Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains

M. A. Leverstein-van Hall[1,2], C. M. Dierikx[3], J. Cohen Stuart[1], G. M. Voets[1], M. P. van den Munckhof[1], A. van Essen-Zandbergen[3], T. Platteel[1,4], A. C. Fluit[1], N. van de Sande-Bruinsma[2], J. Scharinga[1], M. J. M. Bonten[1,5] and D. J. Mevius[3,6]; on behalf of the national ESBL surveillance group*

1) *Department of Medical Microbiology, University Medical Centre Utrecht, Utrecht,* 2) *Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven,* 3) *Department of Bacteriology and TSEs, Central Veterinary Institute of Wageningen UR, Lelystad,* 4) *SALTRO, Primary Health Care Laboratory, Utrecht,* 5) *Julius Centre for Health Sciences and Primary Care, University Medical Centre, Utrecht and* 6) *Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands*

Abstract

Intestinal carriage of extended-spectrum beta-lactamase (ESBL) -producing bacteria in food-producing animals and contamination of retail meat may contribute to increased incidences of infections with ESBL-producing bacteria in humans. Therefore, distribution of ESBL genes, plasmids and strain genotypes in *Escherichia coli* obtained from poultry and retail chicken meat in the Netherlands was determined and defined as 'poultry-associated' (PA). Subsequently, the proportion of *E. coli* isolates with PA ESBL genes, plasmids and strains was quantified in a representative sample of clinical isolates. The *E. coli* were derived from 98 retail chicken meat samples, a prevalence survey among poultry, and 516 human clinical samples from 31 laboratories collected during a 3-month period in 2009. Isolates were analysed using an ESBL-specific microarray, sequencing of ESBL genes, PCR-based replicon typing of plasmids, plasmid multi-locus sequence typing (pMLST) and strain genotyping (MLST). Six ESBL genes were defined as PA ($bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{SHV\text{-}2}$, $bla_{SHV\text{-}12}$, $bla_{TEM\text{-}20}$, $bla_{TEM\text{-}52}$): 35% of the human isolates contained PA ESBL genes and 19% contained PA ESBL genes located on IncI1 plasmids that were genetically indistinguishable from those obtained from poultry (meat). Of these ESBL genes, 86% were $bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}52}$ genes, which were also the predominant genes in poultry (78%) and retail chicken meat (75%). Of the retail meat samples, 94% contained ESBL-producing isolates of which 39% belonged to *E. coli* genotypes also present in human samples. These findings are suggestive for transmission of ESBL genes, plasmids and *E. coli* isolates from poultry to humans, most likely through the food chain.

Keywords: Antibiotic resistance, *Escherichia coli*, human, multi-locus sequence typing, plasmid, poultry, zoonosis
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Corresponding author: M. A. Leverstein-van Hall, Department of Medical Microbiology, Internal post number G04-614, University Medical Center Utrecht, Heidelberglaan 100, 3584CX Utrecht, the Netherlands
E-mail: m.leversteinvhall@umcutrecht.nl
*The national ESBL surveillance group are listed in the Appendix.

Introduction

There is a worldwide increase in infections caused by Gram-negative bacteria producing extended spectrum beta-lactamases (ESBL), even in a low-resistance country such as the Netherlands [1]. This is remarkable because the Netherlands have low levels of antibiotic usage and have been successful in controlling nosocomial spread of other multi-resistant bacteria [2–4].

In contrast to human antibiotic use, antibiotic use in the poultry industry is higher in the Netherlands than in any other European country [5]. The prevalence of ESBL-producing *Escherichia coli* in the gastrointestinal tract of healthy food-producing animals, especially poultry, increased from 3% in 2003 to 15% in 2008 and in 2009 ESBL-producing bacteria were detected in all 26 of 26 broiler farms studied [6,7]. Furthermore, contamination of retail chicken meat

with ESBL-producing Gram-negative bacteria has been documented in several countries [8–10].

For these reasons the poultry industry has been considered a potential reservoir of ESBL-producing Gram-negative bacteria that may be acquired by humans through handling or consumption of contaminated meat. We therefore determined the distribution of ESBL genes, plasmids and strain genotypes in *E. coli* obtained from poultry and retail chicken meat in the Netherlands and defined these as 'poultry associated' (PA). Subsequently, we quantified the proportion of *E. coli* isolates with PA related ESBL genes, plasmids and strains in a large and representative sample of clinical *E. coli* isolates from Dutch patients.

Methods

Isolates

Retail chicken meat. Between April and June 2010, 98 fresh raw chicken breasts were purchased in 12 stores in Utrecht, the Netherlands. Seventy-eight of the samples were purchased at nine stores belonging to six supermarket chains (Dutch market share of 90%) and 20 from three different butcheries. Information about the region where the chickens were raised was available for 30 supermarket samples (27% Netherlands, 73% Benelux). For culture methods see Supplementary material Data S1.

