 distances calculated from the core genome constituted of 2275 genes. Source (LTCF: long-  
463 term care facility; WWTP: wastewater treatment plant), city of isolation (TUB, Tübingen;  
464 BES, Besançon; SEV, Sevilla; UTR, Utrecht; GEN, Geneva), STs, and *bla*<sub>ESBL</sub> gene are  
465 indicated for each isolate. The yellow sectors indicate clusters of isolates retrieved from the  
466 same LTCF discharge sewer at different time points. The green sectors indicate clusters of  
467 isolates which genomes had <40 different core genes.

468

469 **Figure 2. Network analysis of the genomes of ESBL-Ec cultured from food and hp-**  
470 **environment (Europe, 2018-2019).** The network connects the sources with ESBL-Ec which  
471 genomes had <10 core genes of difference. The rectangles represent the sources of the  
472 samples with hp-environments in green, food in blue (n = number of isolates of the source  
473 linked with isolate(s) of other sources). Only sources linked with  $\geq 1$  other source are shown.  
474 Figures indicate the number of ESBL-Ec genomes of the corresponding source linked to the  
475 other source. STs and *bla*<sub>ESBL</sub> genes of the isolates linked are indicated.

476

477 **Figure 3. Maximum likelihood tree based on SNPs in the core genes (n=2279) of 39**  
478 ***K. pneumoniae* isolates producing extended-spectrum  $\beta$ -lactamase cultured from food**  
479 **samples and hp-environment (Europe, 2018-2019).** Source (LTCF: long-term care facility;  
480 WWTP: wastewater treatment plant), city of isolation (TUB, Tübingen; BES, Besançon; SEV,  
481 Sevilla; UTR, Utrecht; GEN, Geneva), STs, and *bla*<sub>ESBL</sub> gene(s) are indicated for each isolate.  
482 \*: only one mutation in allele *phoE* (A314G) differentiates ST5366 from ST219.

483 **Table 1. Contamination of food and human-polluted environment with ESBL-Ec and**  
484 **ESBL-Kp (Europe, 2018-2019).** The Table shows the number and nature of food and hp-  
485 environment samples collected in the five European cities (Besançon, Geneva, Sevilla,  
486 Tübingen and Utrecht), the number and proportion of samples positive for extended-spectrum  
487  $\beta$ -lactamase producing *E. coli* (ESBL-Ec) or *K. pneumoniae* (ESBL-Kp) and the number of  
488 ESBL-Ec and ESBL-Kp isolated from each type of sample. The distribution of the samples  
489 according to the city of isolation is detailed in the Table S1.

490

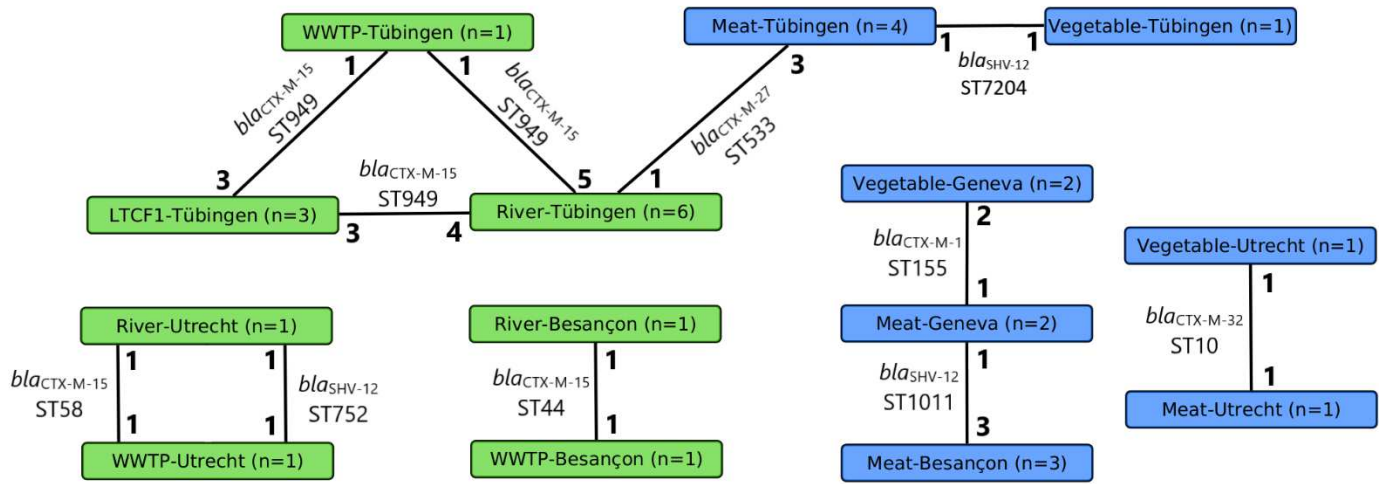
491 **Table 2. Distribution of STs among extended-spectrum  $\beta$ -lactamase producing *E. coli***  
492 **found in the human-polluted environment and food (Europe, 2018-2019).** Hp-  
493 environment, human-polluted environment; STs, sequence types.





