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Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers

A. E. van den Bogaard, N. London, C. Driessen and E. E. Stobberingh*

University Hospital Maastricht, Department of Medical Microbiology, PO Box 5800, 6202 AZ Maastricht, The Netherlands

The percentage of faecal samples containing resistant *Echerichia coli* and the proportion of resistant faecal *E. coli* were determined in three poultry populations: broilers and turkeys commonly given antibiotics, and laying hens treated with antibiotics relatively infrequently. Faecal samples of five human populations were also examined: turkey farmers, broiler farmers, laying-hen farmers, broiler slaughterers and turkey slaughterers. The MICs of antibiotics commonly used in poultry medicine were also determined. Ciprofloxacin-resistant isolates from these eight populations and from turkey meat were genotyped by pulsed-field gel electrophoresis (PFGE) after *Sma*I digestion. The proportion of samples containing resistant *E. coli* and the percentages of resistant *E. coli* were significantly higher in turkeys and broilers than in the laying-hen population. Resistance to nearly all antibiotics in faecal *E. coli* of turkey and broiler farmers, and of turkey and broiler slaughterers, was higher than in laying-hen farmers. Multiresistant isolates were common in turkey and broiler farmers but absent in laying-hen farmers. The same resistance patterns were found in turkeys, turkey farmers and turkey slaughterers and in broiler, broiler farmers and broiler slaughterers. The PFGE patterns of the isolates from the eight populations were quite heterogeneous, but *E. coli* with an identical PFGE pattern were isolated at two farms from a turkey and the farmer, and also from a broiler and a broiler farmer from different farms. Moreover, three *E. coli* isolates from turkey meat were identical to faecal isolates from turkeys. The results of this study strongly indicate that transmission of resistant clones and resistance plasmids of *E. coli* from poultry to humans commonly occurs.

Introduction

Antibiotic usage is considered the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine.[1,2] Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans) or populations.[3–8] Antibiotics are used in animals as in humans for therapy and control of bacterial infections. In intensively reared food animals, antibiotics may be administered to whole flocks rather than individual animals. In addition, antimicrobial agents may be continuously fed to food animals such as broilers and turkeys as antimicrobial growth promoters (AMGP). Therefore the antibiotic selection pressure for resistance in bacteria in poultry is high and consequently their faecal flora contains a relatively high proportion of resistant bacteria.[9] However, as most AMGP commonly used in The Netherlands until recently are effective mainly against Gram-positive bacteria, most resistance in faecal *Escherichia coli* of food animals is to antibiotics used on veterinary prescription.

At slaughter, resistant strains from the gut readily soil poultry carcasses and as a result poultry meats are often contaminated with multiresistant *E. coli*;[10–16] likewise eggs become contaminated during laying.[17] Hence, resistant faecal *E. coli* from poultry can infect humans both directly and via food. These resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora.

However, the mechanism of spread of antibiotic resistance from food animals to humans remains controversial.

*Corresponding author. Tel: +31-43-3874644; Fax: +31-43-3876643; E-mail: est@lmib.axm.nl

Colonization of the intestinal tract with resistant *E. coli* from chicken has been shown in human volunteers.[18] Evidence that animals are a reservoir for *E. coli* found in humans was published by Cooke *et al.*[19] in the early 1970s. Spread of an antibiotic resistance plasmid, pSL222-6, in *E. coli* from chickens to human handlers was described by Levy *et al.*[20] Others have also presented evidence of spread of antibiotic-resistant microorganisms from poultry to humans in various countries. Linton *et al.*[13,18] found the same O serotype in chickens from a commercial rearing centre, in oven-ready birds and in humans. Ojeniyi[21,22] described direct transmission of *E. coli* resistant to streptomycin, sulphonamides and tetracycline from poultry to poultry attendants in Nigeria. Chickens have also been described as a source of antibiotic resistance in humans in northern India,[23] Morocco[24] and Saudi Arabia.[25] Recently, Bass *et al.*[26] described a high incidence of integrons encoding multidrug resistance among chicken isolates as part of transposon Tn*21*. In addition, they described the dissemination of Tn*21* among pathogenic poultry isolates and suggest that Tn*21* may transfer between pathogenic microorganisms in humans as well as in poultry.

