Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers

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The prevalence of resistance in enterococci to antibiotics, commonly used for therapy in poultry or as antimicrobial growth promoters (AMGPs), was determined in faecal samples of two chicken populations: broilers in which antibiotic and AMGP use is common and laying-hens with a low antibiotic usage. In addition faecal samples were examined from three human populations: broiler farmers, laying-hen farmers and poultry slaughterers. MICs of an extended panel of antibiotics for a randomly chosen gentamicin- or vancomycin-resistant enterococcal isolate from each faecal specimen were also determined. The prevalence of resistance for all antibiotics tested was higher in broilers than in laying-hens. Resistance in faecal enterococci of broiler farmers was for nearly all antibiotics higher than those observed in laying-hen farmers and poultry slaughterers. The overall resistance in broilers was correlated with the resistance in broiler farmers and in poultry slaughterers. No correlation between the results obtained in the laying-hens with any of the other populations was found. The 27 gentamicin-resistant isolates all showed high-level resistance to gentamicin and two of these isolates, both *Enterococcus faecium*, were resistant to all antibiotics tested, except vancomycin. The 73 vancomycin-resistant enterococci (VRE) isolated from the five populations belonged to four different species and in all isolates the *vanA* gene cluster was detected by blot hybridization. The pulsed-field gel electrophoresis (PFGE) patterns of these vancomycin-resistant enterococci were quite heterogeneous, but *Enterococcus hirae* isolates with the same or a closely related PFGE pattern were isolated at two farms from the broiler farmer and from broilers. Molecular characterization of *vanA*-containing transposons of these isolates showed that similar transposon types, predominantly found in poultry, were present. Moreover, similar *vanA* elements were not only found in isolates with the same PFGE pattern but also in other VRE isolated from both humans and chickens. The results of this study suggest transmission of resistance in enterococci from animals to man. For VRE this might be clonal transmission of animal strains, but transposon transfer seems to occur more commonly.

Introduction

Acquired resistance against commonly used antibiotics has been observed ever since these agents were introduced in human and veterinary medicine. However, the rate of development of resistance appears to have accelerated in the past decade[1] and today multiple resistant bacteria constitute a global problem.[2,3] In the modern poultry industry antibiotics are used in high quantities not only for therapy

and prevention of bacterial diseases, but also as antimicrobial growth promoters (AMGPs) in animal feeds.[4] In 1990 in The Netherlands 80 000 kg of antibiotics (active substance) were used in humans and 300 000 kg on veterinary prescription in animals.[5] This was in both populations equivalent to *c.* 100 mg of active substance/kg body weight/year. However, *c.* 26% of the veterinary used antibiotics were intended for poultry, mainly broilers, resulting in a yearly exposure of *c.* 430 mg of antibiotics/kg/year for poultry.

This was considerably higher than the antibiotic usage in other food animal populations. In addition to these therapeutic antibiotics, food animals received more or less the same amount of antibiotics in their feeds as AMGP. These amounts have not changed much during subsequent years and similar figures have been published for 1997.[6] This high antibiotic usage in poultry may compromise veterinary therapy but is also of public health concern. Antibiotic use selects not only for resistance in pathogenic bacteria, but also in the endogenous flora of exposed animals.

Enterococci belong to the endogenous flora of man and other animals and are intrinsically resistant to various antibiotics including cephalosporins, penicillinase-resistant penicillins, and clinically available levels of lincosamides and aminoglycosides. Enterococci are not important pathogens for animals; in humans, however, they have been implicated in infective endocarditis and urinary tract infections for nearly a century.[7] Over the last 10 years, enterococci have emerged as major nosocomial pathogens. Approximately 12% of all nosocomial infections in the USA are caused by enterococci.[8,9] The emergence of enterococci as nosocomial pathogens has very likely been caused by the increasing numbers of immunocompromised patients and their exposure to antibiotics against which enterococci are intrinsically resistant such as third- and fourth-generation cephalosporins.

