Original article

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

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ABSTRACT

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

Methods: We sampled 122 human polluted (hp)-environments (sewers and polluted rivers, as a proxy of human contamination) and 714 food items in Besançon (France), Geneva (Switzerland), Sevilla (Spain), Tübingen (Germany) and Utrecht (The Netherlands). A total of 254 ESBL-Ec and 39 ESBL-Kp isolates were cultured. All genomes were fully sequenced to compare their sequence types (ST) and core genomes, along with the distribution of bla_{ESBL} genes and their genetic supports (i.e. chromosome or plasmid).

Results: Sequence data revealed that ESBL-Ec and ESBL-Kp isolates from hp-environments were genetically different from those contaminating food items. ESBL-Ec ST131 was widespread in the hp-environment (21.5% of the isolates) but absent from the food items tested. ESBL-Ec ST10 was in similar proportions in hp-environments and food items (15 and 10 isolates, respectively) but mostly carried reservoir-specific bla_{ESBL}. $bla_{CTX-M-1}$ and bla_{SHV-12} predominated in food-related *E. coli* isolates (32% and 34% of the isolates, respectively), whereas $bla_{CTX-M-15}$ and $bla_{CTX-M-27}$ predominated in isolates from hp-environments (52% and 15% of the isolates, respectively).

Conclusions: We found a very limited connection between ESBL-Ec and ESBL-Kp populations retrieved in food items and from hp-environments and bla_{ESBL}. This suggests that human-to-human contamination,

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Introduction

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

Most studies neglect ESBL-Kp, which also contaminates food items [13] and is responsible for an important burden in humans [14] with a rate of human-to-human transmission higher than that of ESBL-Ec [15].

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

To estimate the contribution of food-borne ESBL-Ec and ESBL-Kp to human colonization, we used whole genome sequences to assess the genetic relationships, and compared the identity of *bla*$_{ESBL}$ between the isolates from retail food and from hp-environments in five European cities.

Materials and methods

Study design

We conducted a multicentre study involving five European cities: Besançon (France), Geneva (Switzerland), Sevilla (Spain), Tübingen (Germany) and Utrecht (The Netherlands). We collected samples of food products and from the hp-environment between January 2018 and August 2019, as part of the multicentre MODERN project. Samples from food products and hp-environment were collected within a prospective cohort study of ESBL-Ec and ESBL-Kp carriage in four LTCFs (manuscript in preparation) and during a 4-month follow up of ESBL-Ec and ESBL-Kp carriers after hospital discharge [15].

Food and environmental samples

The contamination by ESBL-Ec or ESBL-Kp was assessed in 714 food samples collected in LTCF kitchens before any processing (representing collective catering) and in 35 supermarkets located in or nearby each study site representing food bought by ESBL-PE carriers [18] (see Supplementary material, Appendix S1). The contamination of the hp-environment by ESBL-Ec or ESBL-Kp was assessed by collecting samples ($n = 122$) eight times over a 32-week period in (a) the LTCF discharge sewer, (b) the inflow of the downstream wastewater treatment plant (WWTP), and (c) the river 200 m downstream of the WWTP outflow and >5 m from the riverbank (see Supplementary materials, Appendix S1).

Microbiological analysis

For food samples, 25 g of meat or vegetables were incubated overnight at 35°C in 250 mL of tryptic soy broth supplemented with 8 mg/L vancomycin and 0.25 mg/L cefotaxime. Then, 100 μL were streaked on ESBL-specific plates (bioMérieux, Marcy-l'Etoile, France) and incubated overnight at 35°C.

Samples from WWTPs were diluted 1:10 in sterile water, 10 μL and 100 μL were streaked on ESBL-specific plates and incubated overnight at 35°C. One hundred millilitres of river and LTCF sewage samples were filtered on a 0.45-μm filter deposited on ESBL-specific plates and incubated overnight at 35°C.