Poultry. The poultry isolates were derived from the Dutch surveillance programme on antibiotic resistance in bacteria isolated in food-producing animals in 2006 [11]. The sampling strategy in this programme aims to obtain annual collections of *E. coli* and *Salmonella enterica*, representative of the Dutch food-producing animal bacterial populations. Twelve percent (22 *E. coli* and 22 *S. enterica*) of all isolates were cefotaxime resistant. ESBL genes were identified in 35 of these: 17 (49%) $bla_{\text{CTX-M-1}}$, ten (29%) $bla_{\text{TEM-52}}$, four (11%) $bla_{\text{TEM-20}}$, three (9%) $bla_{\text{CTX-M-2}}$, and one (3%) $bla_{\text{SHV-2}}$ [12]. The 27 $bla_{\text{CTX-M-1}}$ and $bla_{\text{TEM-52}}$ positive isolates were included.

Human. From 1 February 2009 until 1 May 2009, 31 Dutch laboratories submitted all *E. coli* with a positive ESBL screen test (MIC > 1 mg/L for cefotaxime or ceftazidime or an ESBL alarm from the Phoenix or Vitek-2 expert system) [13]. For each isolate the following data were collected: age, gender, material and institution (hospital, general practitioner (GP), long-term care facility (LTCF)). From each laboratory the first 25 consecutive isolates (if available), one isolate per patient, were included. The participating laboratories are geographically dispersed over the Netherlands and represent a mixture of secondary and tertiary care hospitals, LTCFs and GPs. The laboratories serve a total of 58 hospitals, covering approximately 45% of all hospital beds in the Netherlands.

Molecular analyses

The presence of ESBL genes was determined by microarray analysis [14] and gene sequencing. All human isolates were investigated by microarray and sequencing was performed on a random selection of 50%. Among the retail isolates all morphologically different ESBL-positive *E. coli* from three meat samples of each available packaging type (whole breast or sliced) per store were analysed by sequencing.

Plasmid analysis was performed on a random selection of human and poultry isolates carrying either a $bla_{\text{CTX-M-1}}$ or a $bla_{\text{TEM-52}}$ gene. All plasmids were characterized using PCR-based replicon typing (PBRT) [12,15]. The association between ESBL gene and plasmids was determined either by Southern blot hybridization or transformation [12]. IncI1 plasmids were typed by plasmid multi-locus sequence typing (pMLST) [16].

Isolates were genotyped by MLST (http://www.mlst.net). Among the human isolates 27 were genotyped: isolates with documented presence of $bla_{\text{CTX-M-1}}$ or $bla_{\text{TEM-52}}$ genes on an IncI1 plasmid (n = 15) and a random selection (n = 12) of all other isolates carrying a $bla_{\text{CTX-M-1}}$ or $bla_{\text{TEM-52}}$.

Among the poultry isolates, all 22 isolates with either $bla_{\text{CTX-M-1}}$ or $bla_{\text{TEM-52}}$ located on IncI1 plasmids were selected for genotyping.

From the retail isolates with a $bla_{\text{CTX-M-1}}$, $bla_{\text{SHV-12}}$ or $bla_{\text{TEM-52}}$ gene, 23 isolates were randomly selected for genotyping.

Results

Distribution of ESBL genes

Retail chicken meat. Of the 98 chicken retail meat samples, 92 (94%) samples contained at least one *E. coli* isolate with an ESBL phenotype, yielding 163 isolates (average number per sample 2; range 1–4). From 48 samples, 81 isolates cultured were further analysed. The array confirmed the presence of an ESBL gene in all isolates: 40 CTX-M-1-group, 21 TEM-3-group, 13 SHV-4-group, three SHV-2-group, three CTX-M-2-group and one TEM-19-group. By sequencing one ESBL gene was identified in each of these six different ESBL groups: $bla_{\text{CTX-M-1}}$, $bla_{\text{TEM-52}}$, $bla_{\text{SHV-12}}$, $bla_{\text{SHV-2}}$, $bla_{\text{CTX-M-2}}$ and $bla_{\text{TEM-20}}$, respectively. These genes were considered as PA. The $bla_{\text{CTX-M-1}}$ and $bla_{\text{TEM-52}}$ accounted for 75% of the genes (Table 1). The $bla_{\text{SHV-12}}$ gene was not detected in poultry in 2006, but has been detected in poultry isolates obtained in 2009 (D. Mevius, personal communication).

TABLE 1. Distributions of extended-spectrum beta-lactamase (ESBL) genes in *Escherichia coli* and Salmonella spp. isolates from poultry, poultry retail meat samples and from human origin based on array results combined with sequence results

Poultry-associated ESBL genes	Poultry *n* = 35	Poultry meat samples[a] *n* = 81	Human[a] *n* = 409
$bla_{CTX\text{-}M\text{-}1}$ (%)	49	49	24
$bla_{TEM\text{-}52}$ (%)	29	26	6
$bla_{SHV\text{-}12}$ (%)	0	16	4
$bla_{SHV\text{-}2}$ (%)	11	4	0.4
$bla_{CTX\text{-}M\text{-}2}$ (%)	9	4	0.2
$bla_{TEM\text{-}20}$ (%)	3	1	0
Total (%)	100	100	35

The number of isolates analysed by array among meat and human isolates was 81 and 409, respectively. The number of isolates analysed by sequencing among poultry, meat and human isolates was 35 (100%), 81 (100%) and 208 (51%), respectively.
[a]Percentages are extrapolations based on array results and sequence results. For calculation of the percentages see also Fig. 1. For example percentage of $bla_{CTX\text{-}M\text{-}1}$ in human isolates = 0.84 × 0.85 × 0.34 = 24%.

