Attributable sources of community-acquired carriage of *Escherichia coli* containing β-lactam antibiotic resistance genes: a population-based modelling study

Lapo Mughini-Gras, Alejandro Dorado-García, Engeline van Duijkeren, Gerrita van den Bunt, Cindy M Dierikx, Marc J M Bonten, Martin C J Bootsma, Heike Schmitt, Tine Hald, Eric G Evers, Aline de Koeijer, Wilfrid van Pelt, Eelco Franz, Dik J Mevius*, Dick J J Heederik*, on behalf of the ESBL Attribution Consortium

Summary

Background Extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC), plasmid-mediated AmpC-producing *E coli* (pAmpC-EC), and other bacteria are resistant to important β-lactam antibiotics. ESBL-EC and pAmpC-EC are increasingly reported in animals, food, the environment, and community-acquired and health-care-associated human infections. These infections are usually preceded by asymptomatic carriage, for which attributions to animal, food, environmental, and human sources remain unquantified.

Methods In this population-based modelling study, we collected ESBL and pAmpC gene data on the Netherlands population for 2005–17 from published datasets of gene occurrences in *E coli* isolates from different sources, and from partners of the ESBL Attribution Consortium and the Dutch National Antimicrobial Surveillance System. Using these data, we applied an established source attribution model based on ESBL-EC and pAmpC-EC prevalence and gene data for humans, including high-risk populations (ie, returning travellers, clinical patients, farmers), farm and companion animals, food, surface freshwater, and wild birds, and human exposure data, to quantify the overall and gene-specific attributable sources of community-acquired ESBL-EC and pAmpC-EC intestinal carriage. We also used a simple transmission model to determine the basic reproduction number (R_0) in the open community.

Findings We identified 1220 occurrences of ESBL-EC and pAmpC-EC genes in humans, of which 478 were in clinical patients, 454 were from asymptomatic carriers in the open community, 103 were in poultry and pig farmers, and 185 were in people who had travelled out of the region. We also identified 6275 occurrences in non-human sources, including 479 in companion animals, 4026 in farm animals, 66 in wild birds, 1430 from food products, and 274 from surface freshwater. Most community-acquired ESBL-EC and pAmpC-EC carriage was attributed to human-to-human transmission within or between households in the open community (60·1%, 95% credible interval 40·0–73·5), and to secondary transmission from high-risk groups (6·9%, 4·1–9·2). Food accounted for 18·9% (7·0–38·3) of carriage, companion animals for 7·9% (1·4–19·9), farm animals (non-occupational contact) for 3·6% (0·6–9·9), and swimming in freshwater and wild birds (ie, environmental contact) for 2·6% (0·2–8·7). We derived an R_0 of 0·63 (95% CI 0·42–0·77) for intracommunity transmission.

Interpretation Although humans are the main source of community-acquired ESBL-EC and pAmpC-EC carriage, the attributable non-human sources underpin the need for longitudinal studies and continuous monitoring, because intracommunity ESBL-EC and pAmpC-EC spread alone is unlikely to be self-maintaining without transmission to and from non-human sources.

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Introduction

Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (ESBL-EC) and plasmid-mediated AmpC (pAmpC)-producing *E coli* (pAmpC-EC) are important causes of treatment failure with β-lactam antibiotics, which include widely used antibiotics of critical importance for human therapy. ESBLs and pAmpCs are a public health concern because these enzymes render bacteria non-susceptible to third-generation cephalosporins, resulting in increased use of last-resort antibiotics like carbapenems.[1] Historically, ESBL-EC and pAmpC-EC infections have been associated with health-care settings,[2] but their acquisition in the community has been increasingly reported in several countries since 2000–10.[3] Although a high prevalence of ESBL-EC and pAmpC-EC has been reported in livestock (particularly poultry),[4] chicken meat,[2] and companion animals,[5] their role as sources of human infection with ESBL-EC and

Research in context

Evidence before this study

Bacteria that produce extended-spectrum β-lactamase (ESBL) and plasmid-mediated AmpC (pAmpC) enzymes can inactivate important antibiotics, such as cephalosporins and monobactams, which then lose their efficacy. Occurrence of ESBLs and pAmpCs is the result of several factors, including antibiotic use or misuse in humans and animals, which promotes ESBL and pAmpC selection and transmission in bacterial populations, and human factors such as mobility of people and trade of goods, as well as environmental contamination. Human exposure to ESBLs and pAmpCs can occur via animals, food, the environment, and human-to-human transmission. However, the relative contributions of these transmission routes are unknown. Source attribution methods have been widely used for foodborne pathogens, allowing the identification of the main sources of human infections. These findings have proven crucial for the development of effective public health interventions. For antimicrobial resistance, few comprehensive microbial subtyping datasets for different sources are available—a cornerstone of some of these methods—preventing their routine application. We did a PubMed search, with no language restrictions, for articles published between database inception and Jan 1, 2019, using the search terms "source attribution" and "antimicrobial resistance", from which we identified only six relevant publications that used microbial subtyping data or exposure assessments for multiple sources of ESBLs and pAmpCs. Despite recognition of the importance of source attribution for foodborne pathogens and the growing use of these approaches, source attribution of antimicrobial resistance as such is still in its infancy. Three of six publications referred to antimicrobial susceptibility patterns as a typing method for *Salmonella* source attribution but did not focus on the sources of specific resistance genes. Two comparative exposure assessments were restricted to estimating the relative contributions of different types of meat to ESBLs and AmpCs in humans. Only one study described similarities in ESBL and pAmpC genes among different sources.

Added value of this study

So far, statements regarding prioritisation of interventions targeting the sources of community-acquired carriage of ESBL-producing and pAmpC-producing *Escherichia coli* have been based on prevalence and similarities in microbial typing data for only a few sources at once. To our knowledge, this is the first study to quantitatively estimate attributions for ESBLs and AmpCs, derived from modelling of large datasets for multiple animal, food, environmental, and human sources. Our analysis provides quantitative links between specific ESBL and pAmpC genes in *E coli* and their human and non-human sources, and shows that most community-acquired carriage of ESBL-producing and pAmpC-producing *E coli* is attributable to human-to-human transmission, followed by food, animal, and environmental sources. Deriving a basic reproduction number also showed that although humans are the main source of community-acquired carriage of ESBL-producing and pAmpC-producing *E coli*, human-to-human transmission within the open community alone might not be self-maintaining without transmission to and from non-human sources.

Implications of all the available evidence

Our study shows the complex links of ESBL-producing and pAmpC-producing *E coli* among humans, animal sources, the food chain, and the environment. Because humans are estimated to be the most important source in the open community, hygiene and responsible use of antibiotics in health care and veterinary medicine remain important pillars of prevention. Although the direct contributions of animals, food, and surface freshwater were estimated to be smaller, they entail large reservoirs of infection, contamination, and dissemination of ESBLs and pAmpCs to which humans remain continuously exposed and subsequently contribute to further spread of ESBLs and pAmpCs among individuals. Therefore, continuous monitoring of antimicrobial resistance in humans, animals, and other sources is crucial to detect changes in trends and dynamics, underpinning the need for longitudinal studies, because carriage of ESBLs and pAmpCs is temporal and the importance of some sources might fluctuate over time.

pAmpC-EC remains controversial.[2,6,7] Human exposure to ESBL-EC and pAmpC-EC might occur via animals, food, the environment, and human-to-human transmission; however, their relative contributions are yet to be quantified.