In contrast, others have concluded that human and poultry isolates belong to two distinct pools of resistant *E. coli*. Smith[27] concluded that the antibiotic resistance transfer between animals and humans was limited and that animal strains colonized the alimentary tract less readily than human ones. He stated that in view of the high prevalence of antibiotic resistance in humans, animals are not an important source of resistant *E. coli* in man. Shooter *et al.*[28] serotyped animal and human *E. coli* isolates using 150 O antisera. Of the animal strains, 289 (36%) of 798 could be serotyped, whereas only two of 1580 human isolates could not be typed.[28] They concluded that O serotypes of animal origin may differ from those of humans. Differences in chloramphenicol and streptomycin resistance between poultry and their attendants in North India has been described[29]—26 versus 58% for chloramphenicol and 69 versus 94% for streptomycin. In female poultry workers exposed to resistant microorganisms of animal origin but who had not received antibiotics, urinary tract infections were infrequently caused by poultry strains. A more detailed analysis using restriction enzyme analysis of plasmid DNA showed that none of the plasmids from human isolates appeared to be related to any of the poultry isolates.[30] Caya *et al.*[31] compared the phenotypes and genotypes of *E. coli* isolates from sick broilers in abattoirs in the province of Quebec with human isolates from hospitalized patients living in the same locality as the abattoir. A higher prevalence of resistance was found among the poultry isolates especially to gentamicin, spectinomycin, tetracycline and sulphamethoxazole. Only two poultry isolates demonstrated a possible relationship with human strains. Comparing *E. coli* from a poultry processing plant in Kenya and isolates from children with diarrhoea living in close contact with poultry, Kariuki *et al.*[32] observed differences in antibiotic resistance patterns and in the levels of multidrug resistance. The authors concluded that human and poultry isolates carry two distinct pools of resistance plasmids. A similar conclusion was drawn by Nijsten *et al.*[33] comparing resistance patterns of faecal *E. coli* isolates of pig farmers and their pigs.

In this study the prevalence of resistance in faecal *E. coli* was analysed in the following populations: broilers and turkeys, both with relatively high antibiotic use, and laying hens with relatively low antibiotic use. To study the possible dissemination of resistant *E. coli* or resistance genes from these poultry populations to humans, the farmers participating in the study were also requested to submit faecal samples. The faecal flora of turkey and poultry slaughterers was also studied. Farmers have daily contact with their animals and are directly exposed to animal faeces, and slaughterers have daily contact with poultry carcasses or meat. All faecal samples were analysed in terms of the prevalence and degree of resistance in *E. coli*.

As the possibility of transfer of ciprofloxacin-resistant bacteria from animals to humans is controversial, ciprofloxacin-resisant *E. coli* isolates from poultry and humans and turkey carcasses were genotyped using PFGE.

Materials and methods

Collection of faecal samples

Fresh faecal samples were collected from farmers keeping either turkeys, broilers or hens producing eggs for human consumption in the south of The Netherlands. The farmers were requested to provide one fresh faecal sample from themselves and a mixed faecal sample from the oldest flock of poultry at the farm and to send these on the day of collection with the completed questionnaire to the bacteriology laboratory. In the questionnaire information was asked about other animals kept at the farm, recent hospital stay and antibiotic usage by themselves, family members or their animals during the 3 months preceding the sample collection. In addition, poultry slaughterers working at a poultry-processing plant in a similar area were asked to submit a faecal specimen and to fill in the same questionnaire. The faecal samples from the turkey farmers, their turkeys and the turkey slaughterers were processed in a manner similar to that described previously.[34] On the day of arrival at the laboratory the samples were diluted (10^{-1}) in 0.9% NaCl containing 20% (v/v) glycerol and stored at −20°C until assayed. Turkey wings were collected immediately after slaughter and sent frozen at −20°C to the laboratory.

