J Antimicrob Chemother 2014; **69**: 2669–2675
doi:10.1093/jac/dku178 Advance Access publication 30 May 2014

Journal of Antimicrobial Chemotherapy

Extended-spectrum and AmpC β-lactamase-producing *Escherichia coli* in broilers and people living and/or working on broiler farms: prevalence, risk factors and molecular characteristics

P. M. C. Huijbers[1,2], E. A. M. Graat[2], A. P. J. Haenen[1], M. G. van Santen[1], A. van Essen-Zandbergen[3], D. J. Mevius[3,4], E. van Duijkeren[1]* and A. H. A. M. van Hoek[1]

[1]*Centre for Infectious Disease Control (CIb), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands;* [2]*Quantitative Veterinary Epidemiology Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, Wageningen, The Netherlands;* [3]*Department of Bacteriology and TSEs, Central Veterinary Institute (CVI) of Wageningen UR, Lelystad, The Netherlands;* [4]*Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

*Corresponding author. Tel: +31-(0)30-2743942; Fax: +31-(0)30-2744434; E-mail: engeline.van.duijkeren@rivm.nl

Received 28 January 2014; returned 18 March 2014; revised 28 April 2014; accepted 30 April 2014

Objectives: The objectives of this study were to: estimate the prevalence of extended-spectrum β-lactamase (ESBL)- and AmpC β-lactamase-producing *Escherichia coli* carriage among broiler farmers, their family members and employees; identify and quantify risk factors for carriage, with an emphasis on contact with live broilers; and compare isolates from humans and broilers within farms with respect to molecular characteristics to gain insight into transmission routes.

Methods: A cross-sectional prevalence study was conducted on 50 randomly selected Dutch broiler farms. Cloacal swabs were taken from 20 randomly chosen broilers. Faecal swabs were returned by 141 individuals living and/or working on 47 farms. ESBL/AmpC-producing *E. coli* were isolated and, for selected isolates, phylogenetic groups, plasmids and sequence types were determined. Questionnaires were used for risk factor analysis.

Results: All sampled farms were positive, with 96.4% positive pooled broiler samples. The human prevalence was 19.1%, with 14.3% and 27.1% among individuals having a low and a high degree of contact with live broilers, respectively. Five pairs of human–broiler isolates had identical genes, plasmid families and *E. coli* sequence types, showing clonal transmission. Furthermore, similar ESBL/AmpC genes on the same plasmid families in different *E. coli* sequence types in humans and broilers hinted at horizontal gene transfer.

Conclusions: The prevalence among people on broiler farms was higher than in previous studies involving patients and the general population. Furthermore, an increased risk of carriage was shown among individuals having a high degree of contact with live broilers. The (relative) contribution of transmission routes that might play a role in the dissemination of ESBL/AmpC-encoding resistance genes to humans on broiler farms should be pursued in future studies.

Keywords: ESBLs, carriage, phylogenetic groups, MLST, plasmids

Introduction

Extended-spectrum β-lactamase (ESBL)- and AmpC β-lactamase-producing Enterobacteriaceae are frequently reported in broilers.[1,2] This raises public health concerns as the intestinal microbiome of these animals might form a reservoir for ESBL/AmpC-encoding resistance genes, capable of being transmitted to humans.[3,4] Transmission via the food chain has been suggested,[5,6] but transmission resulting from close contact between humans and animals on livestock farms is also plausible.[2] Contact with live broilers has already been identified as a risk factor for methicillin-resistant *Staphylococcus aureus* (MRSA) carriage among humans.[7,8]

In the Netherlands, the prevalence of carriage of ESBL/AmpC-producing *Escherichia coli* (hereafter referred to ESBL/AmpC carriage) among broiler farmers was 33.3% (6/18), and β-lactamase genes located on identical plasmid families were detected in isolates from both farmers and their animals.[2] Furthermore, the prevalence among farmers was higher than that found among patients (4.9%)[5] and among humans not living on farms (5.1%)[9] in the Netherlands. This suggests that contact

with broilers, and/or the farm environment, could be a risk factor for ESBL/AmpC carriage among humans.