Cross-sectional studies in the Netherlands report a prevalence of approximately 5% for ESBL-EC and pAmpC-EC intestinal carriage in the open community.[8–12] Because ESBL-EC and pAmpC-EC human infections are often preceded by asymptomatic carriage,[13] a crucial step towards breaking the transmission chain is to identify their sources. Here, we applied an established source attribution model based on nationally representative ESBL-EC and pAmpC-EC gene, prevalence, and human exposure data to quantify the relative contributions of

several sources to community-acquired ESBL-EC and pAmpC-EC intestinal carriage in the Netherlands.

Methods

Study design and data collection

In this population-based modelling study, we collected data on ESBL and pAmpC gene occurrences in *E coli* isolates in the Netherlands for 2005–17. We used a published dataset containing ESBL and pAmpC gene occurrences among 5808 *E coli* isolates from different sources.[7] This dataset, which was derived from 35 studies, comprises over 27 000 samples; full details of these studies have been reported previously.[7] Briefly, peer-reviewed publications reporting ESBL and pAmpC gene data in the Netherlands during 2000–15 were

systematically reviewed and studies were included only if they examined *E coli* with data for ESBL genes of *CTX-M*, *TEM*, or *SHV* families, with restrictions on whether the studies were in humans or non-humans (full details of the eligibility criteria have been reported previously).[7] Partners of the ESBL Attribution (ESBLAT) Consortium[12] and the Dutch National Antimicrobial Surveillance System (MARAN) provided additional data. For this study, additional ESBL-EC and pAmpC-EC gene data for 2016–17 were included from the ESBLAT Consortium and MARAN. We categorised frequencies of ESBL genes of the *CTX-M*, *TEM*, or *SHV* families associated with phenotype 2be,[14] and frequencies of pAmpC genes of the *CMY*, *ACC*, *ACT*, *MIR*, or *DHA* families by source. Because multiple gene occurrences in the same isolate are rare, we arranged them in a mutually exclusive way.

For occurrences of ESBL-EC and pAmpC-EC genes in humans, those in patients with clinical ESBL-EC or pAmpC-EC infections or requiring admission to hospital or stay in long-term care facilities, or both, in the past 12 months for any cause were considered as being from a high-risk group (according to the most common ESBL-EC and pAmpC-EC carriage persistence period in health-care-associated and clinically infected individuals[15,16]) and separated from those identified in the samples of asymptomatic individuals in the open community—ie, carriers. Other high-risk groups were farmers occupationally exposed to poultry and pigs[17] and people who had returned from travelling to Africa, Asia, or South or Central America in the past 4 weeks (defined according to destinations posing an increased risk for ESBL-EC and pAmpC-EC carriage and the most common period for carriage persistence among Dutch travellers[10,18]). Hence, the open community was defined as any individual who had not travelled to these regions in the past 4 weeks, who had not had a clinical infection caused by ECBL-EC or pAmpC-EC or another health condition requiring medical attention in the past 12 months, and who had not engaged in farming activities involving direct occupational contact with poultry or pigs.

We also identified several non-human sources of ESBL-EC and pAmpC-EC gene occurrences and the number of samples that were positive and negative for ESBL-EC and pAmpC-EC in these sources: companion animals (ie, dogs, cats, horses), farm animals (ie, cattle, pigs, chickens, and sheep and goats), wild birds, meat products (ie, chicken meat, turkey meat, bovine meat, pork, and sheep and goat meat), raw vegetables, seafood, and surface freshwater (appendix pp 1–4). The magnitude of exposure of the open community to each of these sources was derived from five large population-based studies in the Netherlands.[10,12,19–21] For food sources, exposure was defined as the probability of a person in the open community having consumed these products at least once in the past 4 weeks. For farm and companion animals, exposure was defined as the probability of a person in the open community having non-occupational (hobby or recreational) direct contact with these animals at least once in the past 4 weeks. For wild birds, exposure was more generally defined as touching or feeding wild birds, cleaning bird feeders or other areas covered with bird droppings, collecting bird feathers, or contact with a dead bird at least once in the past 4 weeks. For surface freshwater, exposure was defined as the probability of a person in the open community having had direct contact with surface freshwater through swimming at least once in the past 4 weeks. For human sources (high-risk groups), exposure was defined as the probability of a person in the open community encountering a traveller who returned from Africa, Asia, or South or Central America fewer than 4 weeks ago; an individual who had a clinical infection caused by ESBL-EC or pAmpC-EC, or another health conditions requiring medical attention (ie, exposed to health-care settings, including hospitals and long-term care facilities) in the past 12 months; or a person who had been engaged in farming activities entailing occupational direct contact with poultry and pigs in the past 4 weeks. Inversely proportional to the exposure to high-risk groups, the exposure to the open community was defined as the probability of an individual belonging to the open community having encountered another individual in the same population. Input parameters and data origins are in the appendix (pp 3–4).

Data analysis

We used a commonly applied source attribution model for pathogens based on typing data, the modified Hald model,[22] with some adaptations for our study compared with its previous applications, for example, for *Salmonella*,[22,23] *Campylobacter*,[22] *Listeria*,[24] and *Shiga* toxin-producing *E coli*.[25] Our analysis was based on ESBL-EC and pAmpC-EC genes in the open community as the group to be attributed to the aforementioned human and non-human sources. Additionally, because ESBL-EC and pAmpC-EC transmission also occurs among people,[16,18] the open community was also set as a potential source of ESBL-EC and pAmpC-EC for the open community itself.

The model assumes that the observed frequency of ESBL-EC or pAmpC-EC gene i (with $i=1$–41) in the open community, denoted as o_i, is given by

$$o_i \sim poisson \left(\textstyle\sum_j \lambda_{ij} \right)$$

with λ_{ij} being the expected frequency of gene i from source j (with $j=1$–20), estimated as

$$\lambda_{ij} = p_{ij} \times m_j \times q_i \times a_j$$

where p_{ij} is the prevalence of gene i in source j, given by $\pi_j \times r_{ij}$, with π_j being the overall ESBL-EC and pAmpC-EC prevalence in source j, and r_{ij} being the relative frequency of gene i in source j. q_i is the gene-dependent factor, which accounts for differences in the success (ie, fitness) of *E coli* carrying ESBL or pAmpC gene i to colonise

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individuals in the open community (eg, persistence, virulence), and a_j is the source-dependent factor, which accounts for the ability of source j to act as a vehicle for ESBL-EC and pAmpC-EC (eg, differences in bacterial concentration, source characteristics influencing bacterial growth and survival, preparation and handling procedures, and differences in sensitivity and randomness of sampling between studies). Both q_i and a_j are unknown and therefore fitted by the model on specification of uninformative priors (appendix pp 3–4). m_j is the exposure weight, a scaling factor for a_j that accounts for the magnitude of exposure to each source j in the open community—ie, the proportion of individuals directly exposed to each source. Details of model parameterisation are in the appendix (pp 3–4). We obtained posterior distributions using a Markov Chain Monte Carlo simulation. Five independent Markov chains were run for 50 000 iterations after a burn-in period (ie, omission of iterations at the beginning of the simulation to minimise the effect of the initial values on the posterior estimates) of 10 000 iterations, which was able to provide convergence according to Gelman-Rubin's method.[26]