Bacterial colonies suspected to be *E. coli* or *K. pneumoniae* were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Microflex LT, Bruker Daltonik GmbH, Bremen, Germany) with a log value ≥2 according to the manufacturer's recommendations. We kept a maximum of three *E. coli* or *K. pneumoniae* isolates with different morphotypes per sample. ESBL production was confirmed by double-disc synergy tests (DDST20 and DDST30) as recommended by EUCAST [19]. All isolates were stored in bead-containing cryotubes (Microbank, PRO-LAB Diagnostics, Richmond Hill, ON, Canada) at −80°C until further analysis.

Genome sequencing and analysis

Bacterial DNA extraction and sequencing, read assembly, determination of sequence types (STs) with *in silico* multi-locus sequence typing (MLST), *bla*$_{ESBL}$ identification and the identification of incompatibility (Inc) groups of plasmids are detailed in the Supplementary material (Appendix S1).

Core genomes of ESBL-Ec and ESBL-Kp isolates were determined using existing schemes (https://www.cgmlst.org/ncs). The home-made pipeline pyMLST analysed the cgMLST (https://github.com/bvalot/pyMLST) by aligning genes present in >95% of the isolates and phylogenetic trees were then constructed (see Supplementary materials (Appendix S1). A network was built with the package IGRAPH on R 3.6.2 software to link genetically related ESBL-Ec, the genomes of which were distant by fewer than ten genes.

Statistical analysis

The proportions of food samples contaminated by ESBL-Ec and ESBL-Kp were compared with non-parametric tests (Kruskal−Wallis test coupled with a post-hoc Dunn test). We then performed a test of equal proportions to determine in which cases the contamination was higher or lower. The distribution and association of bla_{ESBL} genes and plasmid incompatibility groups was also assessed. The α value was set to 0.05. All analyses were performed with R 3.6.2 software (R Core Team Package 2019).

Results

ESBL-Ec and ESBL-Kp isolated from food and hp-environment

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

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

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

We assessed the genetic relatedness of ESBL-Ec isolates retrieved from food and hp-environment by constructing a Bio Neighbour Joining tree from the 254 genomes. It showed that although the genomes of isolates cultured from food and hp-environment were intermixed in the tree, the cgMLST distances were generally high between the two reservoirs (Fig. 1). We identified only five clusters that contained ESBL-Ec isolates from both hp-environment and food, having a cgMLST distance <40 genes. They were represented by ten genomes of ESBL-Ec from the hp-environment and seven genomes of ESBL-Ec from food (see the green sectors in Fig. 1).

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

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

bla_{ESBL} distribution in E. coli cultured from food and hp-environment

Thirteen different bla_{ESBL} were identified. bla_{SHV-2} was specific to food-related isolates while $bla_{CTX-M-3}$, $bla_{CTX-M-8}$, $bla_{CTX-M-24}$ and $bla_{CTX-M-65}$ were specific to isolates from hp-environments (see Supplementary material, Fig. S2). bla_{SHV-12} and $bla_{CTX-M-1}$ were

Table 1
Contamination of food and human-polluted environment with ESBL-Ec and ESBL-Kp (Europe, 2018−2019)

	No. of samples	Positives (%)	ESBL-Ec	ESBL-Kp
Food samples				
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
Overall meat/fish	349	93 (26.7)	90	11
Vegetables	365	7 (1.9)	6	1
Food total	714	100 (14.0)	96	12
Hp-environment				
LTCF sewages	30	22 (73.3)	19	10
WWTP inflows	40	40 (100)	66	8
Rivers downstream WWTPs	52	47 (90.4)	73	9
Hp-environment total	122	109 (89.3)	158	27
Total	836	211	254	39

Abbreviations: ESBL-Ec, extended-spectrum β-lactamase-producing *Escherichia coli*; ESBL-Kp, extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*; hp-, human-polluted; LTCF, long-term care facility; WWTP, wastewater treatment plant.
The table shows the number and nature of food and hp-environment samples collected in the five European cities (Besançon, Geneva, Sevilla, Tübingen and Utrecht), the number and proportion of samples positive for ESBL-Ec or ESBL-Kp and the number of ESBL-Ec and ESBL-Kp isolated from each type of sample. The distribution of the samples according to the city of isolation is detailed in the Supplementary material (Table S1).

