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High occurrence and persistence of antibiotic-resistant enterococci in poultry food samples in Portugal

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***Objectives*: We determined the presence of antibiotic-resistant enterococci (ARE) in commercialized poultry samples from Portugal and analysed their clonal diversity and the resistance genes harboured by these strains.**

***Methods*: Ninety-nine retail poultry samples of 10 widely commercialized brands were studied (1999–2001). Samples were enriched and plated on selective media with and without vancomycin, gentamicin, streptomycin or kanamycin. Antibiotic susceptibility was established following standard criteria. Identification and detection of genes coding for resistance were determined by PCR. Clonal relatedness was established by PFGE.**

***Results*: A high percentage of samples contained vancomycin-resistant enterococci (VRE) (48%), or enterococci highly resistant (HLR) to gentamicin (34%), streptomycin (32%) or kanamycin (30%). Co-resistance to tetracycline, erythromycin, ciprofloxacin and quinupristin/dalfopristin was observed in most of these isolates. VRE were classified as VanA phenotype-*vanA* genotype (38% of samples), VanB phenotype-*vanA* (13%) or VanC phenotype-*vanC1* (23%). All HLR to gentamicin isolates contained *aac(6′)-Ie-aph(2″)-Ia*. We detected *erm*(B) in both erythromycin-resistant and -susceptible isolates. Some VRE and HLR to gentamicin strains were recovered from different samples and brands. Long-term persistence of particular VRE strains (>2 years), exhibiting different Van phenotypes, was observed.**

***Conclusions*: High occurrence of ARE suggests maintenance of selective pressure by the use of antibiotics/other substances in the Portuguese poultry environment. Persistence of a number of widespread PFGE types containing different resistance genes might reflect environmental/host-adapted enterococcal strains that might contribute to the maintenance of antibiotic resistance, thus constituting a resistance reservoir that is non-sensitive to banning interventions.**

Keywords: VRE, persistent clones, macrolide resistance, high-level gentamicin resistance

Introduction

The increase in antibiotic-resistant enterococci (ARE) recovered from animal production, food products and non-hospitalized patients during the 1990s raised concern about the medical consequences of selection of resistance caused by the use of antibiotics for growth enhancement in animal production. This fear has led to the progressive ban of antibiotic growth promoters in the European Union.[1]

Dissemination of ARE occurs through the spread of particular strains and/or different mobile elements carrying antibiotic resistance genes.[1,2] In poultry production, the risk of spread of such enterococci is enhanced as only a few different genetic lines of primary breeding flocks are sold by a reduced number of suppliers. This limits the host diversity and, because of the increased market globalization, the spread of specific clones and elements is facilitated.

Local maintenance of specific antibiotic-resistant strains and/or genetic units associated with poultry has been demonstrated in countries without apparent antibiotic selection.[3] However, little is known about the occurrence of genetically characterized ARE from animals after the growth promoter ban in most European

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countries. Our objective was to analyse the presence and diversity of ARE in poultry samples from Portugal, a country with high antibiotic use in veterinary medicine and with the highest rate of clinical vancomycin-resistant enterococci (VRE) in the European Union.

Materials and methods

Bacterial strains and sample processing

Between September 1999 and March 2001, 99 swabs or meat samples from the inner carcass of retail poultry (93 chicken lots and 6 turkey lots) were collected on 42 different days from two different butcher shops and one market in the Porto area. Samples corresponded to 10 different brands widely commercialized throughout Portugal: Brand A ($n = 20$), B ($n = 1$), C ($n = 10$), D ($n = 12$), E ($n = 12$), F ($n = 3$), G ($n = 8$), H ($n = 1$), I ($n = 5$) and J ($n = 4$). The brand of 23 samples was unknown. They were collected before butcher manipulation or contact with butcher shop surfaces and processed on the same day. Samples were pre-enriched (37°C for 48 h) in buffered peptone water without antibiotics or brain heart infusion (BHI) broth (Oxoid, Basingstoke, UK) with and without 6 mg/L vancomycin. A 100 μL aliquot was plated onto M-Enterococcus agar (Difco, Detroit, MI, USA) or Slanetz-Bartley media (Oxoid, Basingstoke, UK) with and without 6 mg/L vancomycin, 125 mg/L gentamicin, 1000 mg/L streptomycin or 500 mg/L kanamycin. Plates were incubated at 37°C for 24–96 h. Antibiotic resistance phenotype was confirmed by subculturing on the same selective plates as described. From each sample, only one colony per morphology type and antibiotic susceptibility profile was selected.