Human samples. In the study period, 1017 *E. coli* were ESBL screen positive, from which 516 were included (Fig. 1). The median number per laboratory was 17 (range 7–25) and per hospital was 10 (range 0–21). The proportion of isolates derived from non-university hospitals was 54%, from GPs was 30%, from university medical centres was 6% and from LTCFs was 5% (5% unknown).

Based on the microarray results, 409 (79%) isolates contained an ESBL gene, and in 344 (84%) of these the ESBL genes were potentially PA (Fig. 1; rows A and B). Sequence results of 208 randomly selected isolates identified five ($bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{TEM\text{-}52}$, $bla_{SHV\text{-}2}$ or $bla_{SHV\text{-}12}$) of the six PA genes (Fig. 1; row C). The $bla_{TEM\text{-}20}$ gene was not detected in any of the human isolates.

The proportion of $bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}52}$ genes among all ESBL genes detected in clinical isolates was similar in five different age groups (0–4, 20–39, 40–59, 60–79, >80 years) and in four different geographic regions. The proportion of $bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}52}$ genes was similar among isolates submitted by GPs (33%; 23/70; 95% CI: 22–44), non-academic hospitals (26%; 27/104; 95% CI: 18–34), LTCFs (26%; 4/14; 95% CI: 5–52) and academic hospitals (37%; 3/8; 95% CI: 4–71). The 27 isolates that were MLST genotyped were obtained from 17 different laboratories. Of these, 23 (85%) were urine isolates, 19 (70%) came from GPs and none came from the same facility.

Plasmid analysis and isolate typing

Human isolates. The PBRT was performed on 15 of 51 human isolates with a $bla_{CTX\text{-}M\text{-}1}$ gene and on six of 14 human isolates with a $bla_{TEM\text{-}52}$ gene (Table 3; Fig. 1; rows C and D). Nine of the 15 $bla_{CTX\text{-}M\text{-}1}$ genes and all six of the $bla_{TEM\text{-}52}$ (i.e. 15/21; 71%) were located on an IncI1 plasmid.

The pMLST demonstrated that seven of the nine $bla_{CTX\text{-}M\text{-}1}$/IncI1 plasmids (78%) belonged to pMLST Clonal Complex CC7 and pMLST sequence type ST7 (CC7/ST7), one to CC3/ST3 and one to CC31/ST35 (Table 3; Fig. 1; row E).

Isolate genotyping demonstrated that six of the seven CC7/ST7 isolates belonged to the PA MLST types: ST10 (*n* = 1), ST58 (*n* = 3), ST117 (*n* = 2) (Table 3; Fig. 1; row F).

The pMLST analysis of the six $bla_{TEM\text{-}52}$/IncI1 plasmids demonstrated that five were ST36 (CC5) and one was ST10 (CC5), which differ in a single locus (one mutation in the *sogS*-gene).

Genotyping revealed that two isolates belonged to PA ST10 (Table 3; Fig. 1; rows E and F).

Typing by MLST of 13 randomly selected isolates demonstrated among ten $bla_{CTX\text{-}M\text{-}1}$ positive isolates three PA genotypes (ST117, ST57, ST354) and among three $bla_{TEM\text{-}52}$ positive isolates one PA genotype (ST23).

Poultry isolates. The PBRT was performed on all 27 $bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}52}$ containing *E. coli* and *Salmonella*. Sixteen (of 17) $bla_{CTX\text{-}M\text{-}1}$ and six (of 10) $bla_{TEM\text{-}52}$ genes were located on an IncI1 plasmid (22/27; 81%) (Table 3).

Plasmid MLST of the 16 $bla_{CTX\text{-}M\text{-}1}$/IncI1 plasmids demonstrated that 12 (75%) (eight *E. coli*, four *Salmonella*) belonged to CC7/ST7 and one to CC7/ST30 (ST30 is a single-locus variant of ST7). One plasmid belonged to CC3/ST3 and two were non-typable.

Genotyping by MLST of the eight CC7/ST7 *E. coli* revealed ST10, ST48, ST58, ST117 and four STs not found among clinical or meat samples.

he pMLST of the six $bla_{TEM\text{-}52}$/IncI1 plasmids demonstrated that all six (two *E. coli*, four *Salmonella*) belonged to CC5/ST10. One of the *E. coli* belonged to genotype ST10.

Retail meat. Isolate genotyping was performed on 23 retail *E. coli* [nine $bla_{CTX\text{-}M\text{-}1}$ (five stores), seven $bla_{TEM\text{-}52}$ (four stores), seven $bla_{SHV\text{-}12}$ (five stores)]. Nine (39%) belonged to MLST types also found in human isolates: ST10 (*n* = 4), ST23 (*n* = 1), ST57 (*n* = 1), ST117 (*n* = 2), and ST354 (*n* = 1). One isolate belonged to ST48, which was like ST10 and ST117 also identified among the poultry isolates.