We identified which genes were present only in the open community, which were only in sources (ie, high-risk humans, food, animals, and environment), and which were present both in the open community and the sources. Genes only found in sources were kept in the model to preserve the within-source gene relative frequencies, as advised in previous studies.[22,25]

To test our primary results, we did several validation analyses. The model uses the similarities of ESBL-EC and pAmpC-EC gene frequencies as anchor points for attribution, using prevalence and human exposure data as scaling factors and q_i and a_j to account for differential gene-specific and source-specific transmission efficiencies. By letting the open community be both the target and one of the sources, we introduced a strong human-to-human linkage in gene frequencies. Although such linkages are factual (ie, two human carriers are more likely to carry the same ESBL-EC or pAmpC-EC gene than, for example, a human and a chicken), we checked whether such linkages influenced the attributions in a way that would mainly reflect gene resemblances using an independent dataset in which the true situation was known. We applied our model to non-typhoid *Salmonella* serotyping data from a previous Dutch study,[23] including humans as a potential source. Humans are not an important source of non-typhoid salmonellosis because this infection is zoonotic in nature, generally short-lived, and has almost no asymptomatic carriage (prevalence of 0·05% in a 2016 Dutch study).[27] By knowing a priori that humans do not contribute substantially to non-typhoid salmonellosis, we checked whether our model reflected this low attribution.

Our model provided attribution estimates only to potential sources of direct transmission for the open community—ie, we quantified only the last step of the transmission chain. Therefore, all sources were treated as routes of direct transmission, not as reservoirs per se. Indeed, in each source, differential growth, fade, or persistence of *E coli* is possible, so selection leads to distinctive ESBL-EC and pAmpC-EC gene profiles, with prevalence and human exposure differing too, which render these sources quite different from one another. Yet, food sources could be argued to be just vehicles of most of the ESBL-EC and pAmpC-EC of their respective animal reservoirs from which food is contaminated (eg, chickens and chicken meat), similarly for surface water and human and animal faeces. Because human groups, food, animals, and environmental sources were included as separate sources, the model treated them independently, which might introduce uncertainty if they are difficult to distinguish between. Therefore, for comparison purposes, we also applied an alternative model with the corresponding animal-food sources grouped together. Moreover, because swimming in surface freshwater occurs almost exclusively during the warm season, whereas exposure to the other sources is not as seasonal, we did an additional source attribution analysis considering only the summer period (June–August).

We used a simple transmission model to determine the basic reproduction number (R_0) in the open community, considering a 5% equilibrium prevalence of community-acquired ESBL-EC and pAmpC-EC, with and without assuming random mixing (eg, the household clustering effect; appendix pp 7–10).[8–12]

Posterior quantities of interest from the Markov Chain Monte Carlo simulation were mean values and 95% Bayesian credible intervals (CrIs) of percent attributions. We also report SDs and median values of the posterior distribution. We used 95% CIs as the measure of precision for R_0.

We used OpenBUGS 3.2 for the Markov Chain Monte Carlo simulation and R and Microsoft Excel for data storage, statistical calculations, and graphics.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
The frequencies of ESBL-EC and pAmpC-EC genes in the Netherlands for 2005–17 are shown in figure 1 and the appendix (pp 1–2). We identified 1220 occurrences of ESBL-EC and pAmpC-EC genes in humans, of which 478 were in clinical patients, 454 were in carriers in the open community, 103 were in poultry and pig farmers, and 185 were in people who had travelled out of the region. We identified 6275 occurrences of ESBL-EC and pAmpC-EC genes in non-human sources, of which 479
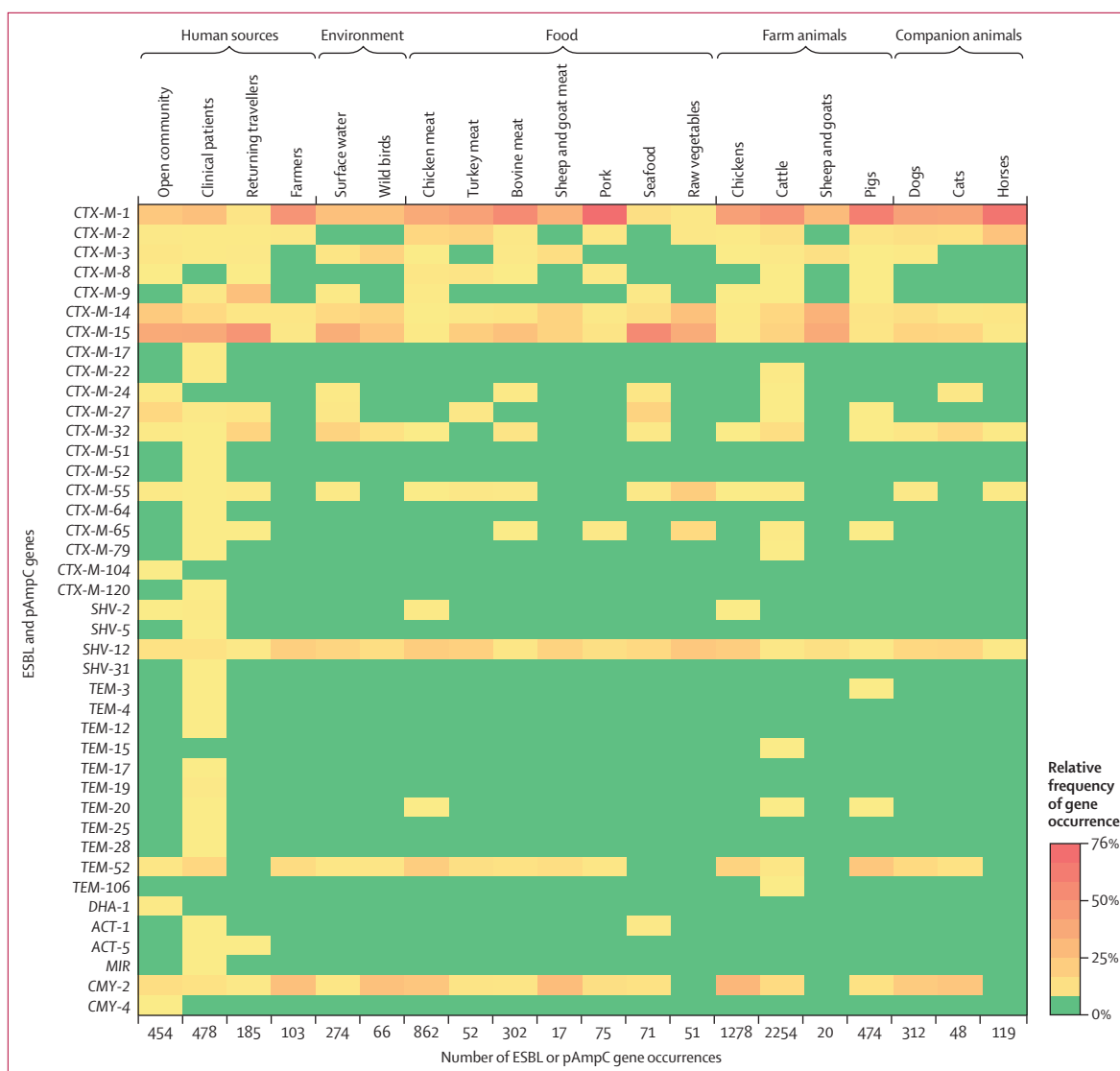
Figure 1: Frequencies of ESBL-encoding and pAmpC-encoding genes detected in *Escherichia coli* isolates from human and non-human sources in the Netherlands, 2005–17
Heat map shows the relative frequencies of ESBL and pAmpC genes occurring in the *E coli* isolates from each source. These data are also reported in extended format in the appendix (pp 1–2). Retuning travellers are defined as having travelled to Africa, Asia, or South or Central America in the past 4 weeks; clinical patients as having an ESBL-producing or pAmpC-producing *E coli* infection or having been admitted to hospital or a long-term care facility, or both, for any reason during the past 12 months; and farmers as people who work occupationally with pigs or poultry. ESBL=extended-spectrum β-lactamase. pAmpC=plasmid-mediated AmpC.