Identification, susceptibility and characterization of antibiotic resistance

Susceptibility to 12 antibiotics (quinupristin/dalfopristin from Rhône-Poulenc, Lisbon, Portugal; vancomycin, teicoplanin, ampicillin, tetracycline, erythromycin, ciprofloxacin, chloramphenicol, gentamicin, streptomycin, kanamycin and nitrofurantoin from Sigma Chemical Co., St Louis, MO, USA) was determined by the agar dilution method.[4] Species identification and detection of genes coding for resistance to glycopeptides, aminoglycosides, macrolides or streptogramins were conducted by PCR.[5]

Mating experiments

Conjugation experiments were performed by filter and/or broth mating methods at a 1:10 donor/recipient ratio using *Enterococcus faecalis* strain JH2-2 and *Enterococcus faecium* strain GE1 as recipients strains (both are fusidic acid- and rifampicin-resistant, plasmid-free).[5] VRE isolates representing each clone/clonal subtype were used as donors ($n = 40$). Transconjugants were selected on BHI agar plates containing fusidic acid (25 mg/L), rifampicin (100 mg/L) and vancomycin (6 mg/L) and incubated at 37°C for 24–48 h. Conjugation frequency was expressed as number of transconjugants per donor cells.

PFGE

Clonal relationship was analysed among enterococci harbouring *vanA* (all species but *Enterococcus gallinarum*) or *aac(6′)-aph(2″)-Ia* (*E. faecium* and *E. faecalis*) by PFGE using *Sma*I as restriction enzyme as previously described.[5]

Results

Bacterial strains and susceptibility background

Enterococci were recovered from 83% of the samples studied (82/99 samples, 409 isolates). A high percentage of samples contained enterococci non-susceptible to vancomycin (48%), or highly resistant to gentamicin (HLR-GEN 34%), streptomycin (HLR-STR 32%) or kanamycin (HLR-KAN, 30%). Lack of susceptibility to other antibiotics was considered as co-resistance to those used in selective plates in order to avoid overestimation of results by repeated counting of isolates on different selective media (Table 1). Co-resistance to tetracycline, erythromycin, ciprofloxacin and quinupristin/dalfopristin was observed in the majority of VRE, HLR-GEN, HLR-STR or HLR-KAN isolates. Interestingly, VRE scarcely showed HLR-GEN or vice versa. *E. faecium* was more often resistant to vancomycin and *E. faecalis* were more often resistant to high levels of gentamicin.

Characterization of antibiotic resistance genes

Enterococci non-susceptible to vancomycin (MIC = 8 to ≥256 mg/L) were classified as: (i) VanA phenotype-*vanA* genotype, (MIC of teicoplanin ≥8 mg/L, 38 samples and 82 isolates); (ii) VanB phenotype-*vanA* genotype (MIC of teicoplanin ≤4 mg/L, 13 samples and 16 isolates); and (iii) VanC phenotype-*vanC1* genotype (MIC of teicoplanin ≤4 mg/L, 23 samples and 41 isolates). Some of the samples contained enterococcal isolates exhibiting different Van types. HLR-GEN was detected in enterococci carrying *aac(6′)-aph(2″)-Ia* (n = 32 isolates, 24 samples) or both *aac(6′)-aph(2″)-Ia* and *aph(3′)-IIIa* (n = 11 isolates, 11 samples). Isolates showing HLR-KAN but not HLR-GEN mainly contained the *aph(3′)-IIIa* gene (30 isolates, 24 samples). We did not detect known genes for HLR-KAN isolates from six samples.

The gene *erm*(B) was found among both erythromycin-resistant and -susceptible isolates (n = 222/391 isolates, MIC = 0.5 to >32 mg/L). We observed *vat*(D) in quinupristin/dalfopristin-resistant isolates (n = 7/259 non-*E. faecalis* isolates tested) and *vat*(E) in both quinupristin/dalfopristin-resistant and -susceptible isolates (n = 14 isolates/259). Simultaneous presence of *vat*(D) and *erm*(B) (one *E. gallinarum* and three *Enterococcus* spp.), *vat*(E) and *erm*(B) (two *E. faecalis*, three *E. faecium* and two *Enterococcus* spp.) or *vat*(D) and *vat*(E) (two *Enterococcus* spp.) was detected. Three isolates susceptible to quinupristin/dalfopristin contained *vat*(E) but did not present *erm*(B). We did not identify *aph(2″)-Ib*, *aph(2″)-Ic*, *aph(2″)-Id*, *erm*(A), *erm*(C) or *mef*(A) genes.