The aim of this study was to analyse the influence of exposure to antibiotics used in The Netherlands as AMGPs or for veterinary therapy in poultry on the resistance of faecal enterococci recovered from poultry, poultry farmers and poultry slaughterers. In addition, the antibiotic susceptibility to several antibiotics for enterococci cultured on antibiotic-free agar plates was determined. The poultry population consisted of two groups with a different usage of antibiotics: broilers, young chickens raised within 8 weeks for slaughter, and laying-hens producing eggs for human consumption. Broilers are fed continuously on AMGP agents and these drugs are not used in laying-hens. Moreover, broilers have a relatively high use of antibiotics on veterinary prescription, while laying-hens are exposed, at least during their productive life, to a relatively low amount of antimicrobial agents.

In addition, the prevalence and degree of resistance against the same antibiotics was assessed in faecal enterococci of three populations of humans with a different risk of exposure to faecal bacteria from chickens: broiler and laying-hen farmers, who have a daily close contact with chickens with a high and low exposure, respectively, to antibiotics, and workers in a poultry processing plant, who handled broilers or broiler products on a daily basis. Finally, possible sharing of vancomycin-resistant enterococci (VRE) between chickens and humans was assessed by genotyping of VRE by pulsed-field gel electrophoresis (PFGE). The similarity of vancomycin resistance elements found in chicken and human isolates was evaluated by comparing Tn*1546* derivatives found in chicken and human isolates of VRE.

Materials and methods

Collection of the faecal samples

From September to December 1997 [*c.* 6 months after the suspension of the use of avoparcin (a glycopeptide antibiotic, like vancomycin) as an AMGP by the European Commission] *c.* 250 farmers in the south of The Netherlands, keeping either broilers or laying-hens were asked by letter to submit one fresh faecal sample from themselves and a mixed sample consisting of fresh faecal droppings of three different chickens from the oldest flock at their farms. In addition 100 poultry slaughterers working at the poultry-processing plant, where the broilers of the participating broiler farmers were slaughtered, were asked to provide one faecal specimen from themselves. All participants were requested to send the samples on the day of collection to the bacteriological laboratory together with a completed questionnaire about recent hospital stay, antibiotic usage by themselves, family members or their animals during the 3 months preceding sample collection and whether they kept food and/or pet animals. The samples (collected in small plastic vials without transport medium) were sent to the laboratory by parcel post. On the day of arrival at the laboratory within 24 h after collection the samples were diluted (10^{-1}) in 0.9% NaCl (w/v) with 20% (v/v) glycerol and stored frozen at $-20°C$ until assayed (3 months maximum).

Isolation of (resistant) enterococci

After thawing the samples 40 µL of 10^{-1} and 10^{-3} dilutions in 0.9% NaCl (w/v) were inoculated on to KF–Streptococcus agar plates (Oxoid CM701, Basingstoke, UK) with and without antibiotics using a spiral plater (Salm en Kip BV, Utrecht, The Netherlands) as described previously.[10,11] The antibiotics were selected because they, or related antibiotics, that are known to show cross-resistance with the tested antibiotics, had been regularly used in poultry either on veterinary prescription or as AMGPs. The antibiotic concentrations incorporated in the agar are shown in Table 1 and are the same as used in previous studies[10–12] to make results comparable. If after 48 h of incubation no VRE were detected on the vancomycin-containing agar plate, 0.5 mL of the 10^{-1} dilution was incubated overnight in nutrient broth containing 10 mg/L vancomycin and 0.4 g/L sodium azide and the next morning 0.5 mL of this broth was plated out on a vancomycin (10 mg/L)-containing agar plate.

Enterococci appeared as typical red or pink colonies on KF–Streptococcus agar. After 48 h incubation at 42°C only the typical pink colonies were counted. The minimum detection level, as assayed with spiked faeces samples, was *c.* 300 cfu/g faeces.

The prevalence of antibiotic resistance (%) in a population was calculated as the number of samples showing

Table 1. Prevalence of antibiotic-resistant enterococci and prevalence of high degree of resistance in faecal samples from broilers, laying-hens, broiler farmers, laying-hen farmers and poultry slaughterers