Risk factors for ESBL/AmpC carriage among people living and/or working on broiler farms have not yet been reported. The objectives were therefore to estimate the prevalence among broiler farmers, their family members and employees, and to identify and quantify risk factors for carriage, with an emphasis on contact with live broilers. To gain an insight into transmission routes between broilers and humans, *E. coli* isolates from humans and broilers from the same farm were compared with respect to ESBL/AmpC genes, plasmid families and sequence types (STs).

Methods

From 5 July 2010 to 11 April 2011, a cross-sectional study of MRSA and ESBL/AmpC-producing bacteria was conducted on 50 Dutch broiler farms with 228 individuals living or working on these farms. Study populations were defined and sampled according to Geenen *et al.*[8] Participating farms had on average of three broiler houses (range 1–6; broilers of one age and using an all-in-all-out system), with a median number of broilers per farm of 78 000 (range 14 400–200 000). The age of broilers at sampling was 21–49 days, with an average of 31 days. In order to accurately estimate the prevalence of ESBL/AmpC carriage among humans, given a prevalence of 33% among broiler farmers, a 95% confidence level and 10% accepted error, at least 85 individuals needed to be sampled.

Each farm was sampled by taking cloacal swabs from 20 broilers in total, the broilers being divided over all the broiler houses present on the farm. This sample size enables the detection of a positive farm at the 95% confidence level with a within-farm prevalence of at least 14%. The study was performed according to Dutch law on studies with animals. Farmers, family members and employees who voluntarily participated in the study returned an informed consent form, a faecal swab and a questionnaire on their lifestyle and health characteristics. For children aged <18 years parental consent was requested. Farmers also completed a questionnaire on the farm (management) characteristics. The median time between the sampling of broilers and the arrival of human samples was 1 day (range 0–28 days).

Broiler samples were pooled into 10 pools of two swabs each; human samples were examined individually. Bacteria were isolated by selective enrichment (Luria–Bertani broth with 1 mg/L of cefotaxime) and cultured on selective plates for 18 h at 37°C (MacConkey agar no. 3 with 1 mg/L of cefotaxime). *E. coli*-like, indole-positive isolates (five isolates per human sample and 10 isolates per broiler farm) were tested phenotypically for ESBL production by combination disc diffusion test according to CLSI guidelines.[10] A cefoxitin disc was used to detect the AmpC phenotypes.

E. coli phylogenetic groups were determined for a minimum of one (range 1–3) isolate per person testing positive as reported by Clermont *et al.*[11] and Escobar-Páramo *et al.*[12] *E. coli* was confirmed in isolates that were negative for all three amplicons as described by Frahm and Obst.[13] Subsequently, phylogenetic groups were determined for a minimum of one (range 1–9) positive broiler isolate from each farm with human ESBL/AmpC carriage. More than one isolate per human or broiler was included if there were differences in the disc diffusion test indicative of the presence of different ESBL/AmpC genes.

β-Lactamase genes were identified by PCR and sequencing in line with Dierikx *et al.*[14] If broiler and human isolates showed similar *E. coli* phylogenetic groups and/or ESBL/AmpC genes within the same farm, multilocus sequence typing (MLST) of *E. coli* was performed as described by Wirth *et al.*[15] Plasmid characterization was performed for a selection of isolates representing the diversity of ESBL/AmpC genes in humans and broilers, and on isolate pairs with the same *E. coli* ST and ESBL/AmpC gene by transformation and PCR-based replicon typing as described by Hordijk *et al.*[16] and Carattoli *et al.*[17]

Prevalences and their exact 95% CIs were calculated based on the binomial probability function. Risk factor analysis could not be conducted at the farm level as all the farms were classified as positive (i.e. bacteria were phenotypically characterized as ESBL/AmpC-producing *E. coli* in at least one broiler sample). To assess the relationship between ESBL/AmpC carriage among humans and possible risk factors, univariable and multivariable logistic regression analyses were performed for the variables in Table 1 as reported by Hosmer and Lemeshow.[18] As observations on the same farm might not have been independent, a random effect of farms was included using an exchangeable covariance structure.