Genetic correlation between human, chicken meat and poultry isolates. These data revealed four sets of *E. coli* isolates of human and animal origin with indistinguishable ESBL genes, plasmids and isolate genotypes: (i) *E. coli* ST10 with $bla_{CTX\text{-}M\text{-}1}$ and IncI1/ST7 as human blood culture isolate and a poultry isolate, (ii) *E. coli* ST58 with $bla_{CTX\text{-}M\text{-}1}$ and IncI1/ST7 as three

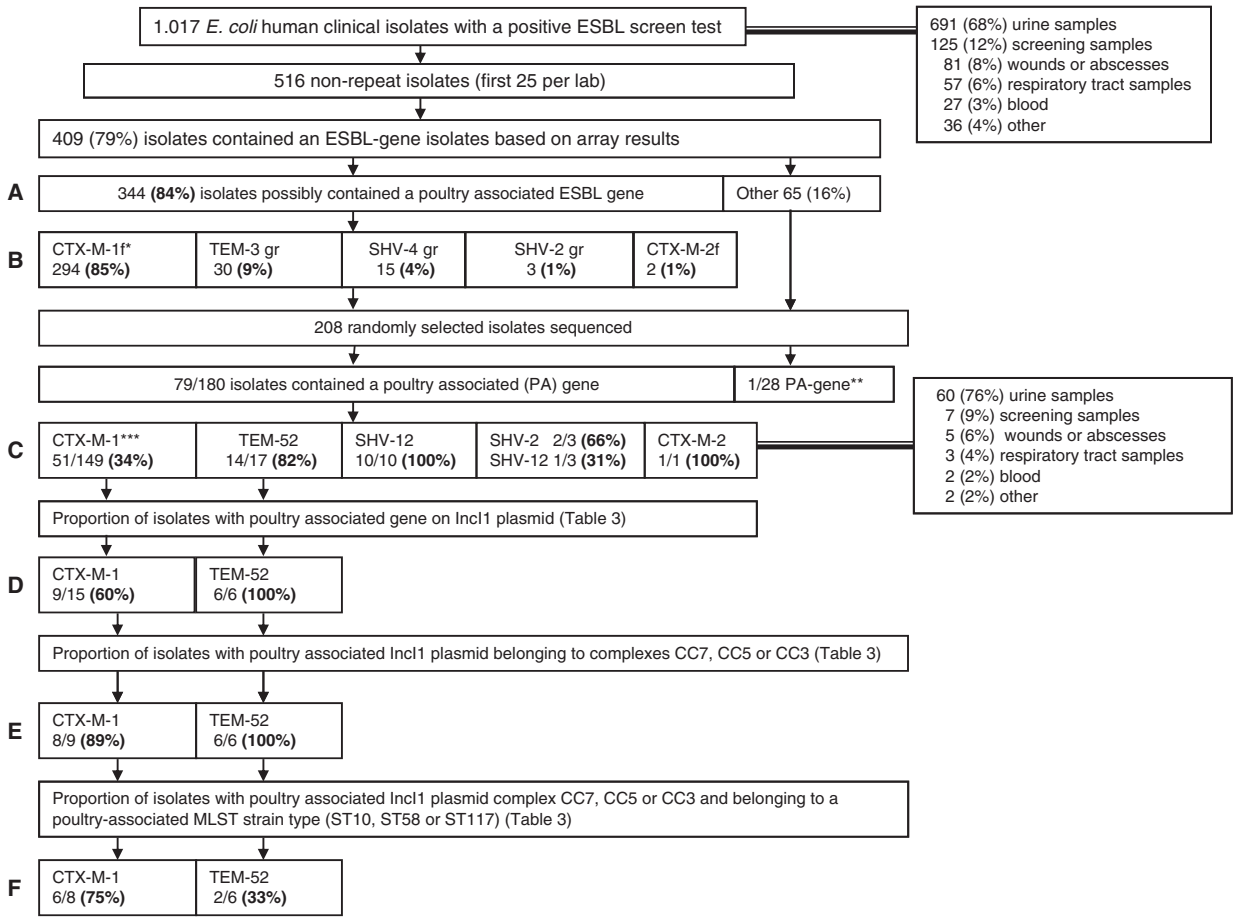

FIG. 1. Schematic view of methods and numbers of isolates used to determine the proportion of human *Escherichia coli* isolates with poultry-associated extended-spectrum beta-lactamase (ESBL) genes, plasmids and isolate genotypes. Percentages in bold were used to determine proportions (Table 2). *Nine isolates harboured next to the CTX-M-1 group another possibly poultry-associated gene: SHV-4-group gene (n = 5), a TEM-3-group gene (n = 3) and SHV-2-group gene (n = 1). **One isolate carried according to the array an ESBL gene of the SHV-31 group but sequencing results indicated the presence of a bla_{SHV-12}. ***Three isolates harboured two poultry-associated genes: $bla_{CTX-M-1}$ plus bla_{SHV-12} (n = 2) and $bla_{CTX-M-1}$ plus bla_{TEM-52} (n = 1).

human urine isolates from three different laboratories and a poultry isolate, (iii) *E. coli* ST117 with $bla_{CTX-M-1}$ and IncI1/ST7 as two human isolates from different laboratories and a poultry isolate, and (iv) *E. coli* ST10 with bla_{TEM-52} and IncI/ST10/36 was detected in two human urine samples from two laboratories and a poultry isolate. These four MLST genotype/ESBL gene combinations were also found in retail meat isolates (Table 3).