Antimicrobial agent	Concentration (mg/L)	Study population (number of faecal samples)									
		broilers (n = 50)		laying-hens (n = 25)		broiler farmers (n = 51)		laying-hen farmers (n = 25)		poultry slaughterers (n = 46)	
		prev.	HD	prev.	HD	prev.	HD	prev.	HD	prev.	HD
Amoxicillin	25	6	0	0	0	4	0	0	0	0	0
Ciprofloxacin	4	28	0	8	0	2	0	4	0	2	0
Gentamicin	500	44	0	40	0	10	0	0	0	2	0
Oxytetracycline	25	94	24	98	0	75	20	76	4	46	10
Quinupristin–dalfopristin	8	92[a]	8	12[a]	0	37[a]	6	8[a]	0	15[a]	0
Vancomycin	10	80 (82)[b]	8	12	0	27 (36)[b]	0	8 (12)[b]	0	13 (17)[b]	2
Erythromycin	8	94	44	98	8	67	16	64	4	50	8

prev. = prevalence of resistance (%).
HD = prevalence of high degree of resistance (%).
[a]At least one *E. faecium* was isolated from each positive sample.
[b]Samples positive after enrichment included.

growth of enterococci on the antibiotic-containing plates, divided by the total number of samples tested × 100.

The degree of antibiotic resistance (%) of each faecal sample tested was determined from the number of enterococcal colonies on the antibiotic-containing plate divided by the total number of enterococcal colonies on the antibiotic-free plate × 100. Two degrees of antibiotic resistance can be distinguished: low degree of resistance, i.e. when <50% of the enterococci present in a faecal sample are resistant and high degree, when 50% or more (thus the majority) are resistant to that particular agent.[11,13] The prevalence (%) of high degree resistance is the number of samples with a high degree of resistance to a particular antibiotic divided by the total number of samples tested × 100.

Because *Enterococcus faecalis* is considered to be intrinsically resistant to dalfopristin–quinupristin, a sample was only considered to contain enterococci resistant to this drug if at least one *Enterococcus faecium* was isolated and identified from the dalfopristin–quinupristin-containing agar plate. For this reason the prevalence (%) of high degree of resistance could only be calculated for the total enterococcal population in the specimen.

Identification and antibiotic susceptibility testing

If growth was observed on a vancomycin- or gentamicin-containing agar plate, one colony from these plates was selected at random for identification and determination of the MIC of an extended panel of antibiotics with a microbroth dilution method in IsoSensitest Broth (Oxoid CM473) using an inoculum size of 5×10^5 cfu/mL. *E. faecalis* ATCC 29212 was used as reference strain. Identification to species level was performed according to the criteria of Devriese *et al.*[14,15]

PFGE analysis of VRE

If VRE were recovered from a chicken and a farmer from the same farm these isolates were genotyped by PFGE, the resistance genes identified and the *vanA* transposons analysed.

PFGE was performed after DNA digestion with *Sma*I as described previously.[16] To assess the similarity of the different patterns the criteria of Tenover *et al.*[17] were used: if an isolate differed from a main type by only three or less bands, it was considered as a subtype. Comparison of the PFGE fingerprints was made using computer assisted analysis (BioNumerics, Applied Maths, Kortrijk, Belgium). Comparisons of patterns were made using the unweighted pair group method using arithmetic averages (UPGMA) clustering method by using the Dice coefficient.

Detection of vanA, vanB and vanC genes

The *vanA*, *vanB* and *vanC* genes were detected by hybridization with specific probes as described previously.[10]

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Molecular characterization of Tn1546 derivatives

Characterization of the *vanA*-containing transposons was performed by means of restriction fragment length polymorphism (RFLP) analysis and DNA sequencing of Tn*1546*-specific PCR products as described previously.[10,18] Tn*1546* derivatives were classified by type in concordance with the nomenclature used previously.[10,18] All VRE isolates were analysed for the presence of the point mutations at positions 1226, 4847, 7658, 8234 and 9692, for left and right end deletions and for the exact integration site and orientation of IS*1216V* downstream of *vanX* in the type B and E transposons.

Statistical analysis

A one-way analysis of variance was used to estimate overall differences between the group means. Group means were compared pairwise using *t*-tests controlled for overall error rate (Bonferroni test) where $P < 0.05$ was regarded as statistically significant. Correlations between populations were calculated using the Pearson's product moment correlation coefficient test.