Table 2
Distribution of STs among extended-spectrum β-lactamase-producing *Escherichia coli* found in the human-polluted environment and food (Europe, 2018−2019)

All sources		Overall 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

Abbreviations: Hp-environment, human-polluted environment; STs, sequence types.

more often associated with food-related isolates (34% and 32% of the isolates, respectively) compared with isolates from hp-environments (3%, p 1e−11 and 12% p 8e−5 of the isolates, respectively). $bla_{CTX-M-15}$ and $bla_{CTX-M-27}$ dominated in ESBL-Ec from hp-environments (52% and 15% of the isolates, respectively) in contrast with food-related ESBL-Ec (11%, p 1e−13 and 6% p 0.043 of the isolates, respectively). Although we found the ST10 clone in similar proportions in the ESBL-Ec populations in food and in the hp-environment (11.5% and 9.5% of the isolates, respectively; p 0.62), ST10 isolates generally harboured different bla_{ESBL} genes (11/ 15 hp-environment samples harboured $bla_{CTX-M-15}$ whereas 1/10 food-related isolates harboured this gene). Moreover, we found only one hp-environment isolate belonging to ST10 that had <40 different core genes with two food isolates, with these three isolates being collected in distinct cities (Geneva, Besancon and Tübingen).

We then analysed the distribution of plasmid incompatibility groups and found that it was homogeneous among the ESBL-Ec isolates of the two reservoirs except for IncB/O/K/Z, which was more often associated with food-related isolates (p 3e−7), and IncFIA groups, which were more frequently found in isolates from the hp-environment (p 0.019) (see Supplementary material, Table S2).

Comparison of genomes and bla_{ESBL} of ESBL-producing K. pneumoniae isolates cultured from food and hp-environment

Among the 39 ESBL-Kp isolates retrieved in this study, 12 were found in food and 27 in the hp-environment. We identified 22 different STs, among which 16 were singletons. Eight STs were specific to the food, 13 were specific to hp-environment, and one ST was shared between the two reservoirs. Indeed, ST219 isolates were found in one river sample of Geneva and Sevilla and in two meat samples (one chicken and one turkey) in Tübingen. We built a maximum-likelihood tree to investigate the relatedness of all ESBL-Kp genomes (Fig. 3). Only the ST219 was found in both food and the hp-environment, but of different cities: two isolates collected from food products in Tübingen had <40 different core genes with one isolate collected in the river in Sevilla. We identified six different ESBL-encoding genes with an overrepresentation of $bla_{CTX-M-15}$, carried by 87.2% of the ESBL-Kp isolates (Fig. 3).