were from companion animals (n=312 dogs, n=48 cats, and n=119 horses); 4026 from farm animals (n=2254 cattle, n=474 pigs, n=1278 chickens, and n=20 sheep and goats); 66 from wild birds; 1308 from meat products (n=862 chicken meat, n=52 turkey meat, n=302 bovine meat, n=75 pork, and n=17 sheep and goat meat); 51 from raw vegetables; 71 from seafood; and 274 from surface freshwater (figure 1; appendix pp 1–2). For all sources, the number of ESBL-positive or pAmpC-positive samples in which these genes occurred is shown in the appendix (pp 1–2) and is slightly smaller or equal to the number of genes. Figure 2 shows the posterior distributions of ESBL-EC and pAmpC-EC prevalence of, and human

exposure to, each source. The final dataset comprised 7495 ESBL and pAmpC gene occurrences belonging to 41 different ESBL and pAmpC genes, 17 of which were found in the open community and in at least one of the sources, whereas the other genes were only found in sources.

Attribution estimates are reported in the table. The ESBL-EC and pAmpC-EC carriers in the open community were estimated to be the primary source of ESBL-EC and pAmpC-EC carriage in the open community itself (60·1%, 95% CrI 40·0–73·5). Secondary transmission from high-risk groups accounted for 6·9% (4·1–9·2) of community-acquired
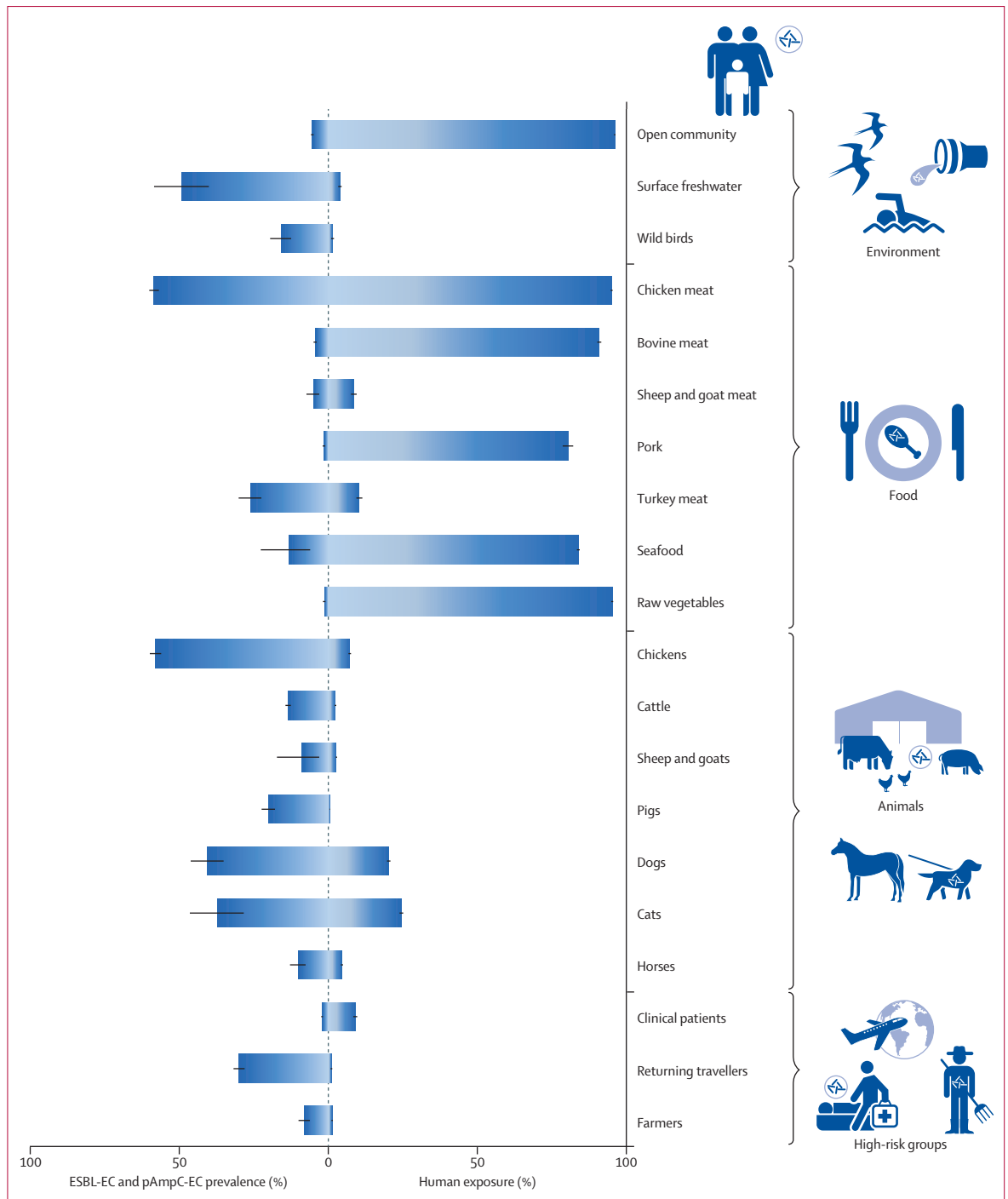
Figure 2: Prevalence of ESBL-EC and pAmpC-EC isolates in each source and probability of exposure of the open community to each source
Error bars are 95% credible intervals. ESBL-EC=extended-spectrum β-lactamase-producing *Escherichia coli*. pAmpC-EC=plasmid-mediated AmpC-producing *E coli*.

carriage of ESBL-EC and pAmpC-EC. Food sources accounted for 18·9% (7·0–38·3) of community-acquired carriage of ESBL-EC and pAmpC-EC (table). Non-occupational (ie, hobby or recreational) contact with farm animals accounted for 3·6% (0·6–9·9) and

companion animals accounted for 7·9% (1·4–19·9) of community-acquired carriage of ESBL-EC and pAmpC-EC. Swimming in surface freshwater and contact with wild birds accounted for 2·6% (0·2–8·7) of community-acquired carriage of ESBL-EC and pAmpC-EC. In

summer, swimming in surface freshwater accounted for 5·7% (0·2–19·7) of community-acquired carriage (appendix pp 11–12).