Transfer of vancomycin resistance was achieved from one *E. faecium* to *E. faecium* GE1 and from one *E. faecalis* to *E. faecalis* JH2-2 at a frequency of 10^{-7} and 10^{-8} transconjugants per donor, respectively.

PFGE

Thirty PFGE types were observed among 98 *vanA*-carrying isolates (PFGE types 1–30). Some strains were identified in different samples (Table 2): *E. faecium* types 1 (n = 10 samples/17 isolates), 4 (n = 8/18), 2 (n = 6/11) and 3 (n = 3/4); *E. faecalis* type 22 (n = 2/3) and *Enterococcus* spp. types 30 (n = 10/12), 28 (n = 3/5), 29 (n = 2/2) and 21 (n = 2/3). Isolates classified as PFGE type 1 showed either VanA phenotype-*vanA* genotype (n = 15 isolates) or VanB phenotype-*vanA* genotype (n = 2). Long-term persistence (2–3 years) was detected in some cases (PFGE types 1, 2, 3, 4, 22 and 29).

High diversity was observed among HLR-GEN isolates (26 types among 42 isolates, PFGE types 31–56). Ten PFGE

Table 1. Resistance and co-resistance of the different species of enterococci to 12 antibiotics

Isolates selected on	No. of resistant isolates of each species (*n*) selected in the different plates	Non-susceptible[a] to											
		VAN MIC ≥8 mg/L	TEC MIC ≥8 mg/L	AMP MIC ≥16 mg/L	TET MIC ≥8 mg/L	ERY MIC ≥1 mg/L	CIP MIC ≥2 mg/L	CHL MIC ≥16 mg/L	GEN MIC ≥500 mg/L	STR MIC ≥2000 mg/L	KAN MIC ≥2000 mg/L	NIT MIC ≥64 mg/L	Q/D MIC ≥2 mg/L
Without antibiotic	*E. faecalis* (*n* = 19)	0/19	0/19	0/19	15/19	12/19	15/19	6/19	3/19	5/19	3/19	0/19	ND
	E. faecium (*n* = 14)	2/14	2/14	2/14	13/14	11/14	11/14	2/14	1/14	4/14	4/14	8/13	4/7
	E. gallinarum (*n* = 2)	1/2	0/2	0/2	2/2	1/2	1/2	0/2	1/2	1/2	1/2	1/2	0/2
	Enterococcus spp. (*n* = 11)	1/11	1/11	0/11	7/11	6/11	8/11	3/11	0/11	4/11	0/11	6/10	5/8
	Total	4/46	3/46	2/46	37/46	30/46	35/46	11/46	5/46	14/46	8/46	15/44	9/17
Vancomycin (6 mg/L)	*E. faecalis* (*n* = 8)	8/8	7/8	0/8	8/8	8/8	3/8	1/8	0/8	5/8	0/8	3/8	6/6
	E. faecium (*n* = 61)	61/61	57/61	0/61	56/61	54/61	48/61	14/61	3/61	25/61	5/61	46/61	50/55
	E. gallinarum (*n* = 41)	41/41	0/41	0/41	38/41	26/41	30/41	6/41	2/41	16/41	6/41	5/41	33/34
	Enterococcus spp. (*n* = 29)	29/29	16/29	0/29	27/29	25/29	21/29	6/29	0/29	17/29	2/29	23/28	20/29
	Total	139/139	80/139	0/139	129/139	113/139	102/139	27/139	5/139	63/139	13/139	77/138	109/124
Gentamicin (125 mg/L)	*E. faecalis* (*n* = 22)	0/22	0/22	0/22	22/22	11/22	16/22	7/22	22/22	5/22	22/22	0/22	1/1
	E. faecium (*n* = 11)	0/11	0/11	2/11	11/11	10/11	9/11	5/11	11/11	7/11	11/11	6/10	9/9
	E. gallinarum (*n* = 6)	1/6	0/6	0/6	6/6	5/6	4/6	1/6	6/6	1/6	6/6	0/6	2/2
	Enterococcus spp. (*n* = 4)	0/4	0/4	0/4	3/4	3/4	1/4	2/4	4/4	1/4	4/4	1/3	2/2
	Total	1/43	0/43	2/43	42/43	29/43	30/43	15/43	43/43	14/43	43/43	7/41	14/14
Streptomycin (1000 mg/L)	*E. faecalis* (*n* = 24)	0/24	0/24	0/24	21/24	24/24	15/24	17/24	0/24	24/24	9/24	6/23	ND
	E. faecium (*n* = 13)	1/13	1/13	4/13	13/13	11/13	12/13	3/13	0/13	13/13	5/13	13/13	9/12
	Enterococcus spp. (*n* = 5)	0/5	0/5	0/5	5/5	5/5	1/5	1/5	0/5	5/5	3/5	2/5	3/5
	Total	1/42	1/42	4/42	39/42	40/42	28/42	21/42	0/42	42/42	17/42	21/41	12/17
Kanamycin (500 mg/L)	*E. faecalis* (*n* = 16)	0/16	0/16	0/16	15/16	12/16	12/16	10/16	5/16	11/16	16/16	0/15	ND
	E. faecium (*n* = 20)	1/20	1/20	7/20	20/20	20/20	17/20	8/20	3/20	20/20	20/20	15/19	12/18
	E. gallinarum (*n* = 3)	1/3	0/3	0/3	3/3	3/3	1/3	0/3	1/3	2/3	3/3	0/3	1/2
	Enterococcus spp. (*n* = 1)	0/1	0/1	0/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	0/1	1/1
	Total	2/40	1/40	7/40	39/40	36/40	31/40	19/40	9/40	34/40	40/40	15/38	14/21
Total	*n* = 310												