4 **Figure 2**

5



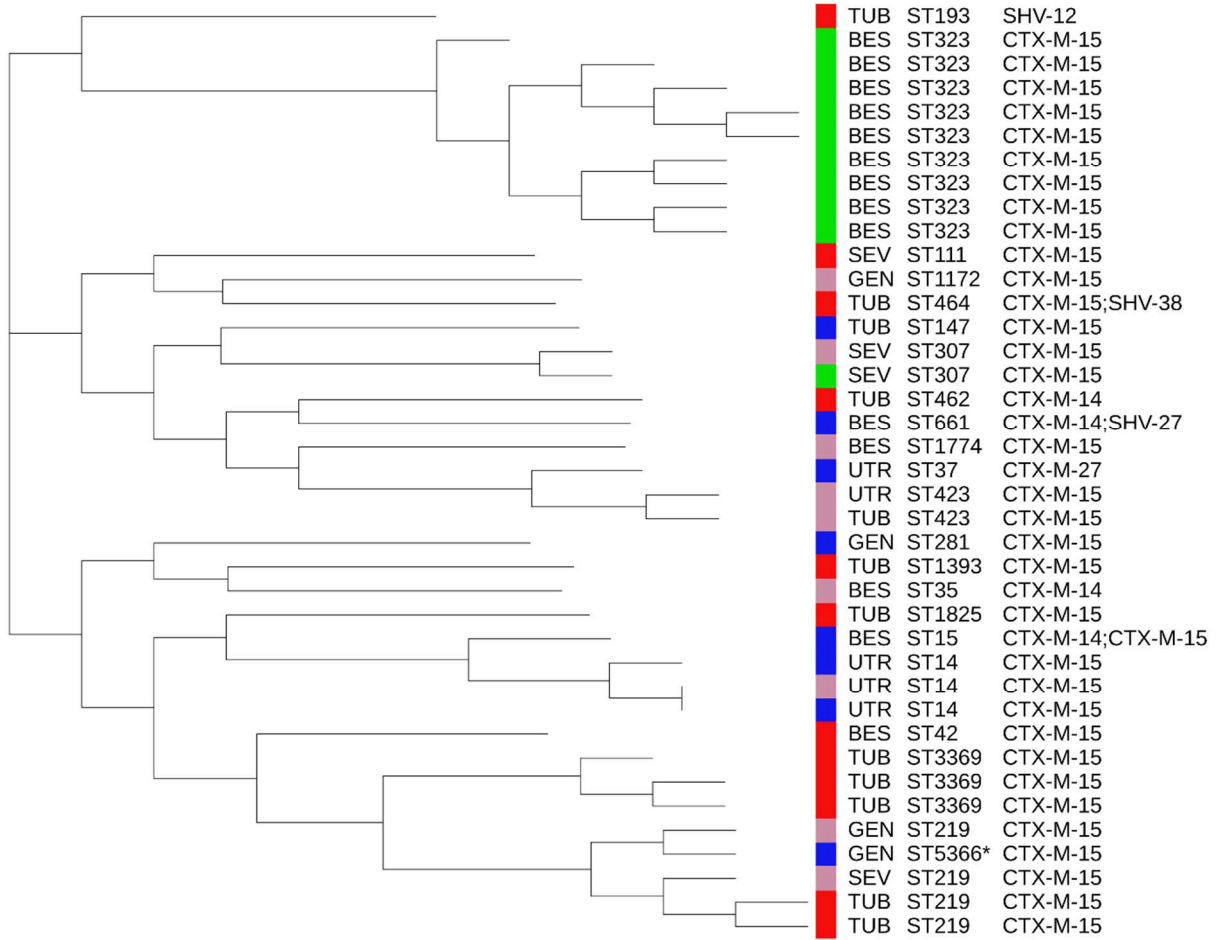
6

**Figure 3**

Tree scale: 0.001

**Source**

- Food
- WWTPs
- River downstream WWTPs
- LTCF discharge sewers



1 **Table 1. Contamination of food and human-polluted environment with ESBL-Ec and**  
2 **ESBL-Kp (Europe, 2018-2019).** The Table shows the number and nature of food and hp-  
3 environment samples collected in the five European cities (Besançon, Geneva, Sevilla,  
4 Tübingen and Utrecht), the number and proportion of samples positive for extended-spectrum  
5  $\beta$ -lactamase producing *E. coli* (ESBL-Ec) or *K. pneumoniae* (ESBL-Kp) and the number of  
6 ESBL-Ec and ESBL-Kp isolated from each type of sample. The distribution of the samples  
7 according to the city of isolation is detailed in the Table S1.  
8

|                             | Number of samples | Positives (%)     | ESBL-Ec    | ESBL-Kp   |
|-----------------------------|-------------------|-------------------|------------|-----------|
| <b>Food samples</b>         |                   |                   |            |           |
| Beef                        | 61                | 4 (6.6)           | 4          | 0         |
| Pork                        | 51                | 3 (8.9)           | 3          | 0         |
| Chicken                     | 190               | 63 (33.2)         | 64         | 3         |