Discussion

We compared the genomes of ESBL-Ec and ESBL-Kp isolates cultured from food items (96 ESBL-Ec and 12 ESBL-Kp) with those from hp-environment (158 ESBL-Ec and 27 ESBL-Kp) in five
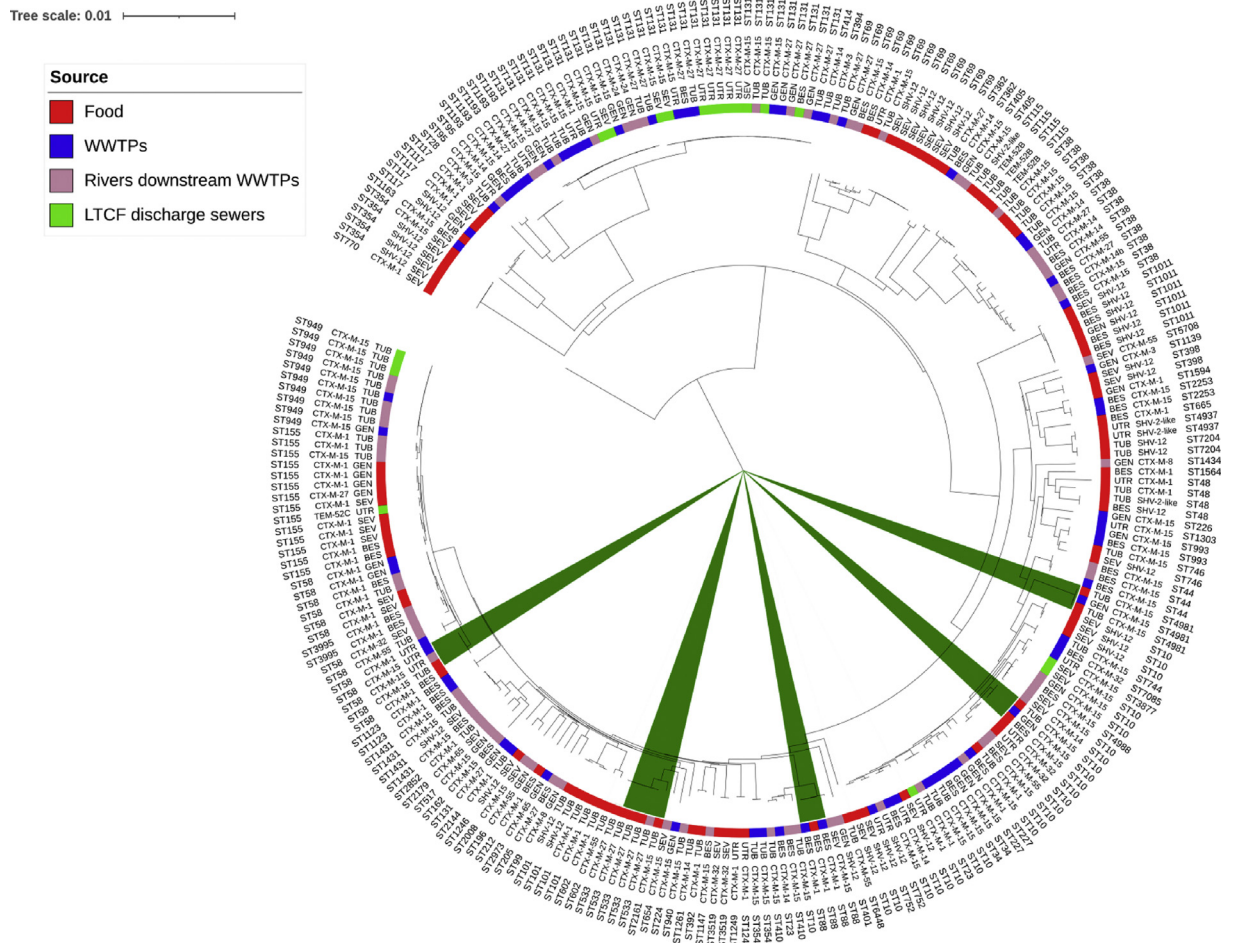
Fig. 1. Bio Neighbour Joining tree based on the core genome of the 254 extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-Ec) isolates cultured from food items and human-polluted (hp-) environment (Europe, 2018−2019). The tree was based on HKY85 distances calculated from the core genome constituted of 2275 genes. Sources: LTCF, long-term care facility; WWTP, wastewater treatment plant; city of isolation: TUB, Tübingen; BES, Besançon; SEV, Sevilla; UTR, Utrecht; GEN, Geneva; sequence types (STs) and *bla*ESBL gene are indicated for each isolate. The yellow sectors indicate clusters of isolates retrieved from the same LTCF discharge sewer at different time-points. The green sectors indicate clusters of isolates which genomes had <40 different core genes.

European cities. The main finding of this international study was that the population of these ESBL-Ec and ESBL-Kp cultured from these two reservoirs were genetically different, and that ESBL-Ec generally had *bla*ESBL genes of different nature.