Results

Study population

From the 100 poultry slaughterers asked to participate in the study 46 responded and 31% from the poultry farmers: 51 of 150 broiler farmers and 26 of 100 laying-hen farmers. One broiler farmer had no animals on the farm at the moment of sample collection so only a human faecal sample was supplied. From 46 faecal samples from slaughterers 41 (89%) yielded enterococci. All 50 broiler samples, all 25 samples of laying-hen farmers and 24 (96%) of the samples of the laying-hens, and 49 (96%) of 51 stools of broiler farmers also yielded enterococci. Sixty-five samples produced growth of enterococci on the vancomycin-containing agar plates and an additional 19 samples yielded enterococci after enrichment (Table 1).

The mean ± S.D. $\log_{10}$ cfu of enterococci per gram of faeces in positive faecal samples was 5.4 ± 1.4 for humans and 7.7 ± 0.4 in chicken faecal samples. In the 3 months preceding the sample collection two poultry slaughterers and one laying-hen farmer had been hospitalized. None of the laying-hen farmers had taken antibiotics, but in four cases one member of their family had; four broiler farmers (8%) and two family members had used antibiotics; and four slaughterers (9%) and two members of their respective families had. In all three groups *c.* 55% kept pet animals, mainly dogs and cats. Thirteen of the laying-hen farmers (52%), 18 of the broiler farmers (35%) and six poultry slaughterers (13%) also kept other food animals, mainly pigs. There were no farmers in the study who kept both laying-hens and broilers. None of the poultry

slaughterers kept poultry. There were no significant differences observed within the same group between people keeping pigs and those who did not (data not shown). From the laying-hen flocks two (8%) and 23 (46%) of the 50 broiler flocks had received antibiotics on veterinary prescription whilst on the farm: one laying-hen flock had been treated with amoxicillin and the other with oxytetracycline. From the broiler flocks 14 had been treated with oxytetracycline, three with co-trimoxazole, two with either amoxicillin or a combination of lincomycin and spectinomycin and one with flumequine. One broiler flock had been treated twice: first with tylosin and subsequently with enrofloxacin. No information was obtained about AMGP use.

Prevalence and degree of resistance

The prevalence of resistance and the prevalence of high degree resistance for the tested antibiotics of the populations studied are presented in Table 1.

The highest percentage of samples with a high degree of resistance was observed in the faecal samples from broilers. The prevalence of resistance was significantly higher in broilers for quinupristin–dalfopristin and vancomycin than in laying-hens ($P < 0.05$). For oxytetracycline, erythromycin and gentamicin the prevalence of resistance was of the same order in both chicken populations, but the prevalence of high degree was significantly higher in broilers than in laying-hens ($P < 0.05$). In the human samples, amoxicillin resistance was only observed in faecal samples of broiler farmers and high-level gentamicin resistance in a few samples of broiler farmers and poultry slaughterers. Tetracycline resistance was high in all three human populations. The prevalence of resistance for vancomycin and quinupristin–dalfopristin was higher in broiler farmers than in laying-hen farmers and poultry slaughterers, but this was only significant for vancomycin ($P < 0.05$).

In general, the prevalence of resistance against all antibiotics correlated well between broilers and broiler farmers (correlation coefficient: 0.95) and between broilers and poultry slaughterers (correlation coefficient: 0.96, $P < 0.01$), while the prevalence of antibiotic resistance of laying-hens showed no significant correlation with any of the three human populations studied.

Resistance of isolates

All 27 isolates that were isolated from the gentamicin-containing agar plates had MIC > 500 mg/L. This high-level resistance (HLR) to gentamicin, was observed in seven isolates from broilers that were identified as *E. faecium*. Three of these were also resistant to amoxicillin and two were additionally resistant to all antibiotics tested: oxytetracycline, erythromycin, quinupristin/dalfopristin and trimethoprim, but not to vancomycin. The other 20 gentamicin HLR isolates were identified as *E. faecalis* except for three isolates from broiler farmers, which were identified as *E. faecium*.