ESBL and pAmpC gene-specific attributions are shown in figures 3 and 4. Of the ESBL-EC and pAmpC-EC genes with more than 20 occurrences in the open community (appendix pp 1–2), *CTX-M-15*, *CTX-M-14*, and *CTX-M-27* were strongly associated with human-to-human transmission, with 70% of *CTX-M-15* occurrences, 83% of *CTX-M-14* occurrences, and 84% of *CTX-M-27* occurrences being attributed to the carriers in the open community themselves (figure 3), while seafood was the second most important source for *CTX-M-15* (10%) and *CTX-M-27* (10%). Approximately 45% of *CTX-M-1* occurrences were attributed to the carriers in the open community, followed by dogs (10%), and bovine meat (9%) and chicken meat (9%; figure 3). *SHV-12* and *CMY-2* were mainly attributed to the carriers in the open community (41% for *SHV-12* and 45% for *CMY-2*), followed by chicken meat (14% for *SHV-12* and 14% for *CMY-2*). Posterior distributions for q_i and a_j are reported in the appendix (pp 5–6).

In our validation analyses, as anticipated, the model for non-typhoid salmonellosis (appendix p 13) including humans as a potential source estimated humans to have a very small contribution (0·054%, 95% CrI 0·001–0·233), supporting the hypothesis that the high resemblance in subtypes did not introduce an artifact into our main analysis. Moreover, the model in which the sources from the same reservoirs were grouped together provided comparable attributions (in terms of ranking, proportionality, and uncertainty) to those of the main analysis differentiating between animal and food sources (appendix p 14), showing that including animals and foods separately had no significant consequences.

The baseline ESBL-EC and pAmpC-EC prevalence of 5% and estimated 60·1% contribution of intra-community transmission corresponded to an R_0 of 0·63 (95% CI 0·42–0·77). Additional structure with household clusters lowered the R_0 (appendix pp 9–10); with up to 50% of first acquisitions occurring within households, the effect of clustering was small.

Discussion

Prioritisation of interventions targeting potential sources of community-acquired ESBL-EC and pAmpC-EC carriage has often been based on prevalence and molecular data. Although highly informative, to our knowledge, no attempts have been made so far to quantify the contributions of different sources in a comparative way. Here, we showed that human-to-human transmission in the open community is estimated to have a greater impact than several putative sources of direct ESBL-EC and pAmpC-EC transmission, such as direct contact with farm and companion animals, some environmental sources, and consumption of meats, seafood, and raw vegetables. Our estimates arose from distinguishable patterns of

	Mean (95% CrI)	Median	SD
Human sources			
Human-to-human transmission in the open community	60·1% (40·0–73·5)	61·3%	8·7
Secondary transmission from high-risk groups	6·9% (4·1–9·2)	6·9%	1·3
Returning travellers	3·9% (2·3–5·5)	3·9%	0·8
Clinical patients	2·0% (1·2–2·6)	2·0%	0·4
Poultry and pig farmers	1·0% (0·5–1·6)	1·0%	0·3
Food consumption and preparation	18·9% (7·0–38·3)	17·6%	8·1
Seafood	6·6% (0·3–21·6)	5·1%	5·8
Chicken meat	4·5% (0·2–13·1)	3·7%	3·5
Bovine meat	3·6% (0·1–12·5)	2·7%	3·3
Turkey meat	1·8% (0–6·1)	1·3%	1·6
Raw vegetables	1·1% (0–3·9)	0·8%	1·1
Pork	0·9% (0–3·3)	0·6%	0·9
Sheep or goat meat	0·4% (0–1·6)	0·3%	0·4
Animals			
Contact with companion animals	7·9% (1·4–19·9)	7·0%	4·9
Dogs	5·1% (0·2–16·3)	3·9%	4·4
Cats	2·4% (0·1–8·0)	1·9%	2·2
Horses	0·5% (0–1·7)	0·3%	0·5
Non-occupational contact with farm animals	3·6% (0·6–9·9)	3·0%	2·5
Chickens	2·8% (0·1–9·0)	2·1%	2·4
Cattle	0·4% (0–1·4)	0·3%	0·4
Sheep or goats	0·3% (0–1·1)	0·2%	0·3
Pigs	0·1% (0–0·5)	0·1%	0·1
Environment	2·6% (0·2–8·7)	1·9%	2·3
Swimming in surface freshwater	2·3% (0·1–8·4)	1·6%	2·3
Contact with wild birds	0·3% (0–1·1)	0·2%	0·3

The percentage attributions of intestinal carriage of ESBL or pAmpC gene detections in *E coli* isolates from individuals of the open community (n=454) to the different human and non-human sources. ESBL=extended-spectrum β-lactamase. pAmpC=plasmid-mediated AmpC. CrI=credible interval.

Table: **Estimated attributions of each considered source of intestinal carriage of ESBL or pAmpC gene-carrying *Escherichia coli* detected in the open community in the Netherlands, 2005–17**

ESBL-EC and pAmpC-EC gene occurrence in different sources.

Livestock, particularly poultry, did not appear to be the primary source of carriage. Poultry has had considerable attention due to its high ESBL-EC and pAmpC-EC prevalence,[2] but its importance as a source for humans is debatable.[6] Although parallel occurrence of ESBL and pAmpC genes, plasmids, or bacterial clones in poultry and humans has been considered as evidence of poultry being an important source of infection,[2] previous whole-genome sequencing has not shown significant clonal transmission from poultry to humans via consumption of chicken meat.[17] Yet, whole-genome sequencing did show the possibility of direct transmission between, for example, pigs and their caretakers via occupational exposure, and the predominance of identical ESBL-carrying IncI1-plasmids in cephalosporin-resistant *E coli* suggested a prominent role of mobile