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

The cgMLST analysis revealed that the populations of ESBL-Ec cultured from food and hp-environment are generally dissociated, with only six isolates out of 254 (four from food products and two from the hp-environment) that could be linked (Figs. 1 and 2). We

found a majority of *bla*CTX-M-15 carriers among ESBL-Kp. The dominance of *bla*CTX-M-15 in wastewater and retail meat has been previously described [16,25]. The population of ESBL-Kp was mostly represented by distinct clones, with 22 different STs and with no linked isolates (i.e. which genomes had fewer than ten different core genes between the reservoirs). Overall, this suggests that transmission of ESBL-Ec and ESBL-Kp strains from food items to humans is a rare event. This supports the idea that *E. coli* ST131 and other B2 clonal groups are not food-related and are mostly transmitted from human to human [10,15,24,26]. ST10 has been previously detected in humans, animals and in the environment [27]. Here, ESBL-Ec ST10 was equally distributed between food and the hp-environment but the isolates were genetically distinct and carried reservoir-specific *bla*ESBL genes, which limits the chance of a common origin [12].

We further compared the distribution of *bla*ESBL genes in isolates from food and hp-environment (see Supplementary material, Fig. S2) and found that 5 out of 13 genes were specific to a compartment. Among the other genes, *bla*SHV-12 and *bla*CTX-M-1 predominated in food isolates, as reported by others [22,26,28]. Overall, distinct *bla*ESBL genes predominated in the two reservoirs. Plasmids carrying *bla*ESBL can spread between phylogenetically distinct *E. coli* populations, possibly contributing to the human contamination with ESBL through the food chain [29]. It has been
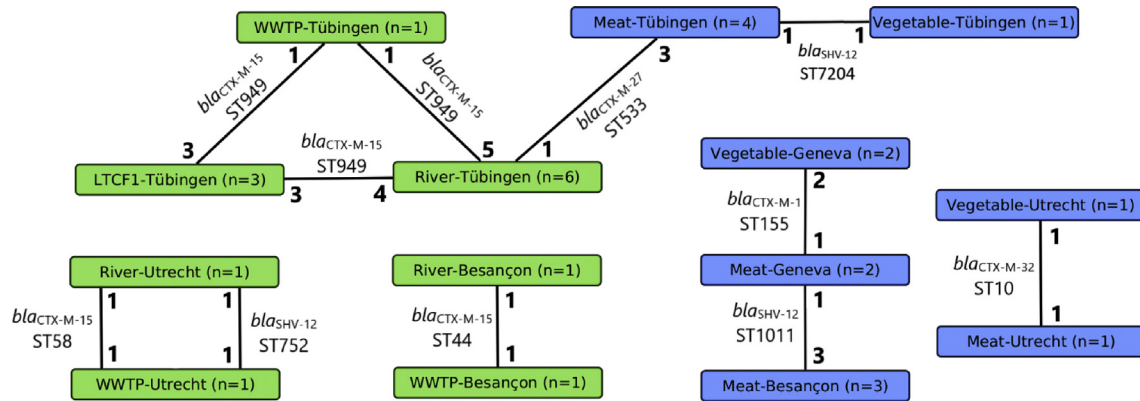
Fig. 2. Network analysis of the genomes of ESBL-Ec cultured from food and hp-environment (Europe, 2018–2019). The network connects the sources with ESBL-Ec which genomes had <10 core genes of difference. The rectangles represent the sources of the samples with hp-environments in green, food in blue (n = number of isolates of the source linked with isolate(s) of other sources). Only sources linked with ≥1 other source are shown. Figures indicate the number of ESBL-Ec genomes of the corresponding source linked to the other source. STs and bla_{ESBL} genes of the isolates linked are indicated.
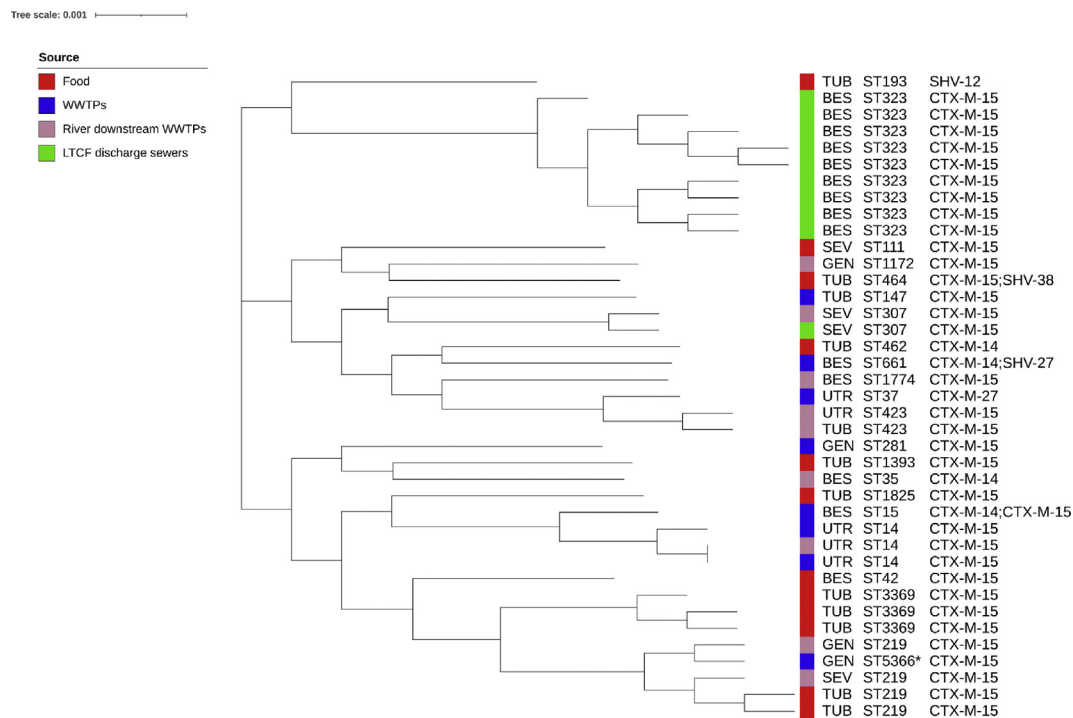
Fig. 3. Maximum likelihood tree based on single nucleotide polymorphisms in the core genes (n = 2279) of 39 *Klebsiella pneumoniae* isolates producing extended-spectrum β-lactamase cultured from food samples and human-polluted (hp-) environment (Europe, 2018–2019). Source: LTCF, long-term care facility; WWTP, wastewater treatment plant; city of isolation: TUB, Tübingen; BES, Besançon; SEV, Sevilla; UTR, Utrecht; GEN, Geneva. Sequence types (STs) and bla_{ESBL} gene(s) are indicated for each isolate. * only one mutation in allele *phoE* (A314G) differentiates ST5366 from ST219.