Results and discussion

This study investigates ESBL/AmpC carriage among not only farmers, but also all individuals living and/or working on broiler farms and is the first study reporting risk factors for carriage. Faecal swabs and informed consent forms were provided by 141/228 individuals from 47 farms (a response rate of 61.8%). An average of three individuals per farm (range 1–9) was included, with an average age of 36.6 years (SD 19.1, range 1–80). Similar to the results of Dierikx *et al.*,[2] ESBL/AmpC-producing *E. coli* were present in broilers on all 50 farms, with a pooled sample prevalence of 96.4% (482/500; 95% CI 94.4%–97.9%). In total, 27 humans originating from 21 farms tested positive (19.1%; 95% CI 13.0%–26.6%). The prevalence in farmers (25.5%; exact 95% CI 13.9%–40.4%) and employees (37.5%; exact 95% CI 8.5%–75.5%) was similar to that of Dierikx *et al.*[2] (33.3%). The prevalence in partners (11.4%; exact 95% CI 3.2%–26.7%) and family members (15.7%; exact 95% CI 7.0%–28.6%) was lower than in farmers and employees, but still higher in comparison with patients and the general population.[5,9]

It was hypothesized that a high degree of contact with live broilers could be a risk factor for human ESBL/AmpC carriage on broiler farms. Information about the number of hours per week present in the broiler house and the number of hours per week having physical contact with live broilers was completed by about half of the participants (by 81 and 77 individuals, respectively). Analysis of variance of these variables showed that farmers (2.8 h and 1.8 h) and employees (1.8 h and 0.6 h) had a significantly higher degree of contact ($P<0.0001$) compared with partners (0.7 h and 0.5 h) and other family members (0.3 h and 0.1 h). In addition individuals reporting the performance of activities in the broiler house (i.e. weighing, vaccination, blood sampling and/or health checks) were more often (χ^2 $P<0.0001$) farmers and employees (98.1%) than partners and family members (33.8%). Based on this information a new variable ('contact with broilers') was created, with farmers and employees in one category and partners and family members in the other. ESBL/AmpC carriage among humans related to possible risk factors are shown in Table 1. Sex, age, hours spent in the broiler house, performance of activities in the broiler house and type of person correlated strongly (>0.5) with the 'contact with broilers' variable. To avoid multicollinearity only the latter variable was included in the multivariable model. Three out of the seven initially included variables (Table 1) remained in the final multivariable model, in which the random farm effect explained 8% of the non-explained variation. In this model, based on 118 individuals with a complete record, farmers and employees were at a higher risk of ESBL/AmpC carriage than partners and family members (27.1% versus 14.3%; OR=2.5; $P=0.08$). It seems that there is an increased risk

Table 1. Prevalence of ESBL/AmpC carriage (Prev) among people living and/or working on 47 Dutch broiler farms in relation to farm-related and individual characteristics ($n=141$),[a] the overall prevalence being 19.1%

		Frequency[b]			
Variable	Category	*n*	%	Prev, %	Overall *P* value[c]
Research question					
contact with broilers	partners, family	86	61.0	14.0	**0.05**
	farmers, employees	55	39.0	27.3	
General characteristics					
sampling period	Jan–May 2011	73	51.8	9.6	**0.002**
	Jul–Dec 2010	68	48.2	29.4	
region	south	48	34.0	22.9	0.84
	east	38	27.0	18.4	
	west	10	7.1	20.0	
	north	45	31.9	15.6	
Farm characteristics					
presence in broiler house	no	22	17.5	13.6	0.40
	yes	104	82.5	21.2	
hours spent in broiler house	0	22	27.2	13.6	**0.02**
	≤2	32	39.5	9.4	
	>2	27	33.3	37.0	
physical contact with broilers	no	28	22.4	14.3	0.38
	yes	97	77.6	19.7	
hours in physical contact with broilers	0	28	36.4	14.3	0.79
	≤1	30	39.0	20.0	
	>1	19	24.7	21.1	
activities in the broiler house[d]	no activity	50	39.7	14.0	**0.17**
	≥1 activity	76	60.3	23.7	
contact with livestock on other farm	no	98	77.2	22.5	**0.13**
	yes	29	22.8	10.3	
changing room in broiler house	no	14	9.9	14.3	0.61
	yes	127	90.1	19.7	
shower present in broiler house	no	78	55.3	24.4	**0.08**
	yes	63	44.7	12.7	
farm size	≤78000 broilers	63	46.7	15.9	0.46
	>78000 broilers	72	53.3	20.8	
Individual characteristics					
type of person	partner	35	24.8	11.4	**0.21**
	family member	51	36.2	15.7	
	farmer	47	33.3	25.5	
	employee	8	5.7	37.5	
age	0–18 years	38	27.0	13.2	0.41
	19–65 years	97	68.8	20.6	
	>65 years	6	4.3	33.3	
sex	male	72	56.3	27.8	**0.01**
	female	56	43.8	8.9	
family members in residence	≤2	25	18.5	16.0	0.57
	>2	110	81.5	20.9	
visit to hospital or polyclinic in past year	no	96	76.8	21.9	0.32
	yes	29	23.2	13.8	
antibiotic use during past 3 months	no	115	92.7	20.9	0.45
	yes	9	7.3	11.1	
having diabetes or skin disease(s)[e]	no	101	85.6	15.8	**0.02**
	yes	17	14.4	41.2	