Table 2. MIC values for enterococcal isolates ($n = 73$) from vancomycin-containing (10 mg/L) selective agar plates

Antibiotic	MIC (mg/L)								
	0.5	1	2	4	8	16	32	64	128
Vancomycin			2		4	4	7	27	29
Avoparcin	2			3	15	32	18	3	
Teicoplanin	16	5	7	12	12	11	9	1	

Table 3. Identification of vancomycin-resistant enterococci ($n = 73$) from broilers, laying-hens, broiler farmers, laying-hen farmers and poultry slaughterers

Species	Broilers ($n = 40$)	Laying-hens ($n = 3$)	Broiler farmers ($n = 19$)	Laying-hen farmers ($n = 3$)	Poultry slaughterers ($n = 8$)
Enterococcus faecium	24	2	10	2	4
Enterococcus faecalis	2	1	–	–	2
Enterococcus hirae	6	–	8	–	2
Enterococcus durans	8	–	1	1	–

The MICs of glycopeptides and the identification of isolates from the vancomycin-containing plates are shown in Tables 2 and 3, respectively. Only 73 isolates were tested as one broiler isolate was lost during storage. In all 73 isolates but two (one broiler and one broiler farmer isolate, which were identified as *E. durans* and *E. faecium* respectively) the *vanA* gene cluster was detected by blot hybridization. The MIC for these *vanA*-containing isolates of vancomycin was 8 mg/L (Table 2). No *vanB* or *vanC* genes were found. Most *vanA*-containing isolates were identified as *E. faecium* ($n = 41$), but five isolates were *E. faecalis*, 16 *E. hirae* and nine *E. durans*.

Pulse-field gel electrophoresis (PFGE)

At 10 farms VRE were isolated from the farmer, and from his poultry. These 10 paired VRE isolates from the farmer and his chickens were analysed by PFGE (Figures 1, 2 and Table 4). In general the PFGE patterns obtained were highly variable. Only at two farms (nos 14 and 31) were the isolates from the farmer and from the broilers of the same farm either identical (100%) or highly similar (93%). In addition, the VRE isolated from broiler farmer 40, showed a high degree of similarity (85%) to the VRE isolates from broiler farmer 14 and his broilers. These five isolates were identified as *E. hirae*.

Tn1546 types among different VRE isolates

Eight different *vanA* transposon types were found among the 18 VRE isolated from poultry and farmers (Figure 1). Tn types A1, A2, B2, E7 and E11 have been described previously.[10,18] In short, type A1 is identical to Tn*1546*.[19] Type A2 is characterized by a G $\rightarrow$ T point mutation at position 8234 and an IS*1216V*–IS*3* insertion at the left end of the transposon (Figure 2). Type B2 contains an IS*1216V* insertion in the *vanX–vanY* intergenic region. Type E7 contains also the IS*1216V* insertion in the same region as in type B2 as well as a large left end deletion encompassing the *orf1* gene and a part of the *orf2* gene, while type E11 contains an additional deletion at the right end of the transposon including the *vanZ* gene. Three new transposon types were found: types E14, E17 and E19. Types E14 and E17 are closely related to type E11. The only difference resides in the size of the small deletion flanking the IS*1216V* insertion site downstream of *vanX*. Type E19 is comparable to type E7, with differences in the size of the left end deletion and in the size of the small deletions adjacent to the IS*1216V* insertion site in the *vanX–vanY* intergenic region. Comparative analysis of the Tn*1546*-like elements of the paired VRE strains revealed that in addition to the two sets of paired strains with comparable PFGE patterns, 14 and 31, the paired strains of broiler and broiler farmer 3 also contained identical transposon types. In the other cases different transposon types were present in chicken and farmer isolates. In conclusion, in three of the eight paired strains that were analysed an identical *vanA* transposon was present in both the animal and farmer isolate.
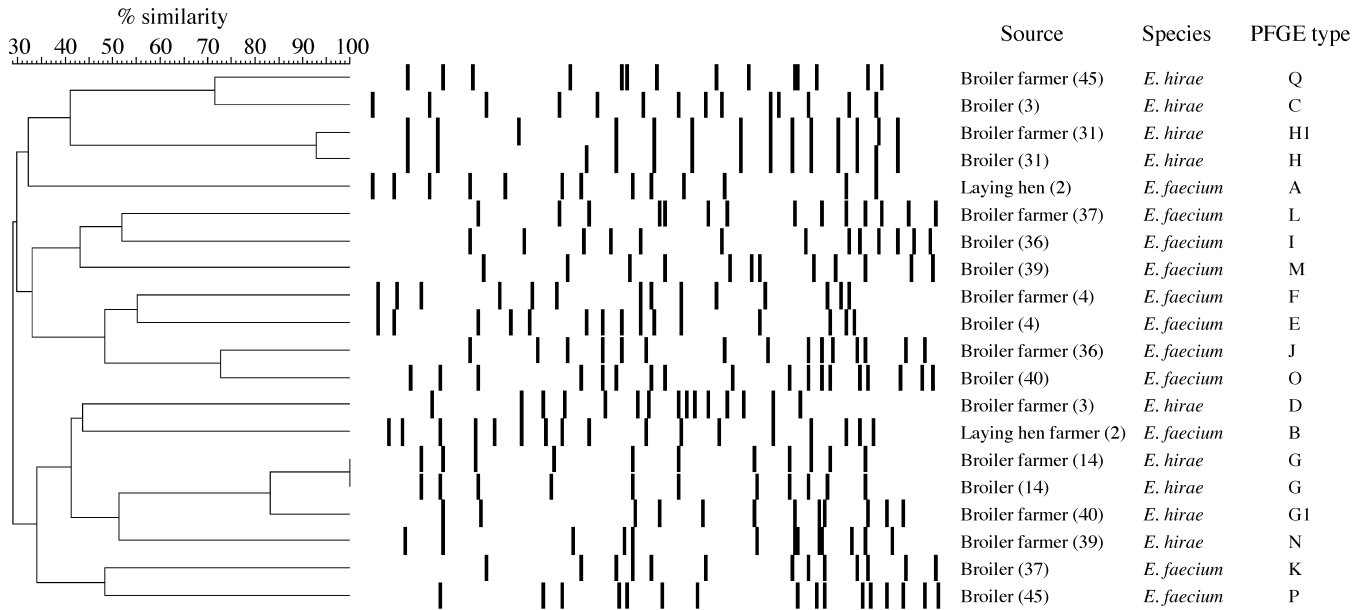
Figure 1. PFGE patterns and dendrogram of 10 paired vancomycin-resistant enterococci isolated from faecal samples of farmers and poultry from the same farm after total DNA digestion with *Sma*I.