Bacteriological analysis

The methods used were as described previously.[35,36] In short, after thawing the samples, 10^{-2} and 10^{-4} dilutions in 0.9% NaCl were inoculated on to Levine agar plates (BBL 11221, Becton Dickenson BV, Etten-Leur, The Nether-

lands) using a spiral plater (Spiral Systems, Lameris Laboratory BV, Breukelen, The Netherlands). The antibiotics and concentrations used in the Levine agar plates are described in Table I. *E. coli* grows on Levine agar as purple colonies with a black centre and metallic shine. Only these colonies were counted after 18–24 h incubation at 37°C. It has been shown that >95% of the presumptively identified colonies are *E. coli*.[37–39] The antibiotics were selected because they, or related antibiotics, have been used regularly in poultry on veterinary prescription and may be active against *E. coli*. The concentrations used to define resistance were similar to those of previous studies.[36–39] For trimethoprim testing 5% lysed horse blood was added to the agar. The turkey wings were defrosted, shaken using a turrax mixer with 20 mL of peptone water and 1 mL of the peptone water was plated only on to a ciprofloxacin-containing agar plate.

Antibiotic susceptibility testing

One *E. coli* colony was chosen randomly from each faecal sample from the control plate without antibiotics, for antibiotic susceptibility testing using a microbroth dilution method in Iso-Sensitest broth (Oxoid CM473, Basingstoke, UK) using an inoculum of 5×10^5 cfu/mL. The antimicrobial agents tested and the breakpoints for resistance were based mostly on the recommendations of the Dutch Working Group for antimicrobial susceptibility testing[40] as follows: amoxycillin (16 mg/L), chloramphenicol (16 mg/L), ciprofloxacin (4 mg/L), flumequine (8 mg/L), gentamicin (8 mg/L), neomycin (16 mg/L), nitrofurantoin (64 mg/L), oxytetracycline (16 mg/L), streptomycin (32 mg/L), sulphamethoxazole (128 mg/L) and trimethoprim (16 mg/L). *E. coli* ATCC 25922 was used as reference strain.

PFGE

Ciprofloxacin-resistant *E. coli* isolates were genotyped using PFGE after *Xba*I digestion. PFGE was performed as described previously with minor modifications.[41] The criteria of Tenover *et al.*[42] were used to assess similarity of the different patterns obtained.

Definitions

The prevalence of antibiotic resistance was defined as the percentage of faecal samples showing *E. coli* on antibiotic-containing agar plates of the total number of samples tested. The percentage of resistance of each sample was calculated as the ratio between the number of colonies on the agar plates with and without antibiotics multiplied by 100. Two degrees were distinguished: a high degree of resistance, i.e. ratio ≥50%, being defined as the majority of the *E. coli* isolates of a sample showing resistance to a particular antimicrobial agent. A ratio of <50% was defined as a low degree of resistance.

Table I. Prevalence (%) and high degree (%) of antibiotic-resistant faecal *E. coli* from poultry, poultry farmers and poultry slaughterers

Antimicrobial agent (concentration mg/L)	Turkeys ($n = 47$)		Broilers ($n = 50$)		Laying hens ($n = 25$)		Turkey farmers ($n = 47$)		Broiler farmers ($n = 51$)		Laying-hen farmers ($n = 25$)		Turkey slaughterers ($n = 47$)		Broiler slaughterers ($n = 46$)	
	prev.	HD	prev.	HD	prev.	HD	prev.	HD	prev.	HD	prev.	HD	prev.	HD	prev.	HD
Amoxycillin (25)	87	32	82	24	68	4	66	19	57	22	32	4	45	9	48	20
Ciprofloxacin (4)	45	0	50	0	0	0	23	4	8	2	0	0	0	0	7	0
Flumequine (16)	66	0	64	0	8	0	26	4	14	2	0	0	0	0	9	0
Neomycin (32)	81	0	72	2	24	0	57	6	20	2	8	0	32	0	54	15
Nitrofurantoin (50)	13	0	0	0	0	0	2	0	2	2	0	0	0	0	0	0
Oxytetracycline (25)	87	51	78	26	76	12	79	28	61	16	36	4	55	19	43	13
Trimethoprim (8)	85	17	80	20	68	12	72	15	47	14	28	4	38	11	37	11

Prev., prevalence.
HD, high degree (i.e. a high proportion of resistant *E. coli*, ≥50% of the total *E. coli* population of each sample).