| Fish                        | 18                | 2 (11.1)          | 3          | 0         |
| Turkey                      | 28                | 21 (75.0)         | 16         | 8         |
| Lamb                        | 1                 | 0                 | 0          | 0         |
| <b>Overall meat/fish</b>    | <b>349</b>        | <b>93 (26.7)</b>  | <b>90</b>  | <b>11</b> |
| <b>Vegetables</b>           | <b>365</b>        | <b>7 (1.9)</b>    | <b>6</b>   | <b>1</b>  |
| <b>Food total</b>           | <b>714</b>        | <b>100 (14.0)</b> | <b>96</b>  | <b>12</b> |
| <b>Hp-environment</b>       |                   |                   |            |           |
| LTCF sewages                | 30                | 22 (73.3)         | 19         | 10        |
| WWTP inflows                | 40                | 40 (100)          | 66         | 8         |
| Rivers downstream WWTPs     | 52                | 47 (90.4)         | 73         | 9         |
| <b>Hp-environment total</b> | <b>122</b>        | <b>109 (89.3)</b> | <b>158</b> | <b>27</b> |
| <b>Total</b>                | <b>836</b>        | <b>211</b>        | <b>254</b> | <b>39</b> |

10 **Table 2. Distribution of STs among extended-spectrum  $\beta$ -lactamase producing *E. coli***  
 11 **found in the human-polluted environment and food (Europe, 2018-2019).** Hp-environment,  
 12 human-polluted environment; STs, sequence types.