Quantification of the proportion of PA genes, plasmids and strains in human isolates

Based on these data we quantified the proportion of human ESBL-producing *E. coli* with PA genes, plasmids and isolates (Fig. 1 and Table 2).

On the level of ESBL genes 35% (95% CI: 30–39%) of the human ESBL isolates contained PA ESBL genes and $bla_{CTX-M-1}$ and bla_{TEM-52} accounted for the majority (30/35; 86%) (Tables 1 and 2).

Plasmid analysis was limited to bla_{TEM-52}-positive and $bla_{CTX-M-1}$-positive isolates. On the level of these two ESBL genes and plasmid family (i.e. IncI1) the proportion of human isolates genetically related to poultry isolates was 20% (95% CI: 17–25%). On the level of these ESBL genes, the presence of IncI1 plasmid and similar plasmid sequence types (CC3, CC5 or CC7), this proportion was 19% (95% CI: 15–23%). Finally, at the level of these ESBL genes, plasmid typing and MLST of the isolate, this proportion was 11% (95% CI: 8–14%) (Table 2).

Of the five ESBL-producing *E. coli* bloodstream isolates that were sequenced two contained a PA ESBL gene: $bla_{CTX-M-1}$ and bla_{TEM-52}. The $bla_{CTX-M-1}$ was located on the same plasmid (IncI1), from the same plasmid sequence type (CC7),

TABLE 2. The proportion of human extended-spectrum beta-lactamase (ESBL) -positive *Escherichia coli* isolates that is genetically related to ESBL-positive poultry isolates on the level of gene, plasmid and strain genotype[a]

Level of genetic typing	% of human isolates with poultry associated genetic element[a]
ESBL genes ($bla_{CTX-M-1}$, bla_{TEM-52}, bla_{SHV-12}, bla_{SHV-2} and $bla_{CTX-M-2}$)	35% (see Table 1)
$bla_{CTX-M-1}$ and bla_{TEM-52} genes	30% (23.7% $bla_{CTX-M-1}$; 6.2% bla_{TEM-52})
$bla_{CTX-M-1}$ and bla_{TEM-52} genes on IncI1 plasmid	20% (14.2% $bla_{CTX-M-1}$; 6.2% bla_{TEM-52})
$bla_{CTX-M-1}$ and bla_{TEM-52} genes on IncI plasmid belonging to complex CC7 or CC3 and CC5 resp.	19% (12.6% $bla_{CTX-M-1}$; 6.2% bla_{TEM-52})
$bla_{CTX-M-1}$ and bla_{TEM-52} genes on IncI plasmid belonging to complex CC7 or CC3 and CC5 resp. in a poultry-associated MLST strain (ST10, ST58 or ST117)	11% (9.5% $bla_{CTX-M-1}$; 2.0% bla_{TEM-52})

MLST, multi-locus sequence typing.
For example percentage of bla_{TEM-52} genes on IncI1 plasmid belonging to complex CC5 in to poultry identical MLST strains = 0.84 (row A) × 0.09 (row B) × 0.82 (row C) × 1 (row D) × 1 (row E) × 0.33 (row F) = 2.0%.
[a]Percentages are extrapolations based on array results, sequence results and results of plasmid characterization and strain typing. For calculation of the percentages see Fig. 1.

and belonged to the same MLST cluster (ST10) as was detected in a poultry isolate (Table 3). No plasmid analysis was performed on the bla_{TEM-52}-positive blood culture isolate, but all other isolates with bla_{TEM-52} that were investigated had the same plasmids as found in poultry isolates (Fig. 1; rows D and E).

Discussion

In a representative sample of human ESBL-positive *E. coli* isolates in the Netherlands, 35% contained ESBL genes and 19% contained ESBL genes located on plasmids that were genetically indistinguishable from those obtained in poultry isolates. The majority of these ESBL genes (86%) were $bla_{CTX-M-1}$ and bla_{TEM-52} genes, also the predominant genes in poultry (77%) and retail chicken meat (75%). Furthermore, 94% of a representative sample of chicken meat was contaminated with ESBL-producing *E. coli*, of which 39% belonged to genotypes also found in human samples.

These findings are suggestive for transmission of ESBL-producing *E. coli* from poultry to humans, most likely through the food chain. Although our findings do not unequivocally prove that the poultry reservoir is the source of infections in humans, there are four lines of circumstantial evidence that do support such a sequence of events.

First, the potential of animal-derived Enterobacteriaceae to cause infections in humans has been established in community outbreaks of *Salmonella* and enteropathogenic *E. coli* [17], and associations between *E. coli* colonization and infection in humans and exposure to retail chicken and other food sources have been reported [18–20]).

Second, the prevalence of $bla_{CTX-M-1}$ genes (24%) and bla_{TEM-52} (6%) among human *E. coli* is higher in the Netherlands than in most other countries [21–26].

Third, the increase of $bla_{CTX-M-1}$ and bla_{TEM-52} genes among human *E. coli* corroborates with an increase of these ESBL genes in poultry isolates in the Netherlands. The prevalence of cefotaxime-resistant *E. coli* in Dutch poultry started to increase in 2003 [6] and in a human surveillance study among 21 laboratories in the Netherlands in 2006, proportions of $bla_{CTX-M-1}$ and bla_{TEM-52} *E. coli* producers were 9% and 3%, respectively (Sandra Bernards; personal communication).