Statistical analysis

The χ^2 test was used to assess significant differences in the prevalence and high degree of antibiotic resistance between the different populations.

Results

Response rates

In total, 47 faecal samples (i.e. *c.* 50% of the major turkey farmers in The Netherlands) and their turkeys were examined. Forty-seven samples from turkey slaughterers were also received. Response rates in poultry slaughterers and poultry farmers were about 50% and 30%, respectively. In total, faecal samples from 46 poultry slaughterers, 51 broiler farmers and 50 broilers, 25 laying-hen farmers and 25 laying hens were received. One broiler farmer had no broilers on the farm at the time of sample collection and only sent his own faecal sample. None of the turkey farmers and slaughterers or their family members had been hospitalized or used antibiotics in the 3 months preceding sample collection. Two poultry slaughterers and one laying-hen farmer had been hospitalized. Antibiotic use was mentioned by four broiler farmers and four slaughterers and by two of their respective family members. None of the laying-hen farmers had used antibiotics but a few family members had.

Prevalence of resistance

Of the three poultry populations, the highest prevalence and degree of resistance to almost all compounds tested was detected in turkey samples, closely followed by those from broilers and distinctly lower in the laying-hen population (Table I). The prevalence of resistance to ciprofloxacin, flumequine and neomycin was significantly higher ($P < 0.005$) in turkeys and broilers than in laying hens. Nitrofurantoin resistance was found only in turkey isolates. For amoxycillin and oxytetracycline the percentage of samples with a high degree of resistance among turkey and broiler isolates was significantly higher ($P < 0.005$), compared with laying hens.

In the human populations the same tendency was observed. Turkey farmers showed the highest percentage resistance to all agents tested both in terms of prevalence and high degree of resistance. The lowest resistance rates were observed in the laying-hen farmers. The prevalence of resistance in turkey and broiler farmers was significantly higher compared with laying-hen farmers for amoxycillin, ciprofloxacin, flumequine, neomycin, oxytetracycline and trimethoprim. In the human populations tested, high-level quinolone resistance was found only in turkey and broiler farmers. The resistance rates in turkey slaughterers and poultry slaughterers were similar, resistance to neomycin excepted, which was significantly lower in turkey slaughterers ($P < 0.05$). The resistance was significantly higher in slaughterers than in laying-hen farmers to neomycin and oxytetracycline. The percentage of high-level resistance was significantly higher to oxytetracycline and trimethoprim and in broiler slaughterers only, also for amoxycillin and neomycin ($P < 0.05$).

Antibiotic susceptibility

In approximately 10% of the human and animal samples no *E. coli* grew on the antibiotic-free control plate, which means that fewer than 300 cfu of *E. coli* (minimum detection level) were present per gram of faeces. Antibiotic susceptibility testing of the *E. coli* isolates showed resistance to five or more antibiotics, with the highest frequency in isolates from turkeys (32%), followed by broilers (23%) and broiler farmers (22%) as shown in Table II. The majority of the isolates from laying hens and laying-hen farmers were susceptible to all compounds tested (65 and 55%, respectively). For the turkey and broiler isolates these percentages were significantly lower (16 and 20%, respectively).

The most prevalent resistance patterns of each population are compared in Table III. The resistance patterns most frequently observed, especially among turkeys, turkey farmers and turkey slaughterers, were resistance to amoxycillin alone and in combination with oxytetracycline, streptomycin, sulphamethoxazole and trimethoprim. Single resistance to oxytetracycline was present in almost all populations studied, laying-hen farmers excepted, but in combination with amoxycillin mainly in turkeys.

The most frequent pattern in laying-hen farmers, i.e. resistance to streptomycin and sulphamethoxazole, was not found among laying-hen isolates. The resistance patterns in turkeys corresponded to those in turkey farmers and slaughterers and those of broilers were in general also present in broiler farmers and slaughterers. No ciprofloxacin resistance was observed in these single isolates of *E. coli*.