Discussion

The overall prevalence of antibiotic resistance between broilers and broiler farmers and poultry slaughterers indicates that contact with broilers is a risk factor for colonization of humans with resistant bacteria. In laying-hens and laying-hen farmers this was only the case for tetracycline and erythromycin resistance, which correlated with the increased risk for humans from animals with a high degree of resistance. The prevalence of 6% amoxicillin-resistant faecal enterococci in broiler flocks and the absence in laying-hens, was most likely a result of the higher overall antibiotic usage in broilers than in laying-hens, which favours the selection of intrinsically less amoxicillin-susceptible *E. faecium* in the intestinal flora. The presence of amoxicillin-resistant enterococci only in the faecal flora of broilers and broiler farmers and not in any other of the studied populations nor in healthy suburban residents in The Netherlands[12] is suggestive of a broiler to farmer transfer of resistant enterococci.

The significantly higher fluoroquinolone resistance observed in broilers compared with the other populations is most likely due to the more common use of enrofloxacin and flumequine, a less potent fluoroquinolone than enrofloxacin, in broilers than in laying-hens. The fluoroquinolone-resistant enterococci isolated from laying-hens might be due to the use of quinolones during the rearing period. The prevalence of resistance in broiler farmers, however, was not different from the other populations studied. As clonal transmission of fluoroquinolone-resistant *E. coli* strains from poultry to humans has been described,[20] this might suggest that colonization of humans by poultry enterococci occurs less readily than colonization
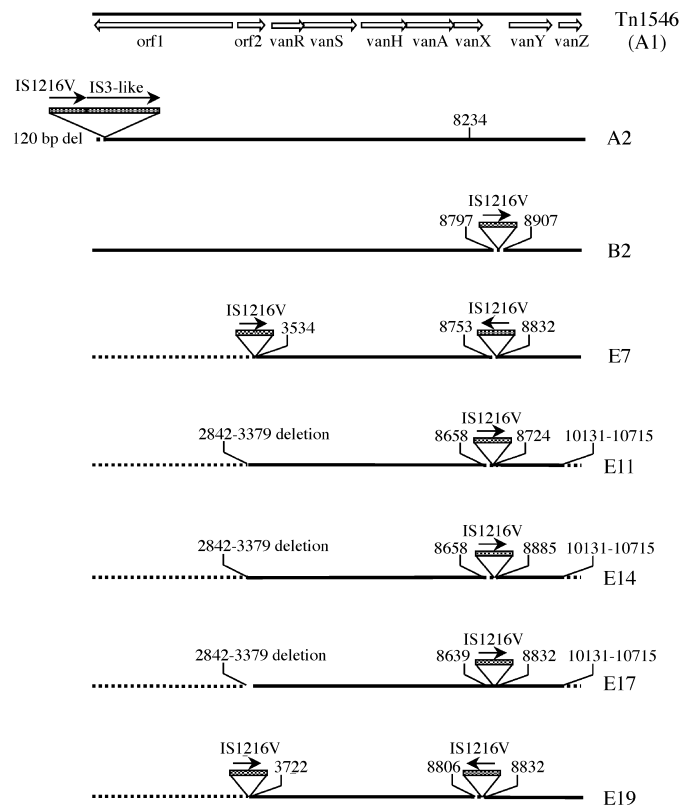
Figure 2. Genetic map of Tn*1546* and four Tn*1546* derivatives. The thick horizontal lines represent Tn*1546* and Tn*1546* types E8–112. The position of genes and open reading frames (orf) and direction of transcription is depicted by open arrows. Dotted boxes represent IS elements. The position of the first nucleotide upstream and downstream from IS insertion sites are depicted. Filled arrows indicate the transcriptional orientation of inserted IS elements. Deletions are indicated by dotted lines.

Table 4. Characteristics of paired GRE isolated from samples of farmers and chickens from the same farm

Source	Species	MIC vancomycin (mg/L)	MIC teicoplanin (mg/L)	PFGE pattern	Transposon type
Laying-hen (2)	*E. faecium*	128	8	A	B2