VAN, vancomycin; TEC, teicoplanin; AMP, ampicillin; TET, tetracycline; ERY, erythromycin; CIP, ciprofloxacin; CHL, chloramphenicol; GEN, high-level resistance (HLR) to gentamicin; STR, HLR to streptomycin; KAN, HLR to kanamycin; NIT, nitrofurantoin; Q/D, quinupristin/dalfopristin.

[a]Resistant and non-susceptible enterococci were considered together.

Table 2. PFGE types, species, antibiotic resistance patterns and antibiotic resistance genes of enterococci resistant to vancomycin and highly resistant to gentamicin found in more than one poultry sample

PFGE	PFGE subtypes (*n*)	Samples (*n*)	Date of isolation (month.year)	Isolates (*n*)	Brand	Antibiotic resistance profile[a]	Antibiotic resistance genes[b]
Vancomycin-resistant isolates							
E. faecium							
1	2	10	09.99–02.01	17	A,B,C,D, E,UN	VAN, (TEC), (TET), (ERY), (CIP), (HLR-STR), (Q/R), (NIT)	*vanA, (erm*(B)*)*
2	3	6	10.99–03.01	11	C,D,F,G	VAN, TEC, TET, (ERY), (CIP), (CHL), (HLR-STR), Q/D	*vanA, (erm*(B)*)*
3	4	3	10.99–03.01	4	A,G,H,UN	VAN, TEC, TET, ERY, (CIP), (HLR-GEN), (HLR-STR), (HLR-KAN), (Q/D), (NIT)	*vanA, (erm*(B)*), aac(6′)-Ie-aph(2″)-Ia*
4	5	8	11.99–03.01	18	A,F,I,UN	VAN, TEC, (TET), (ERY), (CIP), (HLR-STR), (HLR-KAN), (Q/D), (NIT)	*vanA, (erm*(B)*)*
E. faecalis							
22	2	2	10.99–02.01	3	A	VAN, TEC, TET, ERY, (CIP), (HLR-STR), Q/D	*vanA, erm*(B)
Enterococcus spp.							
21	3	2	10.99	3	D,F	VAN, Q/D (NIT)	*vanA, erm*(B)
28	2	3	10.99	5	F,UN	VAN, TEC, TET, (ERY), (HLR-KAN), (Q/D), (NIT)	*vanA, (erm*(B)*)*
29	2	2	10.99–07.00	2	D,F	VAN, TET, ERY, HLR-STR, (Q/D)	*vanA, erm*(B), (*vat*(D))
30	4	10	02–03.01	12	A,C,D,E, G,UN	VAN, TET, ERY, (CIP), (HLR-STR), (Q/D), (NIT)	*vanA, (erm*(B)*)*, (*vat*(E))
Highly resistant gentamicin isolates							
E. faceium							
36	2	2	02–03.01	2	J	AMP, TET, ERY, CIP, HLR-GEN, HLR-STR, HLR-KAN, Q/D, NIT	*aac(6′)-Ie-aph(2″)-Ia, (aphIII), (erm*(B)*), vat(E)*
38	1	2	03.01	2	A,G	TET, ERY, (CIP), HLR-GEN, (HLR-STR), HLR-KAN, Q/D	*aac(6′)-Ie-aph(2″)-Ia, erm*(B)
39	2	2	02.01	2	A,D	TET, (CIP), HLR-GEN, (HLR-STR), HLR-KAN, Q/D	*aac(6′)-Ie-aph(2″)-Ia*
E. faecalis							