PFGE patterns

The PFGE patterns obtained with the ciprofloxacin-resistant isolates from turkeys ($n = 21$), turkey farmers ($n = 11$), broilers ($n = 25$) and broiler farmers ($n = 4$) were quite heterogeneous. Of the isolates from the turkey and turkey farmer populations, 27 patterns could be discriminated. Five patterns were found both in turkeys and turkey farmers. The pattern of three farmer isolates were similar to those of the turkeys from the same farm (Figure). Some broilers and broiler isolates showed similar patterns but the isolates were not from the same farm.

Discussion

In The Netherlands *c.* 300 000 kg of antibiotics are used yearly on veterinary prescription in animals, of which 10% is used in poultry.[7,43] The exposure to therapeutic anti-

Table II. Prevalence of multiresistant *E. coli* (%)

Number of antibiotics resistant	Turkeys ($n = 43$)	Broilers ($n = 45$)	Laying hens ($n = 20$)	Turkey farmers ($n = 45$)	Broiler farmers ($n = 42$)	Laying-hen farmers ($n = 20$)	Turkey slaughterers ($n = 45$)	Poultry slaughterers ($n = 39$)
0	7 (16)	9 (20)	13 (65)	15 (33)	17 (40)	11 (55)	15 (33)	16 (41)
1	8 (19)	5 (11)	3 (15)	13 (29)	7 (17)	1 (5)	15 (33)	8 (20)
2	7 (16)	7 (16)	0	4 (9)	3 (7)	5 (25)	1 (2)	5 (13)
3	2 (5)	7 (16)	3 (15)	2 (4)	3 (7)	0	3 (7)	1 (3)
4	5 (12)	7 (16)	1 (5)	5 (11)	2 (7)	2 (10)	4 (9)	5 (13)
$\geqslant 5$	14 (32)	10 (23)	0	6 (14)	9 (22)	1 (5)	7 (15)	4 (10)

Table III. Number of most prevalent resistant patterns found in *E. coli* isolates

Antibiotic(s)	Number of isolates							
	turkeys	broilers	laying hens	turkey farmers	broiler farmers	laying-hen farmers	turkey slaughterers	poultry slaughterers
Amoxycillin	5	2	–	8	2	–	11	1
Oxytetracycline	2	2	2	3	2	–	4	1
Amoxycillin + oxytetracycline	5	–	–	2	1	–	1	1
Streptomycin + sulphamethoxazole	–	–	–	–	1	4	–	1
Amoxycillin + oxytetracycline + streptomycin + sulphamethoxazole + trimethoprim	6	2		3	1		2	1
Amoxycillin + oxytetracycline + sulphamethoxazole			2					
Amoxycillin + streptomycin + sulphamethoxazole + trimethoprim					1			2
Amoxycillin + oxytetracycline + streptomycin + trimethoprim		1			2			