Laying-hen farmer (2)	*E. faecium*	128	8	B	A2
Broiler (3)	*E. hirae*	64	4	C	E11
Broiler farmer (3)	*E. hirae*	64	4	D	E11
Broiler (4)	*E. faecium*	64	8	E	ND[a]
Broiler farmer (4)	*E. faecium*	1	0.5	F	A1
Broiler (14)	*E. hirae*	64	4	G	E11
Broiler farmer (14)	*E. hirae*	64	0.5	G	E11
Broiler (31)	*E. hirae*	64	4	H	E11
Broiler farmer (31)	*E. hirae*	32	1	H1	E11
Broiler (36)	*E. faecium*	64	4	I	A1
Broiler farmer (36)	*E. faecium*	64	16	J	E7
Broiler (37)	*E. faecium*	128	32	K	A1
Broiler farmer (37)	*E. faecium*	128	16	L	A2
Broiler (39)	*E. faecium*	32	0.5	M	E19
Broiler farmer (39)	*E. hirae*	64	1	N	E14
Broiler (40)	*E. faecium*	32	1	O	A1
Broiler farmer (40)	*E. hirae*	64	0.5	G1	E11
Broiler (45)	*E. faecium*	32	2	P	ND[a]
Broiler farmer (45)	*E. hirae*	64	0.5	Q	E17

[a]ND = not determined.

by poultry *E. coli*. As resistance to fluoroquinolones is caused by chromosomal mutations and is not transferable, this could explain the observed lack of correlation in the prevalence of ciprofloxacin-resistant enterococci between broilers and broiler farmers and suggests that for other antibiotics the exchange of resistance genes between poultry enterococci and endogenous human enterococci is a more important phenomenon for resistance transfer than colonization of the human intestinal tract by poultry enterococci alone.

The high prevalence of HLR to gentamicin in the enterococcal faecal flora of both broilers and laying-hens in this study was unexpected. Thal *et al*. found no high-level gentamicin-resistant enterococci in faecal samples of poultry despite their presence in wild birds[21–23] and environmental samples.[24] In Denmark only 1% of the *E. faecalis* isolated from healthy broilers at slaughter were resistant to gentamicin and no gentamicin-resistant *E. faecium* were isolated.[25] In this study in both species HLR to gentamicin has been observed. Gentamicin is not registered for use in poultry in The Netherlands, but the observed resistance might have been due to regular off label use in young chickens. High-level gentamicin resistance in enterococci has been reported in several studies from human infections,[26–28] but in faecal enterococci from healthy Dutch suburban residents in 1997 no high-level gentamicin-resistant enterococci were detected.[12] The prevalence of 10% in broiler farmers is most likely derived from their animals

as gentamicin is practically only used in hospitals in The Netherlands and none of the farmers or family members had been in hospital in the 3 months before the study.