Fourth, in one study people working with poultry seemed to have a higher risk for intestinal carriage of ESBL-producing bacteria [7].

Our study was restricted to Dutch patients, poultry and poultry meat products. Yet, ESBL carriage by poultry and contamination of retail meat with ESBL-producing bacteria has also been demonstrated in other European countries [8,9,25–29].

Our study has limitations. First, the spectrum of PA ESBL genes was based on a single study in poultry in 2006 and the analysis of 98 retail chicken meat samples in 2010, and this spectrum was compared with human isolates obtained between February and May 2009. Naturally, it is impossible to directly link carriage among poultry in 2006 to contaminated meat samples in 2010 to infected humans in 2009. Yet, although the ESBL epidemiology is rapidly evolving, it seems unlikely that the spectrum of genes present in these three compartments has changed dramatically over the period of 4 years. In fact, the five genes identified in poultry in 2006 were all identified on meat in 2010, in both compartments $bla_{CTX-M-1}$ and bla_{TEM-52} genes accounted for 78% and 75% of ESBL genes and in both compartments strains with the same genotype were detected.

Second, the plasmid analysis was limited to a small selection of isolates with $bla_{CTX-M-1}$ and bla_{TEM-52} genes, only. The latter was a consequence of the extreme labour-intensity of these analyses.

Strengths of our study include the detailed molecular analyses and the inclusion of human isolates from a nationwide surveillance programme covering all aspects of the healthcare system and with an unbiased selection of isolates allowing, for the first time, the possibility to quantify the association between genetic relationships and incidence of infections in humans.

For example, during the study period 27 patients had an *E. coli* bacteraemia with a positive ESBL screen test. If,

TABLE 3. **Results of strain (multi-locus sequence typing) and plasmid typing (Inc-group and plasmid multi-locus sequence typing) of *bla*$_{CXT\text{-}M\text{-}1}$- and *bla*$_{TEM\text{-}52}$-producing *Escherichia coli* isolates from human patients and *E. coli* and *Salmonella enterica* isolates from poultry sources**

					Plasmid typing		IncI1 typing		*E. coli* strain typing
ESBL-gene	Strain code	Origin	Species	Material	ESBL localization	Plasmid size (kb)	Clonal complex	Sequence type	Sequence type
bla$_{CTX\text{-}M\text{-}1}$	148	Human	*E. coli*	Blood	IncI1	100	**CC7**	ST7	10
	38.27	Poultry	*E. coli*	Caecum	IncI1	88	**CC7**	ST7	10
	53a, 54a	Retail	*E. coli*	Chicken meat	n.d.	n.d.	n.d.	n.d.	10 (*n* = 2)
	1365	Human	*E. coli*	Urine	IncI1	100	**CC7**	ST7	58
	1350	Human	*E. coli*	Urine	IncI1	100	**CC7**	ST7	58
	1240	Human	*E. coli*	Urine	IncI1	95	**CC7**	ST7	58
	38.16	Poultry	*E. coli*	Caecum	IncI1	100	**CC7**	ST7	58
	1240	Human	*E. coli*	Urine	n.d.	n.d.	n.d.	n.d.	58
	897	Human	*E. coli*	Respiratory tract	IncI1	100	**CC7**	ST7	117
	1047	Human	*E. coli*	Rectal swab	IncI1	100	**CC7**	ST7	117
	38.52	Poultry	*E. coli*	Caecum	IncI1	100	**CC7**	ST7	117
	623	Human	*E. coli*	Urine	n.d.	n.d.	n.d.	n.d.	117
	39.26	Poultry	*E. coli*	Caecum	IncI1	100	**CC7**	ST7	48
	38.53	Poultry	*E. coli*	Caecum	IncI1	100	**CC7**	ST7	155
	38.49	Poultry	*E. coli*	Caecum	IncI1	97	**CC7**	ST7	641
	39.02	Poultry	*E. coli*	Caecum	IncI1	110	**CC7**	ST7	665
	39.05	Poultry	*E. coli*	Caecum	IncI1	97	**CC7**	ST7	752
	1247	Human	*E. coli*	Urine	IncI1	100	**CC7**	ST7	767
	162.03	Poultry	*S.* Java[b]	Unknown	IncI1	97	**CC7**	ST7	n.d.
	175.77	Poultry	*S.* Infantis	Unknown	IncI1	100	**CC7**	ST7	n.d.
	187.45	Poultry	*S.* Infantis	Caecum	IncI1	100	**CC7**	ST7	n.d.
	187.46	Poultry	*S.* Infantis	Caecum	IncI1	100	**CC7**	ST7	n.d.
	39.51	Poultry	*E. coli*	Caecum	IncI1	95	**CC7**	ST30	155
	691	Human	*E. coli*	Urine	IncI1	90	CC31	ST35	131
	1503	Human	*E. coli*	Urine	n.d.	n.d.	n.d.	n.d.	131
	39.47	Poultry	*E. coli*	Meat	IncI1	97	n.d.	Non-typable[a]	117
	186.74	Poultry	*S.* Java[b]	Caecum	IncI1	97	n.d.	Non-typable[a]	n.d.
	450	Human	*E. coli*	Urine	IncI1	95	**CC3**	ST3	167
	186.27	Poultry	*S.* Agona	Caecum	IncI1	110	**CC3**	ST3	n.d.
	990	Human	*E. coli*	Urine	IncB/O	95	n.d.	n.d.	n.d.
	1198	Human	*E. coli*	Urine	IncB/O	95	n.d.	n.d.	n.d.
	312	Human	*E. coli*	Urine	IncB/O	100	n.d.	n.d.	n.d.
	60	Human	*E. coli*	Urine	IncN	30	n.d.	n.d.	n.d.
	1455	Human	*E. coli*	Urine	IncN	35	n.d.	n.d.	n.d.
	627	Human	*E. coli*	Urine	Non-typable	30	n.d.	n.d.	n.d.
	13, 591, 416, 152, 179	Human	*E. coli*	Urine	n.d.	n.d.	n.d.	n.d.	69 (*n* = 2), 57, 162
	666, 152, 387	Human	*E. coli*	Urine	n.d.	n.d.	n.d.	n.d.	354, 453, 545
	52a, 54a, 72a, 71	Retail	*E. coli*	Chicken meat	n.d.	n.d.	n.d.	n.d.	23 (*n* = 2), 624, 1564
	60, 61, 63a, 69, 39b	Retail	*E. coli*	Chicken meat	n.d.	n.d.	n.d.	n.d.	1594 (*n* = 2), 1901, n.t. (*n* = 2)
bla$_{TEM\text{-}52}$	38.34	Poultry	*E. coli*	Caecum	IncI1	97	**CC5**	ST10	10
	320	Human	*E. coli*	Urine	IncI1	95	**CC5**	ST36	10
	681	Human	*E. coli*	Urine	IncI1	95	**CC5**	ST36	10
	85b	Retail	*E. coli*	Chicken meat	n.d.	n.d.	n.d.	n.d.	10
	68	Human	*E. coli*	Urine	IncI1	95	**CC5**	ST10	156
	39.76	Poultry	*E. coli*	Caecum	IncI1	90	**CC5**	ST10	752
	166.01	Poultry	*S.* Java[b]	Meat	IncI1	82	**CC5**	ST10	n.d.
	166.22	Poultry	*S.* Java[b]	Meat	IncI1	82	**CC5**	ST10	n.d.
	162.19	Poultry	*S.* Infantis	Unknown	IncI1	82	**CC5**	ST10	n.d.
	173.44	Poultry	*S.* Infantis	Caecum	IncI1	90	**CC5**	ST10	n.d.
	85	Human	*E. coli*	Urine	IncI1	90	**CC5**	ST36	131
	91	Human	*E. coli*	Urine	IncI1	90	**CC5**	ST36	Non-typable[c]
	1362	Human	*E. coli*	Urine	IncI1	90	**CC5**	ST36	453
	229, 194	Human	*E. coli*	Urine	n.d.	n.d.	n.d.	n.d.	23, 744
	45a, 47a, 83a, 90, 95a	Retail	*E. coli*	Chicken meat	n.d.	n.d.	n.d.	n.d.	23, 48, 117, 1403, n.t.