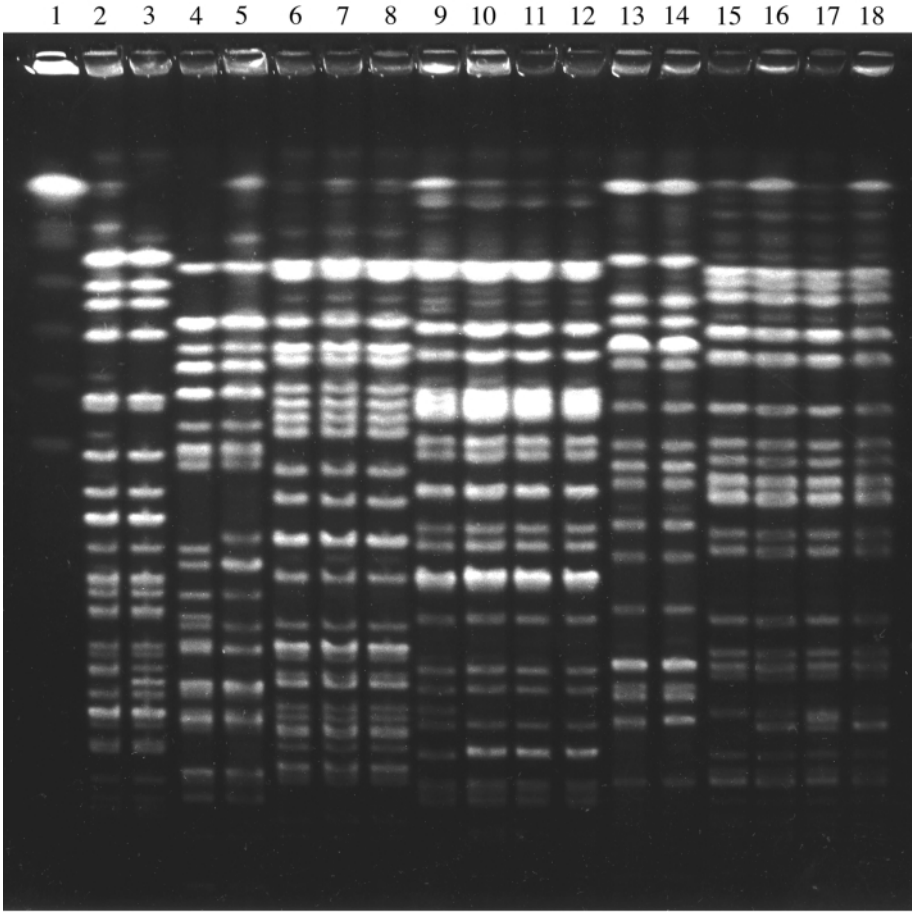

Figure. PFGE patterns of ciprofloxacin-resistant *E. coli* isolated from turkeys (T), turkey farmers (TF) and turkey wings (TW) after digestion of total DNA with *Xba*. Lane 1, molecular weight marker; lane 2, T1; lane 3, TF1; lane 4, T12; lane 5, TF12; lane 6, T47; lane 7, TF47; lane 8, T27; lane 9, T24; lane 10, TF26; lane 11, TF29; lane 12, TF45; lane 13, T28; lane 14, TF10; lane 15, TF28; lane 16, TW; lane 17, TW; lane 18, TW.

biotics per year and per kg is *c*. 100 mg for humans and animals alike. However, for poultry it is more than 400 mg/kg/year, which is considerable higher than in other animals.[7]

The major factor selecting for antimicrobial resistance in bacteria is antibiotic use, and additionally, crowding and poor sanitation. These three factors are typical of intensive poultry farming and explain the high prevalence and degree of resistance in faecal *E. coli* of poultry in this and other studies.[9] Ojeniyi[21] found all 3444 *E. coli* isolates from battery hens to be multiresistant but none of 2284 isolates from free-range chickens. Antibiotics are seldom given to laying hens producing eggs for human consumption: bacterial infections occur less frequently in these than in young broilers as farmers are reluctant to give antibiotics because of the possibility of antibiotic residues in eggs. However, during rearing, antibiotics are commonly used and resistance in *E. coli* in the avian intestinal tract may persist for a long time even in the absence of antibiotics.[44] Faecal samples were collected from the oldest birds because these reflect the chance of contamination of eggs and poultry meat during slaughtering.

Therefore, recent antibiotic exposure explained differences in antimicrobial resistance between the turkey and broiler populations and laying hens as for the five human populations tested. The prevalences and degrees of antibiotic resistance for nearly all antibiotics tested were significantly higher in the turkey and broiler populations, compared with laying hens and also for resistance in single isolates. In these isolates resistance to five or more antibiotics occurred commonly in turkey and broiler isolates and not in laying-hen isolates. These differences clearly reflected more recent antibiotic usage in broilers and turkeys.

Similarly, a higher prevalence of resistance was found among faecal samples and single isolates from both turkey and broiler farmers and slaughterers compared with those from laying-hen farmers. Moreover, the same resistance patterns were found in turkeys, turkey farmers and slaughterers and in broilers, broiler farmers and broiler slaughterers. Dissemination of resistant bacteria and/or resistance plasmids from turkeys and broilers to their respective farmers is the most likely explanation for the high antimicrobial resistance observed in the faecal *E. coli* of the farmers and

slaughterers, as there had been no significant difference in antibiotic consumption in these populations. None of the turkey farmers and slaughterers and four broiler farmers and slaughterers had used antibiotics in the 3 months preceding sample collection. Neomycin resistance (prevalence and level) was very high in the broiler slaughterers compared with other human populations. As neomycin resistance was higher in turkeys than in broilers it is unlikely that the high prevalence in broiler slaughterers was caused by direct contact with poultry meat products. The same phenomenon has been observed in a previous study in pig slaughterers:[45] the common use of neomycin-containing ointments for treatment of minor occupational cuts and skin lesions had most likely generated resistance. However, no information about the use of ointments could be obtained from the two abattoirs in the present study.