genetic elements.[17] Moreover, a previous analysis of molecular linkages in a large collection of ESBL-EC and pAmpC-EC isolates (which were included in our study) showed that ESBL-EC and pAmpC-EC gene distribution in the open community does not have a high level of similarity with livestock and food.[7] Risk assessments have also shown that human exposure to ESBL-EC and pAmpC-EC via meat consumption and swimming in
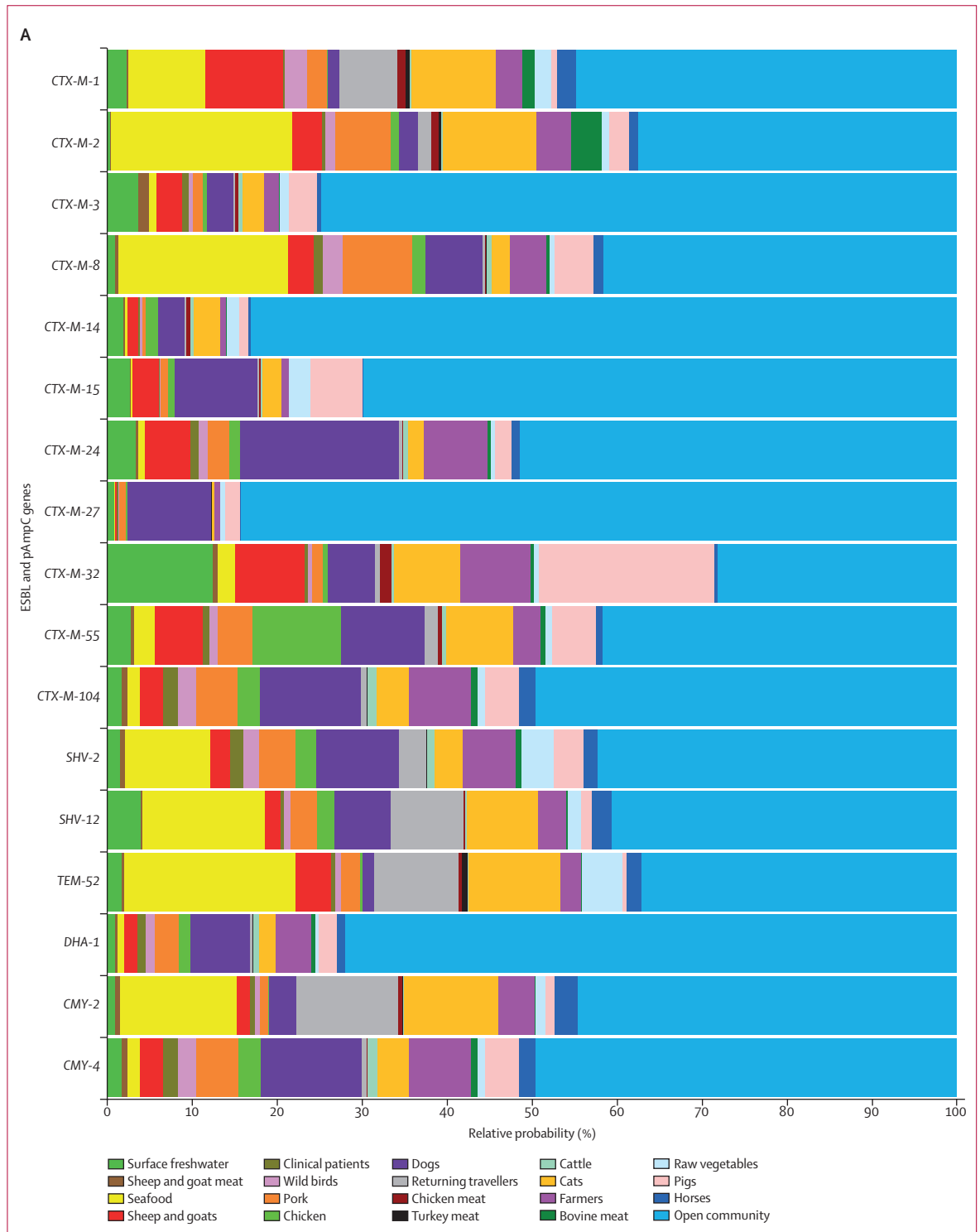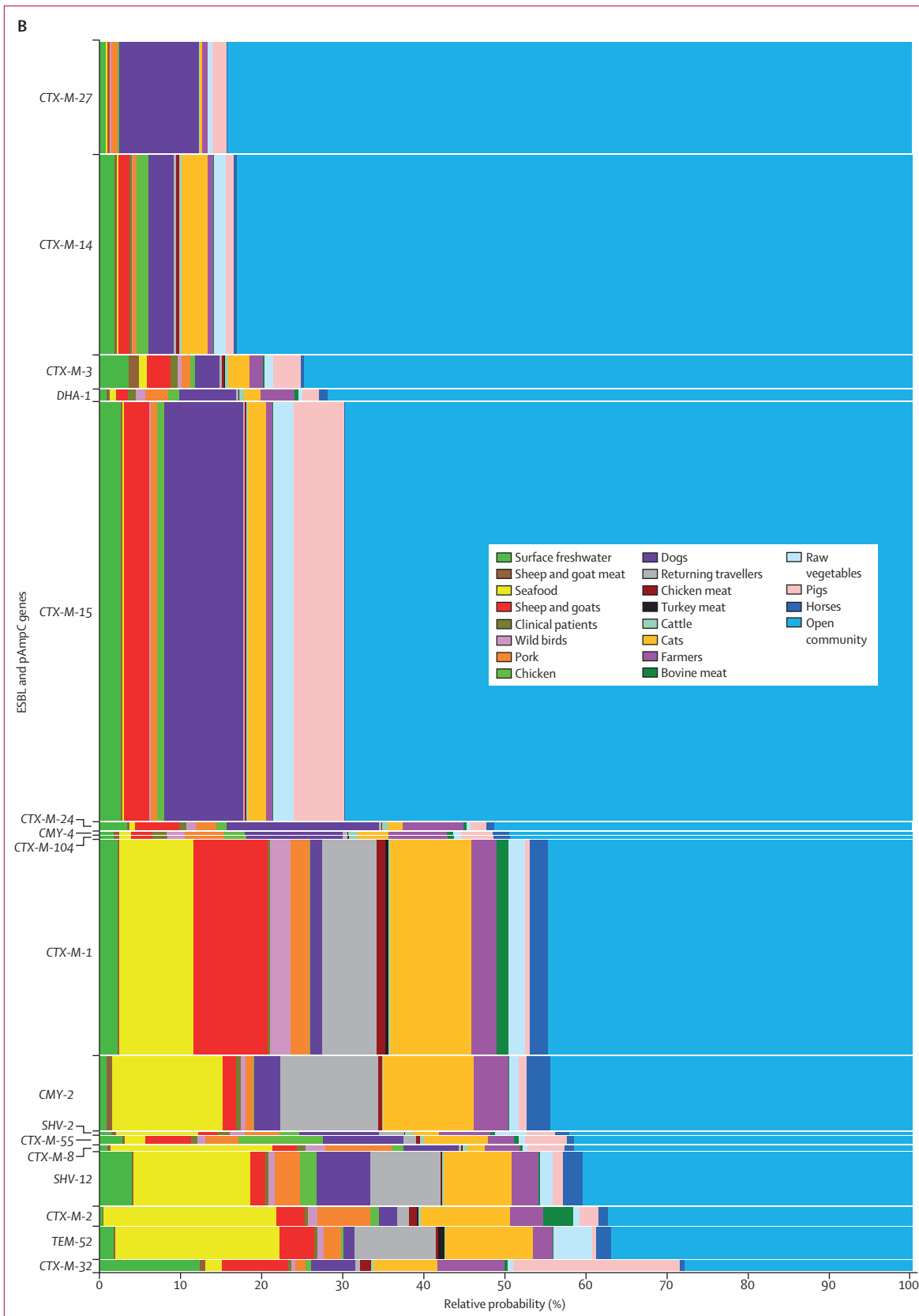