Continued

Table 1. *Continued*

Variable	Category	Frequency[b]		Prev, %	Overall *P* value[c]
		n	%		
MRSA-positive in this study	no	133	94.3	17.3	**0.04**
	yes	8	5.7	50.0	
shared use of towels	no	48	39.7	14.6	**0.23**
	yes	73	60.3	23.3	
playing team sports	no	83	66.4	18.1	0.45
	yes	42	33.6	23.8	
travel abroad during past year	no	60	48.8	20.0	0.93
	yes	63	51.2	20.6	

[a]In an 'intercept only' model (without explanatory variables) the random farm effect was not significant and explained only 1.9% of the non-explained variation.
[b]A number of questionnaires were not complete, resulting in variables with missing values.
[c]Variables with a *P* value (based on the likelihood ratio test) in bold ($P<0.25$) were considered for multivariable modelling.
[d]Activities in the broiler house include weighing, vaccination, blood sampling and health checks.
[e]Skin diseases include psoriasis, eczema, impetigo, infected skin, infected wounds and boils.

of exposure to ESBL/AmpC-producing *E. coli* for humans on broiler farms and that this risk is larger for individuals in close contact with broilers. Two other factors associated with higher risk of ESBL/AmpC carriage in humans were having diabetes or a skin disease (41.2% versus 15.8%; OR=16.5; $P=0.002$) and sampling in July–December 2010 (33.3% versus 7.8% in January–May 2011; OR=13.0; $P=0.002$). Risk factors reported in the literature such as travel abroad[19] and antibiotic use[20] were not identified in the current study. The similarity of answers to the questionnaire, which resulted in categories with <10% of available data, makes it difficult, however, to draw statistically valid conclusions on these risk factors.

The distribution of phylogenetic groups, ESBL/AmpC genes and plasmids for a selection of 43 human and 90 broiler *E. coli* isolates from 21 farms with humans testing positive is summarized in Table 2. The molecular characteristics of all the isolates are presented in Table S1 (available as Supplementary data at *JAC* Online). Phylogenetic groups A_0, A_1, $B1_1$, and D_2 predominated both in humans and in broilers. The most prevalent genes in isolates from humans as well as broilers were bla_{CMY-2}, $bla_{CTX-M-1}$ and bla_{SHV-12}, and the most recovered plasmid family was IncI1 (Table 2). The similarity of distribution of the phylogenetic groups, ESBL/AmpC genes and plasmid families in human isolates compared with broiler isolates suggests an exposure to a local pool of resistance genes related to broilers and the farm environment. This is further supported by the fact that $bla_{CTX-M-15}$, which is one of the most prevalent genes found in humans in the Netherlands,[5,21] was not found in the present study. In addition, bla_{SHV-12} and bla_{CMY-2} predominated but these are only found sporadically in isolates from patients[5,6] and the community.[21]