hypothesized that other food-related clones might serve as the source for plasmids or mobile genetic elements with resistance determinants to supply the major clone ST131, which is wide open for plasmid exchange [30]. Even if distinct genes predominated in the two reservoirs, plasmid reconstruction with long-read sequencing could help to identify transfer between the two reservoirs for the other genes.

We found here a limited concordance between the food and hp-environment reservoirs. This is in line with previous studies [10,12] but might appear as contrasting with a monocentric modelling study of the source distribution in a high-income country [9] where food products accounted for 18.9% of human contamination. However, this contribution could have been overestimated because

a model based on identity of *bla* genes was being used, while we also considered their bacterial vehicle. Although we cannot exclude the possibility of human colonization with food-borne ESBL-Ec and ESBL-Kp, our data suggest that, in Western Europe, human-to-human transmission plays a more important role in colonization of humans rather than contamination by food items. However, the situation may be radically different in low- and middle-income countries where interconnections between humans, animals and the environment are stronger [8].

Our study has several limitations. Although we isolated the two most relevant ESBL producers in the community and in hospitals (ESBL-Ec and ESBL-Kp, respectively), we have neglected bla_{ESBL} borne by other species [22,31]. The ultimate identification of

horizontal gene transfer events requires plasmid reconstruction from long-read sequencing data and the comparison of plasmids carrying identical bla_{ESBL} gene (manuscript in preparation). However, long-read sequencing would not have changed the main conclusions because there is a limited similarity between bla_{ESBL} genes carried by the isolates of the two reservoirs.