(Figure 3 continues on next page)

B

Figure 3: **ESBL and pAmpC gene-specific attributions for intestinal carriage of *Escherichia coli* in the open community**
(A) Relative probabilities (%) for each ESBL-EC and pAmpC-EC gene in the open community originating from each source as estimated by the source attribution model; the number of occurrences for each gene is reported in figure 1. (B) Relative probabilities (%) for all ESBL-EC and pAmpC-EC genes in the open community originating from each source as estimated by the source attribution model, with the number of gene occurrences in the open community illustrated by the height of the row. ESBL=extended-spectrum β-lactamase. pAmpC=plasmid-mediated AmpC. ESBL-EC=ESBL-producing *E coli*. pAmpC-EC=pAmpC-producing *E coli*.
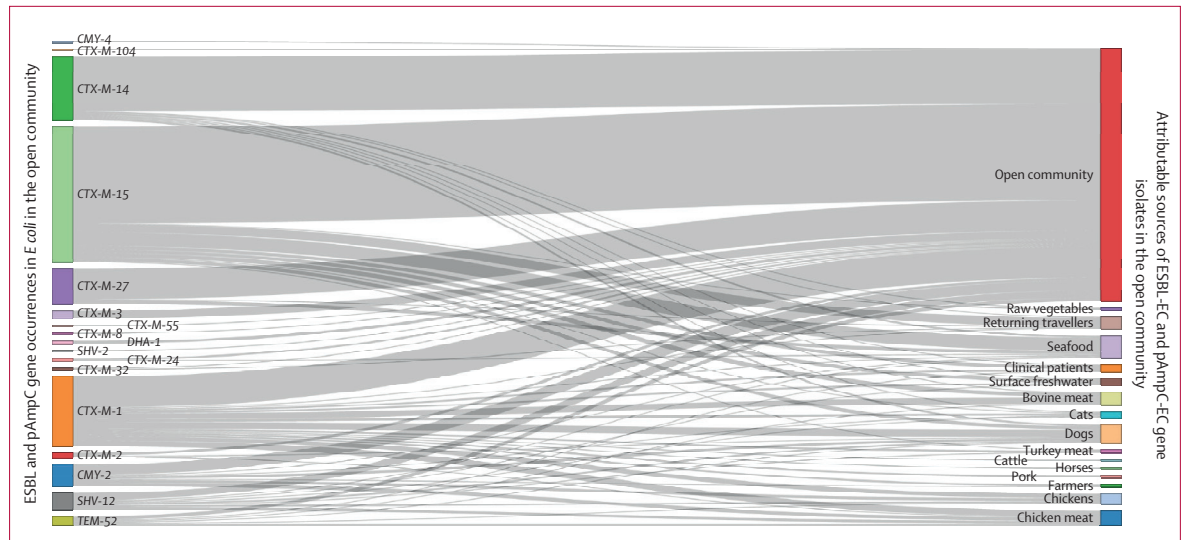
Figure 4: **Sankey diagram of the flow of ESBL and pAmpC genes found in *Escherichia coli* isolates in the open community to and from their attributable sources**
The width of the coloured boxes and their connecting grey bands are directly proportional to the frequency of gene occurrence in the open community (left side) and flow quantities of these genes to the attributable sources (right side). Only sources with at least one full gene occurrence attributed to them are included. ESBL=extended-spectrum β-lactamase. pAmpC=plasmid-mediated AmpC. ESBL-EC=ESBL-producing *E coli*. pAmpC-EC=pAmpC-producing *E coli*.

surface freshwater is generally low.[12,28] In a 2018 study,[29] a large collection of *E coli* genomes (including ESBL-EC and pAmpC-EC) from livestock and retail meat in the UK were compared with those from bloodstream infections. Core-genome analysis showed that livestock and patient isolates were genetically distinct, and accessory genome analysis (ie, the genome containing genes that are not present in all strains of a species) identified a virulence cassette associated previously with cystitis and neonatal meningitis that was only present in humans. We also found little evidence that most ESBL-EC and pAmpC-EC in humans originated from livestock. Moreover, a Dutch study[16] on household transmission of ESBL-producing bacteria suggested that, although no single dominant acquisition route in the community exists, the estimated probability of transmission from an index patient to a household contact is 67%. Studies of children have also shown that the only factors predicting carriage of ESBL-producing and pAmpC-producing bacteria were related to hygiene and cleaning practices,[8,9] further indicating that anthroponotic transmission of ESBL-EC and pAmpC-EC contributes substantially to total transmissions. Our finding of a relatively large attributable fraction of ESBL-EC and pAmpC-EC recirculation in the open community led to a higher R_0 than previously estimated.[16,18] However, these previous estimates were only based on in-household transmission from patient or returning traveller index cases and ignored other modes of anthroponotic transmission. When adding clustering to such transmission (as increased human-to-human contact is expected to occur in, for example, households, workplaces, and schools), the R_0 decreased, suggesting that our estimate was actually an upper bound. Moreover, we assumed ESBL-EC and pAmpC-EC prevalence to be

at quasiequilibrium—ie, adapting reasonably fast to changes in source populations, which is supported by the lack of differences in resistance to third-generation and fourth-generation cephalosporins among Dutch clinical isolates in 2013–17.[30] However, before this time period, prevalence showed periods of increase,[30,31] so our R_0 estimate might be conservative. Because we could only attribute human ESBL-EC and pAmpC-EC to sources and not vice versa, hypothetically, the R_0 in either group could be less than 1, whereas the R_0 in the whole system could be more than 1, meaning that the leading transmission route is still anthroponotic, albeit not self-maintaining without transmission to and from non-human sources.

Herein, we only considered ESBL-EC and pAmpC-EC genes. These genes can be acquired horizontally through mobile genetic elements like plasmids, independently of cell division, in a variety of intestinal and extraintestinal environments. Thus, full understanding of ESBL-EC and pAmpC-EC spread depends on the characterisation of both ESBL and pAmpC genes and the plasmids carrying them, and the *E coli* sequence types. Human-adapted *E coli* variants, such as sequence type 131, which is often reported to carry *CTX-M* genes (especially *CTX-M-15*),[12] might then be more favourable for transmission than other strains. Other source attribution studies might therefore provide additional insights if they include plasmid data, and possibly sequence types too. Indeed, although different sources have different *E coli* populations, they might carry identical ESBLs and pAmpCs because of horizontal gene transfer via plasmids. Therefore, bacterial variants might be less important than plasmids for source attribution. Additionally, resistance genes can be transferred between the chromosome and

plasmids and between different plasmids, although some genes are associated with specific plasmid families and might therefore mirror one another.[7] The resistance gene itself was therefore the most robust infective unit for this analysis.