MLST was performed for a selection of human and broiler isolates from 12 farms (Table 3). On the other nine farms with humans testing positive (Table S1), ESBL/AmpC genes and/or *E. coli* phylogenetic groups were not similar between humans and broilers so MLST was not performed. In five cases, broiler–human isolate pairs showed the same ESBL/AmpC gene, plasmid family and *E. coli* ST (Table 3). Given the epidemiological relatedness of isolates collected in this study, a clonal transfer of ESBL/AmpC-producing *E. coli* between broilers and humans is likely. The ST diversity observed in the current study and by Dierikx *et al.*[2] further suggests that finding these broiler–human isolate pairs is not a coincidence. Clonal transfer is only the starting point for transmission as horizontal gene transfer[3,4] may occur within bacterial populations, e.g. between *E. coli* with different STs and between *E. coli* and other bacterial species. Focusing on clonal transfer alone will therefore underestimate the frequency of transfer. In the current study identical ESBL/AmpC genes located on the same plasmid family were found in different *E. coli* STs (Table 3; farms 10, 12 and 16), suggesting a horizontal transfer of plasmids via conjugation between different *E. coli* strains.

Transmission between humans and broilers has been shown but knowledge about the (relative) contribution of transmission routes that might play a role in the dissemination of ESBL/AmpC-encoding resistance genes is lacking. Direct contact between humans and live broilers seems to play a major role, given the highest prevalence in farmers and employees. It is important, however, also to consider transmission via the farm environment and between humans within the household, given the relatively high prevalence in family members. *E. coli* have a high survival rate in the environment, which might lead to the accumulation of these bacteria,[22] both inside and outside the broiler house, and indirect transmission to individuals living on farms. Environmental samples were not collected in the current study. A high rate of intestinal colonization with ESBL-producing organisms was shown for the household members of patients suffering from community-acquired urinary tract infections, and up to 66.6% of isolates from case patients and their corresponding household members had indistinguishable PFGE patterns.[23] In the current study two isolates were found, one from a farmer and one from a family member, from the same farm with bla_{CMY-2}, located on a plasmid from the incI1 family, in an *E. coli* with ST117, which might indicate human-to-human transmission. The family member reported no contact with live broilers and rarely entered the broiler house.

This study contributes to the growing body of evidence supporting the risk for humans working and/or living on broiler

Table 2. Distribution of phylogenetic groups, ESBL/AmpC genes and plasmids among *E. coli* isolates from broilers and humans, representing the 21 farms with human carriage

	Broiler		Human		Total	
Molecular characteristic (PCR and sequencing)	*n*	%	*n*	%	*n*	%
Phylogenetic groups						
D_2	29	32.2	8	18.6	37	27.8
A_0	19	21.1	10	23.3	29	21.8
$B1_1$	18	20.0	8	18.6	26	19.5
A_1	12	13.3	9	20.9	21	15.8
$B2_3$	8	8.9	2	4.7	10	7.5
D_1	4	4.4	4	9.3	8	6.0
$B2_2$	0	0.0	2	4.7	2	1.5
total	**90**	**100**	**43**	**100**	**133**	**100**
ESBL/AmpC genes[a]						
bla$_{CMY-2}$	34	38.6	14	32.6	48	36.6
bla$_{CTX-M-1}$	25	28.4	12	27.9	37	28.2
bla$_{SHV-12}$	15	17.0	10	23.3	25	19.1
bla$_{TEM-52}$[b]	8	9.1	4	9.3	12	9.2
bla$_{CTX-M-2}$	2	2.3	1	2.3	3	2.3
AmpC promoter mutants[c]	0	0.0	2	4.7	2	1.5
bla$_{CTX-M-1}$, *bla*$_{CTX-M-9}$	1	1.1	0	0.0	1	0.8
bla$_{CTX-M-1}$, *bla*$_{SHV-12}$	1	1.1	0	0.0	1	0.8
bla$_{CTX-M-14}$	1	1.1	0	0.0	1	0.8
bla$_{CTX-M-32}$	1	1.1	0	0.0	1	0.8
total[d]	**88**	**100**	**43**	**100**	**131**	**100**
Plasmid, ESBL/AmpC gene combinations						
IncI1, *bla*$_{CTX-M-1}$	16	37.2	8	30.8	24	34.8
IncK, *bla*$_{CMY-2}$	6	14.0	4	15.4	10	14.5
IncI1, *bla*$_{SHV-12}$	6	14.0	3	11.5	9	13.0
IncI1, *bla*$_{CMY-2}$	3	7.0	5	19.2	8	11.6
IncX1, *bla*$_{TEM-52}$	2	4.7	2	7.7	4	5.8
IncX1, *bla*$_{SHV-12}$	2	4.7	0	0.0	2	2.9
IncN, *bla*$_{SHV-12}$	1	2.3	1	3.8	2	2.9
IncA/C, *bla*$_{CMY-2}$	1	2.3	0	0.0	1	1.4
IncB/O, *bla*$_{SHV-12}$	0	0.0	1	3.8	1	1.4
IncFIB, *bla*$_{CMY-2}$	1	2.3	0	0.0	1	1.4
IncFII, *bla*$_{SHV-12}$	1	2.3	0	0.0	1	1.4
IncHI2_IncP, *bla*$_{CTX-M-2}$	1	2.3	0	0.0	1	1.4
IncI1, *bla*$_{CTX-M-2}$	0	0.0	1	3.8	1	1.4
IncI1, *bla*$_{CTX-M-14}$	1	2.3	0	0.0	1	1.4
IncI1, *bla*$_{TEM-52}$	1	2.3	0	0.0	1	1.4
IncX1, *bla*$_{CTX-M-32}$	1	2.3	0	0.0	1	1.4
untypeable, *bla*$_{SHV-12}$	0	0.0	1	3.8	1	1.4
total[e]	**43**	**100**	**26**	**100**	**69**	**100**