The prevalence of erythromycin resistance was in all populations high, but the high degree of resistance was significantly higher ($P < 0.05$) in broilers than in the three human populations, indicating transfer from broilers to humans. Erythromycin is fully cross-resistant with tylosin, a commonly used antibiotic for poultry as AMGP, and on veterinary prescription. Several studies have shown that in chickens raised on tylosin-containing feed not only the percentage tylosin-resistant enterococci in the faecal flora of exposed animals increased over time but also the relative numbers of *E. faecium* to the total numbers of enterococci present in the flora.[29–32] Quinupristin–dalfopristin is like virginiamycin a mixture of two pristinamycins that act synergistically. In the EU virginiamycin has only been used sparsely for human therapy of staphylococcal infections and only in a few member states, but has been used extensively as AMGP for many years. Both compounds are cross-resistant. At the time of the study quinupristin–dalfopristin was not registered for any use in the EU. The high prevalence of quinupristin–dalfopristin resistance observed in broilers is therefore most likely caused by the common use of virginiamycin in broiler feeds as AMGP.

VRE were most common in the faecal samples from broilers. In broiler farmers a significantly higher prevalence

and degree of VRE was observed than in the other two human populations in this study and in Dutch suburban residents.[5] Because avoparcin does not select for *vanB* resistance the most common resistance gene cluster in animal isolates is of the *vanA* type, which confers resistance to both vancomycin and teicoplanin. In this study, however, >50% of the VRE were phenotypically susceptible to teicoplanin. This was due to the fact that the most prevalent transposon variant was a Tn*1546* derivative, which contained a large left end deletion including the *orf1* gene, an IS*1216V* insertion in the *vanX–vanY* intergenic region and a deletion of the *vanZ* gene, which in the normal transposon confers teicoplanin resistance. These features found in the homologous types E11, E14 and E17 were present in half of the transposon types analysed. Interestingly, type E11 and the homologous types E9 and E10 were also predominantly present (9/13) in isolates recovered from turkeys and in almost half of the isolates (4/9) recovered from turkey farmers in The Netherlands.[10] All Tn*1546* variants found in the isolates from broilers and laying-hens contained at position 8234 the 'G', which is consistent with the results reported previously.[10,18,33] This variant is also found in humans although the majority of human isolates and all isolates recovered from pigs contain a 'T' at this position.[10,18,33] The finding that human isolates analysed in this study contain *vanA* transposon types predominantly found in poultry and not the most prevalent human transposon type is again an indication that poultry farmers have acquired *vanA* resistance genes from their animals. Since the isolates recovered from poultry and poultry farmers were in most cases genetically different, it is most likely that vancomycin resistance was transmitted from poultry enterococci to enterococci of the intestinal flora of the farmers by horizontal transfer of Tn*1546* variants. That this was only found in three cases does not mean that it is a rarely occurring event since only one isolate was tested from each sample and, therefore, the method used is likely to have a very low sensitivity. Other studies have also pointed to a similarity between *vanA*-containing elements in animals and humans.[10,18,34–38] It was interesting to note that all *E. hirae* isolates, irrespective of whether they originated from animal or human sources, contained the highly homologous *vanA* transposon types E11, E14 and E17. This could suggest a level of enterococcal species specificity of *vanA* transposon types and is consistent with the results of Butaye *et al.*[39] who found that 50% of VRE from boilers and nearly all VRE from laying-hens were *E. hirae*.

In conclusion the results of this study provide evidence for dissemination of resistant enterococci from animals to man and, probably more importantly the exchange of resistance genes between poultry and human enterococci. As antibiotic resistance is not restricted to *E. faecium*, but also occurs regularly in other enterococcal species, monitoring of resistance should not be restricted to *E. faecium* or *E. faecium* and *E. faecalis* isolates alone.

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