41	3	6	01–03.01	6	A,D,G,J	TET, (ERY), CIP, HLR-GEN, (HLR-STR), HLR-KAN, (Q/D)	*aac(6′)-Ie-aph(2″)-Ia, (aphIII), (erm*(B)*)*
44	1	2	02–03.01	2	C,E	TET, HLR-GEN, HLR-KAN	*aac(6′)-Ie-aph(2″)-Ia, aphIII, (erm*(B)*)*
46	1	2	03.01	2	A,G	TET, ERY, CIP, HLR-GEN, HLR-KAN	*aac(6′)-Ie-aph(2″)-Ia, (erm*(B)*)*
49	1	2	03.01	2	C,E	TET, (ERY), (CIP), HLR-GEN, HLR-KAN	*aac(6′)-Ie-aph(2″)-Ia, (erm*(B)*)*
50	1	2	11.99	2	A,I	TET, ERY, HLR-GEN, HLR-STR, HLR-KAN, Q/D	*aac(6′)-Ie-aph(2″)-Ia, aphIII, (erm*(B)*)*
51	1	2	11.99	2	A	TET, ERY, HLR-GEN, HLR-STR, HLR-KAN, Q/D	*aac(6′)-Ie-aph(2″)-Ia, aphIII, (erm*(B)*)*
56	2	4	02–03.01	4	A,C,D,E	TET, (CIP), HLR-GEN, HLR-KAN	*aac(6′)-Ie-aph(2″)-Ia, (aphIII), (erm*(B)*)*

VAN, vancomycin; TEC, teicoplanin; AMP, ampicillin; TET, tetracycline; ERY, erythromycin; CIP, ciprofloxacin; CHL, chloramphenicol; HLR-GEN, high-level resistance (HLR) to gentamicin; HLR-STR, HLR to streptomycin; HLR-KAN, HLR to kanamycin; NIT, nitrofurantoin; Q/D, quinupristin/dalfopristin; UN, unknown.

Variable presence of a given resistance phenotype among isolates belonging to the same PFGE type appears in parentheses.

[a]Antibiotic resistance or [b]resistance genes were not transferred by conjugation.

types were observed in different samples, *E. faecalis* types 41 and 56 being isolated from six and four samples, respectively. It is of note that 19 samples presented isolates with distinct PFGE types (2–4 types per sample). Twenty-three (VRE) and 16 (HLR-GEN) PFGE types were represented by a single isolate (data not shown).

Discussion

The frequency of VRE in our study was high but within the range reported in other studies after ≥2 years of the avoparcin ban.[1,6] Although most of our VRE isolates presented VanA phenotype-*vanA* genotype, a relatively high percentage of samples showed the VanB phenotype-*vanA* genotype (13%) which has been mainly described in enterococci from poultry samples.[7] The occurrence of enterococci HLR-GEN was higher than that reported in other European studies.[6] Different genes responsible for this phenotype have been described in poultry from America and Asia,[8] and *aac(6′)-Ie-aph(2″)-Ia* is the only one found in the European Union to date.