Overall, we found a very limited connection between ESBL-Ec and ESBL-Kp populations and bla_{ESBL} genes retrieved in retail food and that retrieved from hp-environment. This suggests that the human-to-human contamination, rather than the food chain, is likely the most frequent route of transmission for ESBL-Ec and ESBL-Kp in high-income countries.

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Transparency declaration

Role of the funding source

Author contributions

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.07.022.

References

[1] Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, et al. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 2007;59:165—74.

[2] Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. Fecal colonization with extended-spectrum beta-lactamase-producing *Enterobacteriaceae* and risk factors among healthy individuals: a systematic review and meta-analysis. Clin Infect Dis 2016;63:310—8.

[3] Tinelli M, Cataldo MA, Mantengoli E, Cadeddu C, Cunietti E, Luzzaro F, et al. Epidemiology and genetic characteristics of extended-spectrum β-lactamase-producing Gram-negative bacteria causing urinary tract infections in long-term care facilities. J Antimicrob Chemother 2012;67:2982—7.

[4] Madec J-Y, Haenni M. Antimicrobial resistance plasmid reservoir in food and food-producing animals. Plasmid 2018;99:72—81.

[5] Freitag C, Michael GB, Li J, Kadlec K, Wang Y, Hassel M, et al. Occurrence and characterisation of ESBL-encoding plasmids among *Escherichia coli* isolates from fresh vegetables. Vet Microbiol 2018;219:63—9.

[6] Randall LP, Lodge MP, Elviss NC, Lemma FL, Hopkins KL, Teale CJ, et al. Evaluation of meat, fruit and vegetables from retail stores in five United Kingdom regions as sources of extended-spectrum β-lactamase (ESBL)-producing and carbapenem-resistant *Escherichia coli*. Int J Food Microbiol 2017;241:283—90.

[7] Bréchet C, Plantin J, Sauget M, Thouverez M, Talon D, Cholley P, et al. Wastewater treatment plants release large amounts of extended-spectrum β-lactamase—producing *Escherichia coli* into the environment. Clin Infect Dis 2014;58:1658—65.

[8] Hernando-Amado S, Coque TM, Baquero F, Martínez JL. Defining and combating antibiotic resistance from One Health and Global Health perspectives. Nat Microbiol 2019;4:1432—42.

[9] Mughini-Gras L, Dorado-García A, van Duijkeren E, van den Bunt G, Dierikx CM, Bonten MJM, et al. Attributable sources of community-acquired carriage of *Escherichia coli* containing β-lactam antibiotic resistance genes: a population-based modelling study. Lancet Planetary Health 2019;3:e357—69.

[10] Day MJ, Hopkins KL, Wareham DW, Toleman MA, Elviss N, Randall L, et al. Extended-spectrum β-lactamase-producing *Escherichia coli* in human-derived and foodchain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study. Lancet Infect Dis 2019;19:1325—35.

[11] Lazarus B, Paterson DL, Mollinger JL, Rogers BA. Do human extraintestinal *Escherichia coli* infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review. Clin Infect Dis 2015;60:439—52.

[12] Nguyen MN, Hoang HTT, Xavier BB, Lammens C, Le HT, Hoang NTB, et al. Prospective One Health genetic surveillance in Vietnam identifies distinct blaCTX-M-harbouring *Escherichia coli* in food-chain and human-derived samples. Clin Microbiol Infect 2021. https://doi.org/10.1016/j.cmi.2021.01.006.

[13] Kim H-S, Chon J-W, Kim Y-J, Kim D-H, Kim M, Seo K-H. Prevalence and characterization of extended-spectrum-β-lactamase-producing *Escherichia*

coli and *Klebsiella pneumoniae* in ready-to-eat vegetables. Int J Food Microbiol 2015;207:83—6.

[14] Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis 2019;19:56—66.

[15] Riccio ME, Verschuuren T, Conzelmann N, Martak D, Meunier A, Salamanca E, et al. Household acquisition and transmission of ESBL-producing *Enterobacteriaceae* after hospital discharge of ESBL-positive index patients. Clin Microbiol Infect 2021. https://doi.org/10.1016/j.cmi.2020.12.024.