ESBL, extended-spectrum beta-lactamase; n.d., not determined; n.t., non-typable.
[a]Four sequences in conformance with pMLST ST7, but for locus ardA no sequence results were obtained.
[b]Salmonella enterica serovar paratyphi B variant Java.
[c]Six sequences in conformance with MLST ST767, but for locus icd no sequence results were obtained.
This table shows the genetic correlation between *E. coli* from patients and retail meat and *E. coli* and *Salmonella* from poultry carrying *bla*$_{CTX\text{-}M\text{-}1}$ or *bla*$_{TEM\text{-}52}$. The *E. coli* isolates were compared by Multi Locus Sequence Typing (http://www.mlst.net). All IncI1 plasmids were compared by pMLST and three genetically related clusters were found, indicated by bold face text: CC7, CC3 and CC5. There were four sets of *E. coli* isolates, of human and animal origin, with indistinguishable ESBL genes, plasmids and isolated genotypes, indicated in the table by different shading patterns (light grey, MLST ST10 (*n* = 2); middle grey, MLST 58; dark grey, MLST 117).

based on our results, 79% of these isolates contained an ESBL gene, this would imply 21 patients with ESBL bacteraemia. The ESBL genes from five of these isolates were sequenced and at least one and possibly two were PA. When extrapolated, at least one of 21 (5%), but possibly eight (38%) patients would have suffered an episode of PA *E. coli* bacteraemia. As the participating laboratories cover nearly half of Dutch hospital beds this would mean between two and 16 patients in the Netherlands between February and May 2009.

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Transparency Declaration

Conflicts of interest: nothing to declare.