[a]A number of genes were found in combination with TEM-1b [CMY-2 ($n=8$), CTX-M-1 ($n=10$), CTX-M-2 ($n=2$), CTX-M-32 ($n=1$), SHV-12 ($n=8$) and TEM-52 ($n=5$)]; see Table S1.
[b]One TEM-52 isolate was found with one mutation (C228T) and 11 TEM-52 isolates were found with three mutations (C18T, C228T and G396T) in this gene. All were synonymous mutations.
[c]One AmpC type 3 promoter mutant [mutations −42T−18A−1T(+23A+51T)+58T+81G] and one AmpC type 4 promoter mutant (mutations +22T+26G+27T+32A+70T+81G) were found.
[d]In two isolates from broilers, only *bla*$_{TEM-1b}$ was found.
[e]Plasmids were not characterized for $n=64$ isolates.

Table 3. *E. coli* STs in isolates from 12 farms where humans and broilers had similar ESBL/AmpC genes and phylogenetic groups within the same farm

Farm	Isolate	Source	Phylogenetic group	*E. coli* ST	Clonal complex	β-Lactamase genes (PCR and sequencing)[a,b]	Replicon type of plasmid with ESBL/AmpC gene
04	HR1_1	farmer	D_2	648		bla_{SHV-12}	incB/O
	C09_1	broiler	D_2	648		bla_{SHV-12}	incI1
07	HR1_1	farmer	D_2	117		bla_{SHV-12}	incI1
	C02_1	broiler	D_2	117		bla_{SHV-12}	incFII
08	GR1_3	family	$B1_1$	351		bla_{CMY-2}, bla_{TEM-1b}	incI1
	C02_1	broiler	$B1_1$	351		bla_{CMY-2}	incI1
10	HR1_3	farmer	A_0	93	ST168	bla_{SHV-12}, bla_{TEM-1b}	incN
	GR2_1	family	A_0	399	ST399	$bla_{CTX-M-1}$	incI1
	C02_1	broiler	A_0	189	ST165	$bla_{CTX-M-1}$, bla_{TEM-1b}	incI1
12	HR1_1	farmer	$B2_3$	131		bla_{CMY-2}	incI1
	HR1_3	farmer	$B1_1$	641	ST86	bla_{CMY-2}	
	HR1_5	farmer	D_2	new[c]		$bla_{CTX-M-1}$	incI1
	C01_2	broiler	D_2	1640		$bla_{CTX-M-1}$, bla_{TEM-1b}	incI1
	C08_1	broiler	$B1_1$	1146		bla_{CMY-2}	
	C09_2	broiler	D_2	1775		bla_{CMY-2}, bla_{TEM-1b}	incI1
	C10_1	broiler	$B2_3$	355		bla_{CMY-2}	
15	HR1_3	farmer	D_2	117		bla_{CMY-2}	incI1
	GR1_4	family	D_2	117		bla_{CMY-2}	incI1
	C03_1	broiler	D_2	57	ST35	bla_{CMY-2}	incK
16	HR1_3	farmer	A_1	48	ST10	$bla_{CTX-M-1}$, bla_{TEM-1b}	incI1
	C03_1	broiler	A_1	10	ST10	$bla_{CTX-M-1}$	incI1
20	GR2_1	family	A_1	10	ST10	$bla_{CTX-M-1}$	incI1
	C04_1	broiler	A_1	10	ST10	$bla_{CTX-M-1}$	incI1
25	GR2_4	family	A_0	1818		bla_{TEM-52}[d]	incX1
	C01_1	broiler	A_0	1818		bla_{TEM-52}[d]	incX1
	GR2_1	family	A_0	373	ST168	bla_{CMY-2}	incK
	C03_1	broiler	A_0	373	ST168	bla_{CMY-2}	incK
	C05_1	broiler	D_2	38	ST38	bla_{CMY-2}, bla_{TEM-1b}	incK
27	HR1_1	farmer	A_1	88	ST23	$bla_{CTX-M-1}$	incI1
	HR1_5	farmer	A_1	88	ST23	$bla_{CTX-M-1}$	incI1
	C06_1	broiler	A_1	88	ST23	$bla_{CTX-M-1}$	incI1
	C08_1	broiler	A_0	2223		$bla_{CTX-M-1}$	incI1
28	HR1_1	farmer	A_0	1324		$bla_{CTX-M-1}$	incI1
	C09_1	broiler	A_0	641	ST86	$bla_{CTX-M-1}$	
37	GR2_1	family	A_1	23	ST23	bla_{SHV-12}, bla_{TEM-1b}	incI1
	GR3_2	family	D_2	1163		bla_{CMY-2}, bla_{TEM-1b}	incI1
	C05_1	broiler	A_0	2509		bla_{TEM-52}[d], bla_{TEM-1b}	
	C07_1	broiler	D_2	57	ST350	bla_{CMY-2}, bla_{TEM-1b}	incK