13

| All sources |           | Overall<br>hp-environment |           | Overall food |           |
|-------------|-----------|---------------------------|-----------|--------------|-----------|
| ST          | n (%)     | ST                        | n (%)     | ST           | n (%)     |
| ST131       | 34 (13.4) | ST131                     | 34 (21.5) | ST10         | 10 (11.5) |
| ST10        | 26 (10.2) | ST10                      | 16 (9.5)  | ST155        | 10 (10.4) |
| ST155       | 14 (5.5)  | ST38                      | 11 (7)    | ST69         | 9 (9.4)   |
| ST38        | 14 (5.5)  | ST949                     | 10 (6.3)  | ST1011       | 6 (6.3)   |
| ST69        | 13 (5.1)  | ST58                      | 9 (5.7)   | ST354        | 5 (5.2)   |
| ST58        | 13 (5.1)  | ST1193                    | 5 (3.2)   | ST58         | 4 (4.2)   |
| ST949       | 10 (3.9)  | ST155                     | 4 (2.5)   | ST533        | 4 (4.2)   |
| ST1011      | 6 (2.4)   | ST69                      | 4 (2.5)   | ST117        | 4 (4.2)   |
| ST354       | 5 (2.0)   | ST1431                    | 4 (2.5)   | ST101        | 4 (4.2)   |
| ST533       | 5 (2.0)   | ST88                      | 3 (1.9)   | ST115        | 4 (4.2)   |
| ST117       | 5 (2.0)   | ST44                      | 3 (1.9)   | ST48         | 4 (4.2)   |
| ST1193      | 5 (2.0)   | ST3995                    | 2 (1.3)   | ST38         | 3 (3.1)   |
| ST101       | 4 (1.6)   | ST405                     | 2 (1.3)   | ST4981       | 2 (2.0)   |
| ST115       | 4 (1.6)   | ST410                     | 2 (1.3)   | ST1249       | 2 (2.0)   |
| ST48        | 4 (1.6)   | ST227                     | 2 (1.3)   | ST3519       | 2 (2.0)   |
| ST88        | 4 (1.6)   | ST23                      | 2 (1.3)   | ST4937       | 2 (2.0)   |
| ST1431      | 4 (1.6)   | ST752                     | 2 (1.3)   | ST602        | 2 (2.0)   |
| ST4981      | 3 (1.2)   | ST95                      | 2 (1.3)   | ST746        | 2 (2.0)   |
| ST44        | 3 (1.2)   | ST1123                    | 2 (1.3)   | ST398        | 2 (2.0)   |
| ST1249      | 2 (0.8)   | ST2253                    | 2 (1.3)   | ST7204       | 2 (2.0)   |
| ST3519      | 2 (0.8)   | ST34                      | 2 (1.3)   | Singletons   | 12 (12.5) |
| ST4937      | 2 (0.8)   | ST3541                    | 2 (1.3)   | -            | -         |
| ST602       | 2 (0.8)   | ST993                     | 2 (1.3)   | -            | -         |
| ST746       | 2 (0.8)   | Singletons                | 32 (20.2) | -            | -         |
| ST398       | 2 (0.8)   | -                         | -         | -            | -         |
| ST7204      | 2 (0.8)   | -                         | -         | -            | -         |
| ST362       | 2 (0.8)   | -                         | -         | -            | -         |
| ST3995      | 2 (0.8)   | -                         | -         | -            | -         |
| ST405       | 2 (0.8)   | -                         | -         | -            | -         |
| ST410       | 2 (0.8)   | -                         | -         | -            | -         |
| ST227       | 2 (0.8)   | -                         | -         | -            | -         |
| ST23        | 2 (0.8)   | -                         | -         | -            | -         |
| ST752       | 2 (0.8)   | -                         | -         | -            | -         |
| ST95        | 2 (0.8)   | -                         | -         | -            | -         |
| ST1123      | 2 (0.8)   | -                         | -         | -            | -         |
| ST2253      | 2 (0.8)   | -                         | -         | -            | -         |
| ST34        | 2 (0.8)   | -                         | -         | -            | -         |
| ST3541      | 2 (0.8)   | -                         | -         | -            | -         |
| ST993       | 2 (0.8)   | -                         | -         | -            | -         |
| Singletons  | 38 (15.0) | -                         | -         | -            | -         |
| Total       | 254       |                           | 158       |              | 96        |