Appendix: Members of the National ESBL Surveillance Group

Gunnar Andriesse, Jan P. Arends, Sandra T. Bernards, Marc J.M. Bonten, Els I.G.B. De Brauwer, Anton G.M. Buiting, James W. Cohen Stuart, Alje P. van Dam, Bram M.W. Diederen, J. Wendelien Dorigo-Zetsma, Andre Fleer, Ad C. Fluit, Arjanne van Griethuysen, Hajo Grundmann, Bea G.A. Hendrickx, Alphons M. Horrevorts, Jan A.J.W. Kluytmans, Maurine A. Leverstein-van Hall, Ellen M. Mascini, Bernard Moffie, Albert J. de Neeling, Tamara N. Platteel, Luc J.M. Sabbe, Nienke van de Sande, Claudia M. Schapendonk, Jelle Scharringa, Joop F.P. Schellekens, Fré W. Sebens, Frans S. Stals, Patrick Sturm, Steven F.T. Thijssen, Jeroen T. Tjhie, Liesbeth Verhoef, Bart J.M. Vlaminckx, Guido M. Voets, Willem H.M.Vogels, Rolf W. Vreede, Karola Waar, Peter C. Wever, Rob G.F. Wintermans, Maurice J.H.M. Wolfhagen.

University Medical Centre Utrecht, Dept of Med. Microbiology, Utrecht (M.A. Leverstein-van Hall, MD PhD, J.W. Cohen Stuart, MD PhD, A.C. Fluit, PhD, G.M. Voets, J. Scharringa, C.M. Schapendonk, T.N. Platteel, MD, Prof. M.J.M. Bonten, MD PhD); Onze Lieve Vrouwe Gasthuis, Dept of Med. Microbiology, Amsterdam (A.P. van Dam, MD PhD); Lievensberg Hospital, Lab. of Med. Microbiology, Bergen op Zoom (G. Andriesse, MD PhD); Amphia Hospital, Dept of Med. Microbiology, Breda (J.A.J.W. Kluytmans, MD PhD); Diagnostic Centre SSDZ, Dept of Med. Microbiology, Delft (R.W. Vreede, MD PhD); Deventer Hospital, Dept of Med. Microbiology and Infection Control Infectious Dis., Deventer (F.W. Sebens, MD); Admiraal De Ruyter Hospital, Dept of Med. Microbiology and Immunology, Goes (L.J.M. Sabbe, MD PhD); Laboratory for Infectious Diseases Groningen, Groningen (J.F.P. Schellekens, MD PhD, W.H.M.Vogels MD); University Medcal Centre Groningen, Lab. of Medical Microbiology, Groningen (Jan P. Arends, MD, H. Grundmann, MD PhD MSC); Central Bact. and Ser. Laboratory Hilversum/Almere, Dept of Med. Microbiology, Hilversum (J.W. Dorigo-Zetsma, MD PhD); Izore, Centre of Infect. Diseases Friesland, Leeuwarden (Karola Waar, MD PhD); St. Antonius Hospital, Dept of Med. Microbiology, Nieuwegein (B.J.M. Vlaminckx, MD PhD); Canisius Wilhelmina Hospital, Dept of Med. Microbiology, Nijmegen (A.M. Horrevorts MD PhD); University Medical Centre St Radboud, Dept of Med. Microbiology, Nijmegen (P. Sturm, MD PhD); Laurentius Hospital, Dept of Med. Microbiology, Roermond (F.S. Stals, MD); Franciscus Hospital, Lab. Of Med. Microbiology, Roosendaal (R.G.F. Wintermans, MD); Vlietland Hospital, Dept of Med. Microbiology, Schiedam (B.G. Moffie, MD); ZorgSam Hospital Zeeuwsvlaanderen, Lab. Of Med. Microbiology, Terneuzen (B.G.A. Hendrickx, MD PhD); Streeklaboratorium voor de Volksgezondheid, Tilburg (A.G.M. Buiting, MD PhD); SALTRO, Primary Health Care Laboratory, Dept of Med. Microbiology, Utrecht (L. Verhoef, MD PhD); Stichting PAMM, Lab. Of Med. Microbiology, Veldhoven (H.T. Tjhie, MD PhD); ISALA Clinics, Lab. of Med. Microbiology and Inf. Diseases (M.J.H.M. Wolfhagen, MD PhD); Streeklaboratorium voor de Volksgezondheid Kennemerland, Haarlem (B.M.W. Diederen, MD PhD); Diakonessenhuis, Dept of Med. Microbiology, Utrecht (S.F.T. Thijssen, MD PhD); Alysis Zorggroep, Dept of Med. Microbiology and Med. Immunology, Velp (E.M. Mascini, MD PhD, A. van Griethuysen, MD PhD); Jeroen Bosch Hospital, Reg. Lab. of Med. Microbiology and Inf. Control, Den Bosch (P.C. Wever, MD PhD); Gelre Hospitals, Dept of Med. Microbiology, Apeldoorn (A. Fleer, MD PhD); Atrium Medisch Centrum Parkstad, Dept of Med. Microbiology, Heerlen (E.I.G.B. De Brauwer); University Medical Centre Leiden, Dept of Med. Microbiology, Leiden (A.T. Bernards, MD PhD); Epidemiology and Surveillance (EPI), Centre for Infectious Disease Control (CIb), National Institute for Public Health and the Environment (RIVM), Bilthoven (M.A. Leverstein-van Hall, MD PhD, N. van de Sande-Bruinsma, PhD); Laboratory for Infectious Diseases and Perinatal Screening (LIS), Centre for Infectious Disease Control (CIb), National Institute for Public Health and the Environment (RIVM), Bilthoven (H. Grundmann, MD PhD MSC, A.J. de Neeling, PhD).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials.

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