[a]Shaded areas indicate human–broiler or human–human isolate pairs from the same farm with the same ESBL/AmpC gene, plasmid family and *E. coli* ST.
[b]With the primers used in this study no distinction was made between TEM-1b and TEM-198; however, for readability of the table, TEM-1b was inserted.
[c]MLST results: adk35, fumC3, gyrB234, icd342 mdh45, purA5, recA95.
[d]Isolates were found with three mutations (C18T, C228T and G396T). All were synonymous mutations.

farms and confirms the transmission of ESBL/AmpC-producing Enterobacteriaceae between broilers and humans. To further elucidate the role of broilers in ESBL/AmpC carriage among humans on broiler farms, future studies should attempt to quantify the transmission between broilers, and between humans and broilers, taking into account indirect (via the environment) and direct transmission routes, as well as clonal spread and horizontal gene transfer.

Acknowledgements

Preliminary results were presented at the Spring Meeting of the Dutch Society for Medical Microbiology (NVMM) and the Royal Netherlands Society for Microbiology (KVvM), Papendal, The Netherlands, 2013 (Abstract O120) and at the Annual Conference of the Netherlands Epidemiology Society (WEON) on Epidemiology in Global Health, Utrecht, The Netherlands, 2013 (Abstract O24).

We would like to thank all farmers, their family members and employees for participating in this study. We are grateful to P. L. Geenen, P. Doornenbal, W. M. van Overbeek, B. V. Lambrechts, C. C. Kappert and G. A. C. Lammers for their valuable assistance.

Funding

This work is supported by: the Netherlands Food and Consumer Product Authority (NVWA); the Dutch Product Board for Poultry and Eggs (PPE); and the Dutch Ministry of Health, Welfare and Sport.

Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Smet A, Martel A, Persoons D *et al*. Diversity of extended-spectrum β-lactamases and class C β-lactamases among cloacal *Escherichia coli* isolates in Belgian broiler farms. *Antimicrob Agents Chemother* 2008; **52**: 1238–43.

2 Dierikx CM, van der Goot J, Fabri T *et al*. Extended-spectrum-β-lactamase- and AmpC-β-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. *J Antimicrob Chemother* 2013; **68**: 60–7.