The ESBL-EC and pAmpC-EC genes in the open community were obtained from large population-based studies in the Netherlands and were representative of the gene landscape in the Dutch general population.[7] This approach implies that we also captured the full spectrum of individuals in the community, including those more susceptible to colonisation with, rather than more exposed to, ESBL-EC and pAmpC-EC because of underlying conditions, unless they were exposed to health-care settings, in which case they belonged to the clinical patient group and acted as potential source of secondary transmission for the open community. While an overall estimate of the relative contributions of the different sources to all the ESBL-EC and pAmpC-EC genes occurring in the open community was possible, variation in gene-specific attributions was substantial. Although our dataset was based on several studies that were subject to strict scrutiny to ensure representativeness, some heterogeneity in terms of spatiotemporality, study design, and analytical methods was present, as addressed and discussed in the previous publication of this dataset.[7] Moreover, differentiating between imported and domestic foods or animals was not possible, and ESBL-EC and pAmpC-EC gene occurrences from sources like chickens, cattle, and meats were more exhaustively represented than sources like seafood, sheep and goats, and surface water. Molecular similarities in ESBL-EC and pAmpC-EC between humans and surface waters,[7] while being suggestive of transmission, might also indicate a contribution of human wastewater to ESBL-EC and pAmpC-EC contamination in surface waters.[32] Similarly, the ESBL-EC and pAmpC-EC genes in wild birds and fish and shellfish are usually exchanged through surface waters. Surface waters might therefore also act as a vector for local transmission between humans and animals. Nevertheless, the contribution of surface freshwater is seasonally dependent, as shown in the higher attribution during summer than across the whole year. We found ESBL-EC and pAmpC-EC to be quite prevalent in seafood, which is compelling because in the Netherlands several seafood products are regularly consumed raw, undercooked, cured, or smoked (eg, oysters, scallops, herrings, salmon, eel, sushi). Because fishery has received relatively little attention regarding ESBL-EC and pAmpC-EC, relies heavily on imports (also from areas where antimicrobial use in aquaculture is widespread), and mirrors the larger problem of antimicrobial resistance in surface waters, further studies in this area are advised. Similarly, further studies are recommended for raw vegetables contaminated via irrigation. ESBL-EC and pAmpC-EC prevalence and concentration in raw vegetables are low,[33] although fashionable culinary trends like decorative sprouts and juices and smoothies might favour transmission. Both seafood and vegetables might be enriched with ESBL-EC and pAmpC-EC genes of human origin via human wastewater. Indeed, seafood is increasingly farmed in coastal aquacultures, close to surface freshwater sources and coastal run-off, which provide opportunities for accumulation of human-borne ESBL-EC and pAmpC-EC genes, especially in filter-feeding organisms. The same applies to surface freshwater-irrigated vegetables and swimming in surface freshwater. Thus, these sources are likely part of a larger human metacycle, whereby humans are re-infected with human-adapted ESBL-EC and pAmpC-EC. If this cycle were taken into account, we expect that the contribution of food to ESBL-EC and pAmpC-EC spread would be lower than estimated here. The same would also apply to companion animals; indeed, dogs and cats have dynamic ESBL-EC and pAmpC-EC shedding patterns and are a source in close vicinity to humans.[5] Contact with farm animals, while posing a risk for those occupationally exposed,[4] has a less important role in the open community owing to the low exposure levels.

We considered a broad spectrum of putative sources; however, our list was far from complete. No data were available for analysis for several animal, food, and environmental sources (eg, non-avian wildlife, pests, pets other than dogs and cats, dairy, cereals, seawater, different fomites, soil, dust, air). Hence, we might have overestimated the contribution of some of the considered sources, including the human ones. Moreover, data were not longitudinal in nature. Consequently, our calculated attributions are a snapshot of a situation that might change over time because carriage is temporal and the importance of some sources might fluctuate. We used an extensively applied source attribution model,[22] with humans also included as sources, which is a deviation from the standard attribution framework for pathogens like *Salmonella* for which unidirectionality of transmission (from animal, food, or environmental sources to humans, with humans as the endpoint) is always an assumption. This deviation allowed for the quantification of the anthroponotic contribution alongside the non-human sources, which were all potential sources of direct transmission, although we did not explicitly account for transmission cycles within or between sources. The modified Hald model is ecological in nature whereby populations are the unit of investigation, making it vulnerable to bias from large unrecognised outbreaks linked to a source, which undermine causal inference. However, ESBL-EC and pAmpC-EC are commensals, and although successful clonal expansions do happen, their epidemiology is different from that of pathogens like *Salmonella*, for which point-source outbreaks occur continuously. Moreover, the studies from which data for the open community were collected were cross-sectional (not longitudinal surveillance data), excluded

recognised outbreaks, contained few household contacts (approximately 7%), and reported a high diversity of *E coli* sequence types where available,[7] indicating that outbreaks were unlikely and supporting the hypothesis that the attributions reflected sporadically acquired ESBL-EC and pAmpC-EC.

ESBL-EC and pAmpC-EC occurrence is the result of several factors, including antibiotic use and misuse in humans and animals. Our analysis showed an important role of human-to-human transmission, indicating that same-species transmission predominates, possibly due to increased chance of horizontal gene transfer or higher fitness of human-associated *E coli* strains carrying the ESBL and pAmpC genes in question. Yet, our analysis could not discern whether positive selection leading to emergence or spread of new variants of ESBL-EC and pAmpC-EC genes in humans mainly takes place in non-human sources, or whether these genes are subsequently propagated in the open community after successful spillover. Indeed, continuous exposure to ESBL-EC and pAmpC-EC from non-human sources could be speculated to only sporadically result in successful transfer of ESBL and pAmpC genes to human-adapted *E coli* or other bacteria, or to successful adaptation (eg, to the human gut) of *E coli* strains carrying the ESBL or pAmpC genes in question. From a broader perspective, our finding of a relatively large fraction of community-acquired carriage of ESBL-EC and pAmpC-EC attributable to anthroponotic sources is actually a reassuring finding, because with an R_0 of less than 1 continuous growth is unlikely. This finding would also explain the relatively stable ESBL-EC and pAmpC-EC prevalence of approximately 5% that has been repeatedly measured across the past two decades in the Netherlands.[8–12] To discern whether the non-human sources might act as natural boosts (eg, new introductions, possibly from abroad) to revive the baseline prevalence (which might not maintain itself with only human-to-human transmission), we need time-dependent source attribution analyses based on longitudinal data.

Although complex dynamics are involved in ESBL-EC and pAmpC-EC transmission, our results provide quantitative links between specific ESBL-EC and pAmpC-EC genes in the open community and their probable human and non-human sources of direct transmission. Approximately two-thirds of community-acquired ESBL-EC and pAmpC-EC carriers are attributable to human-to-human transmission, with the considered non-human sources accounting for the other third. While anthroponotic sources prevail, our findings underpin the need for longitudinal studies and warrant continuous monitoring in both human and non-human populations, because intracommunity ESBL-EC and pAmpC-EC spread alone seems unlikely to be self-maintaining without transmission to and from non-human sources. Transmission routes of antibiotic resistance are complex, with numerous interconnected cycles and subcycles involving different hosts and environments. Because resistant bacteria might pass into humans from animals and food, and via environment-mediated and human-to-human transmission, a One Health approach is needed that values interdisciplinarity and stresses the connections between public, animal, and environmental health, and provides an integrated framework for improving our understanding of the global threat of antimicrobial resistance.

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