[16] Dolejska M, Frolkova P, Florek M, Jamborova I, Purgertova M, Kutilova I, et al. CTX-M-15-producing *Escherichia coli* clone B2-O25b-ST131 and *Klebsiella* spp. isolates in municipal wastewater treatment plant effluents. J Antimicrob Chemother 2011;66:2784—90.

[17] Kwak Y-K, Colque P, Byfors S, Giske CG, Möllby R, Kühn I. Surveillance of antimicrobial resistance among Escherichia coli in wastewater in Stockholm during 1 year: does it reflect the resistance trends in the society? J Antimicrob Agents 2015;45:25—32.

[18] Peregrin T. Understanding millennial grocery shoppers' behavior and the role of the registered dietitian nutritionist. J Acad Nutr Diet 2015;115:1380—3.

[19] EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, version 2.0 n.d.

[20] Schürch AC, Arredondo-Alonso S, Willems RJL, Goering RV. Whole genome sequencing options for bacterial strain typing and epidemiologic analysis based on single nucleotide polymorphism versus gene-by-gene-based approaches. Clin Microbiol Infect 2018;24:350—4.

[21] Chattaway MA, Jenkins C, Ciesielczuk H, Day M, DoNascimento V, Day M, et al. Evidence of evolving extraintestinal enteroaggregative *Escherichia coli* ST38 clone. Emerg Infect Dis 2014;20:1935. —7.

[22] Doi Y, Iovleva A, Bonomo RA. The ecology of extended-spectrum β-lactamases (ESBLs) in the developed world. J Travel Med 2017;24:S44—51.

[23] Nicolas-Chanoine M-H, Bertrand X, Madec J-Y. *Escherichia coli* ST131, an intriguing Clonal Group. Clin Microbiol Rev 2014;27:543—74.

[24] Ludden C, Raven KE, Jamrozy D, Gouliouris T, Blane B, Coll F, et al. One Health genomic surveillance of *Escherichia coli* demonstrates distinct lineages and mobile genetic elements in isolates from humans versus livestock. MBio 2019;10. e02693—18.

[25] Ludden C, Moradigaravand D, Jamrozy D, Gouliouris T, Blane B, Naydenova P, et al. A One Health study of the genetic relatedness of *Klebsiella pneumoniae* and their mobile elements in the east of England. Clin Infect Dis 2020;70:219—26.

[26] Dorado-García A, Smid JH, van Pelt W, Bonten MJM, Fluit AC, van den Bunt G, et al. Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food and the environment: a pooled analysis. J Antimicrob Chemother 2018;73:339—47.

[27] Blaak H, de Kruijf P, Hamidjaja RA, van Hoek AHAM, de Roda Husman AM, Schets FM. Prevalence and characteristics of ESBL-producing E. coli in Dutch recreational waters influenced by wastewater treatment plants. Vet Microbiol 2014;171:448—59.

[28] Kluytmans JAJW, Overdevest ITMA, Willemsen I, Kluytmans-van den Bergh MFQ, van der Zwaluw K, Heck M, et al. Extended-spectrum β-lactamase-producing *Escherichia coli* from retail chicken meat and humans: comparison of strains, plasmids, resistance genes, and virulence factors. Clin Infect Dis 2013;56:478—87.

[29] Been M de, Lanza VF, Toro M de, Scharringa J, Dohmen W, Du Y, et al. Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. PLoS Genet 2014;10:e1004776.

[30] Lanza VF, Toro M de, Garcillán-Barcia MP, Mora A, Blanco J, Coque TM, et al. Plasmid flux in Escherichia coli ST131 sublineages, analyzed by plasmid constellation network (PLACNET), a new method for plasmid reconstruction from whole genome sequences. PLoS Genet 2014;10: e1004766.

[31] Wyres KL, Lam MMC, Holt KE. Population genomics of *Klebsiella pneumoniae*. Nat Rev Microbiol 2020;18:344—59.