3 Smet A, Martel A, Persoons D *et al*. Comparative analysis of extended-spectrum-β-lactamase-carrying plasmids from different members of Enterobacteriaceae isolated from poultry, pigs and humans: evidence for a shared β-lactam resistance gene pool? *J Antimicrob Chemother* 2009; **63**: 1286–8.

4 Smet A, Rasschaert G, Martel A *et al*. *In situ* ESBL conjugation from avian to human *Escherichia coli* during cefotaxime administration. *J Appl Microbiol* 2011; **110**: 541–9.

5 Overdevest I, Willemsen I, Rijnsburger M *et al*. Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. *Emerg Infect Dis* 2011; **17**: 1216–22.

6 Voets GM, Fluit AC, Scharringa J *et al*. Identical plasmid AmpC β-lactamase genes and plasmid types in *E. coli* isolates from patients and poultry meat in the Netherlands. *Int J Food Microbiol* 2013; **167**: 359–62.

7 Mulders MN, Haenen APJ, Geenen PL *et al*. Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughterhouse personnel in The Netherlands. *Epidemiol Infect* 2010; **138**: 743–55.

8 Geenen PL, Graat EAM, Haenen APJ *et al*. Prevalence of livestock-associated MRSA on Dutch broiler farms and in people living and/or working on these farms. *Epidemiol Infect* 2013; **141**: 1099–108.

9 Huijbers PMC, de Kraker M, Graat EAM *et al*. Prevalence of extended-spectrum β-lactamase-producing Enterobacteriaceae in humans living in municipalities with high and low broiler density. *Clin Microbiol Infect* 2013; **19**: E256–9.

10 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement M100–21*. CLSI, Wayne, PA, USA, 2011.

11 Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; **66**: 4555–8.

12 Escobar-Páramo P, Le Menac'h A, Le Gall T *et al*. Identification of forces shaping the commensal *Escherichia coli* genetic structure by comparing animal and human isolates. *Environ Microbiol* 2006; **8**: 1975–84.

13 Frahm E, Obst U. Application of fluorogenic probe technique (TaqMan PCR) to the detection of *Enterococcus* spp. and *Escherichia coli* in water samples. *J Microbiol Meth* 2003; **52**: 123–31.

14 Dierikx CM, van Duijkeren E, Schoormans AHW *et al*. Occurrence and characteristics of extended-spectrum-β-lactamase and AmpC-producing clinical isolates derived from companion animals and horses. *J Antimicrob Chemother* 2012; **67**: 1368–74.

15 Wirth T, Falush D, Lan R *et al*. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol* 2006; **60**: 1136–51.

16 Hordijk J, Wagenaar JA, Kant A *et al*. Cross-sectional study on prevalence and molecular characteristics of plasmid mediated ESBL/AmpC-producing *Escherichia coli* isolated from veal calves at slaughter. *Plos One* 2013; **8**: e65681.

17 Carattoli A, Bertini A, Villa L *et al*. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

18 Hosmer DW, Lemeshow S. *Applied Logistic Regression*, 2nd edn. New York: John Wiley & Sons, Inc., 2000.

19 Paltansing S, Vlot JA, Kraakman MEM *et al*. Extended-spectrum β-lactamase-producing Enterobacteriaceae among travellers from the Netherlands. *Emerg Infect Dis* 2013; **19**: 1206–13.

20 Ben-Ami R, Rodríguez-Baño J, Arslan H *et al*. A multinational survey of risk factors for infection with extended-spectrum β-lactamase-producing Enterobacteriaceae in nonhospitalized patients. *Clin Infect Dis* 2009; **49**: 682–90.

21 Reuland AE, Overdevest ITMA, al Naiemi N *et al*. High prevalence of ESBL-producing Enterobacteriaceae carriage in Dutch community patients with gastrointestinal complaints. *Clin Microbiol Infect* 2012; **19**: 542–9.

22 Van Bunnik BAD, Ssematimba A, Hagenaars TJ *et al*. Small distances can keep bacteria at bay for days. *PNAS* 2014; **111**: 3556–60.

23 Valverde A, Grill F, Coque TM *et al*. High rate of intestinal colonization with extended-spectrum-β-lactamase-producing organisms in household contacts of infected community patients. *J Clin Microbiol* 2008; **46**: 2796–9.