A striking observation was the difference in resistance to ciprofloxacin—respectively 45 and 50% for turkey and broiler isolates, 25 and 8% for their respective farmers and 7% for broiler slaughterers. These relatively high percentages especially among the animal isolates were very probably due to the therapeutic use of fluoroquinolones in poultry. The use of flumequine and enrofloxacin accounts for 14% of all antibiotic use in poultry, especially in turkeys and broilers.[46] Enrofloxacin is a methylester of ciprofloxacin and both agents are completely cross-resistant. Flumequine, the first fluoroquinolone developed, selects for low-level resistance to ciprofloxacin, as does nalidixic acid.[47,48] In The Netherlands, approximately 10% of clinical *E. coli* poultry isolates are resistant to ciprofloxacin and 35% to flumequine. This low resistance rate is consistent with the absence of ciprofloxacin resistance in single isolates of *E. coli* and a low degree of resistance. In The Netherlands, fluoroquinolone use in humans is low and resistance in faecal *E. coli* in the general population is extremely unusual.[37,39,45] Similarly, because no formulation for mass medication of pigs is available in The Netherlands, enrofloxacin use in pigs is unusual and the prevalence of resistance in pig faecal *E. coli* is low (approximately 2%; in pig farmers 1% and in pig slaughterers <1%).[36,46,49]

As resistance to fluoroquinolones occurs by chromosomal mutations, large populations of bacteria probably contain small numbers of spontaneously resistant mutants, which may then undergo clonal expansion under the selective pressure of fluoroquinolone use. This will initially occur only within the population, but as the numbers of resistant bacteria within that population (degree of resistance) increase, the chance of spilling over to other populations becomes greater, as has probably occurred in the Dutch poultry and poultry farmer populations. A similar course of events has been described in Spain, where the increased use of fluoroquinolones for therapy in humans was followed by an increase (up to 18%) in resistant *E. coli* from urinary tract infections.[50,51] Prior exposure of a patient to a fluoroquinolone was the single most important risk factor for a fluoroquinolone-resistant *E. coli* infection. Analysis of fluoroquinolone-resistant *E. coli* in the intestinal tract of healthy persons showed a prevalence of 24% in adults and of 26% in children. Carriage in the healthy population did not correlate with previous quinolone use; a strong argument for the pre-existence of resistant strains in the community. As the prevalence of fluoroquinolone resistance in faecal *E. coli* from Spanish chickens and pigs is very high, 90 and 45%, respectively, it was postulated that food animals were the primary reservoir of fluoroquinolone resistance in humans. Additionally, human fluoroquinolone use might have caused further selection in the intestinal tract and secondary dissemination in the human population. However, resistant isolates were not genotyped and clonal transmission could not be proved.[51]

In this study PFGE of ciprofloxacin-resistant isolates from turkey and turkey farmers, and broiler and broiler farmers showed a variety of patterns; in a turkey farmer and his turkeys and a broiler farmer and a broiler, identical patterns were observed, which proved that identical clones were present in humans and in poultry. Moreover, the ciprofloxacin-resistant *E. coli* isolates from the turkey wing tips showed patterns similar to those from turkey farmers. Because of the low sensitivity of the method used, only one *E. coli* was tested from each sample that grew *E. coli* in the ciprofloxacin-containing agar plates; one might expect that clonal transmission of resistant bacteria from humans to animals is more common. The results in this study strongly suggest a spread of antibiotic-resistant *E. coli* from animals to people—not only to farmers but also at a lower level to the consumers of poultry meats, and hence the low incidence of fluoroquinolone-resistant *E. coli* in the Dutch human population. Further increase in fluoroquinolone use in human primary care medicine will be followed by clonal spread of resistant commensal bacteria and an increase in fluoroquinolone-resistant *E. coli* pathogens, as documented for *E. coli* isolates from urinary tract infections.[52]

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