High frequency of resistance to erythromycin, tetracycline, ciprofloxacin and quinupristin/dalfopristin was observed in enterococci from all selective plates. High values of resistance to these antibiotics have been related to the extensive use of quinolones, tetracyclines and streptogramins in animal husbandry.[1] Resistance values to chloramphenicol or nitrofurantoin was unexpected because these antibiotics are not available for veterinary use in Portugal and also enterococci are usually susceptible to these molecules.[1] However, the Portuguese authorities recently detected the illegal use of nitrofurans by several poultry producers which might have selected a resistant population. Genetic linkage to Tn*1546* has only been demonstrated in genes coding for macrolides of copper sulphate resistance[1] although co-selection by other agents cannot be ruled out. Since genes encoding resistance to erythromycin, tetracycline, quinupristin/dalfopristin or chloramphenicol are frequently located on multiresistance gene clusters, selection of one or more of these specific genetic elements could explain the persistence of different multiresistance patterns.[2] The lack of linkage between resistance to glycopeptides and gentamicin could be due to a species specificity of particular resistance-mediating elements and the remarkable low transferability of vancomycin resistance due to the adaptation of certain transferable elements to particular hosts. Hasman *et al*.[9] have recently described differences in the stability of plasmids coding for glycopeptide resistance among *E. faecium* strains, which may explain why some resistance plasmids are not successfully disseminated among animal enterococcal populations.

A relatively high number of enterococcal strains carried *erm*(B) that was not phenotypically expressed. The antibiotic banning policy is based on the concept that antibiotic-resistant strains should have a biological cost (low fitness) if compared with their susceptible counterparts that consequently would prevail without selection. Compensatory mutations reducing this cost[10] may include loss of expression of the involved genes, explaining the observed phenomenon.

The persistence (>2 years) of particular strains from different commercial brands suggests that a certain number of widely disseminated poultry clones might be relevant in the emergence and successful spread of ARE in animals. A reduced number of different genetic lines of primary breeding flocks might also facilitate the spread of particular host-adapted strains.

In summary, the high occurrence of ARE suggests maintenance of selective pressure by the use of different antibiotics or other substances. The relatively high number of widespread strains might reflect particular environmental/host-adapted enterococcal strains that could contribute to the persistence of antibiotic resistance, constituting a reservoir of non-susceptible isolates non-sensitive to banning interventions.

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Transparency declarations

No declarations were made by the authors of this paper.

References

1. Phillips I, Casewell M, Cox T *et al*. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *J Antimicrob Chemother* 2004; **53**: 28–52.

2. Weaver KE, Rice LB, Churchward G. Plasmids and transposons. In: Gilmore MS, ed. *The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance*. Washington, DC: American Society for Microbiology, 2002; 219–65.

3. Johnsen PJ, Østerhus JI, Sletvold H *et al*. Persistence of animal and human glycopeptide-resistant enterococci on two Norwegian poultry farms formerly exposed to avoparcin is associated with a widespread plasmid-mediated *vanA* element within a polyclonal *Enterococcus faecium* population. *Appl Environ Microbiol* 2005; **71**: 159–68.

4. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Sixth Edition: Approved Standard M7-A56*. NCCLS, Wayne, PA, USA, 2003.

5. Novais C, Coque TM, Ferreira H *et al*. Environmental contamination with vancomycin resistant enterococci from hospital sewage in Portugal. *Appl Environ Microbiol* 2005; **71**: 3364–8.

6. Busani L, Del Grosso M, Paladín C *et al*. Antimicrobial susceptibility of vancomycin-susceptible and -resistant enterococci isolated in Italy from raw meat products, farm animals and human infections. *Int J Food Microbiol* 2004; **97**: 17–22.

7. Ozawa Y, Tanimoto K, Nombra T *et al*. Vancomycin-resistant enterococci in humans and imported chickens in Japan. *Appl Environ Microbiol* 2002; **68**: 6457–61.

8. Harada T, Mito Y, Otsuki K *et al*. Resistance to gentamicin and vancomycin in enterococcal strains isolated from retail broiler chickens in Japan. *J Food Prot* 2004; **67**: 2292–5.

9. Hasman H, Villadsen AG, Aarestrup FM. Diversity and stability of plasmids from glycopeptide-resistant *Enterococcus faecium* (GRE) isolated from pigs in Denmark. *Microb Drug Resist* 2005; **11**: 178–84.

10. Andersson DI. Persistence of antibiotic resistant bacteria. *Curr Opin Microbiol* 2003; **6**: 452–6.