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The susceptibility to growth-promoting antibiotics of *Enterococcus faecium* isolates from pigs and chickens in Europe

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***Objectives*: To establish the susceptibility of *Enterococcus faecium* isolates from pigs and chickens to antimicrobial growth promoters that either were or had been in use in the European Union.**

***Methods*: Samples were taken at abattoirs in two successive years (mid-1998–mid-1999, year 1; mid-1999–mid-2000, year 2) from chickens (France, The Netherlands, Sweden, UK) and pigs (Denmark, The Netherlands, Spain, Sweden). *E. faecium* was isolated from faecal samples at national laboratories and sent to a central laboratory where MICs of avilamycin, avoparcin, bacitracin, flavophospholipol, spiramycin, tylosin and virginiamycin were determined. Microbiological breakpoints were allocated on the basis of MIC distributions, and comparison was made between host species, country of origin and year of sample.**

***Results*: In total, 2567 isolates were obtained from chickens and 1742 from pigs. In all countries, resistance to avoparcin (banned in 1997) was uncommon, but resistance to bacitracin and flavophospholipol was common (and was probably largely intrinsic). The prevalence of resistance was similar in chicken and pig isolates, with the exception of avilamycin, to which resistance was commoner among chicken isolates. The removal of four compounds as growth promoters (bacitracin, spiramycin, tylosin, virginiamycin) between years 1 and 2 appeared to result in a significant decrease in resistance to three of them—spiramycin, tylosin and virginiamycin, with no change in resistance to bacitracin, but an increase in resistance to avilamycin (not discontinued). Associated resistance was shown between some of the compounds.**

***Conclusions*: Resistance prevalence declined rapidly following removal of growth promoters in pigs and chickens, suggesting that in the absence of selective pressure, a susceptible population began to replace phenotypically resistant strains. Associated resistance between different compounds, where seen, could have resulted from either shared resistance mechanisms or from carriage of resistance genes on the same plasmid. Multiresistance to streptogramins, macrolides and glycopeptides was rare.**

Keywords: *E. faecium*, growth promoters, pigs, chickens, animals, surveillance, resistance

Introduction

Enterococci in the normal faecal flora of animals have been widely accepted as indicator bacteria for the detection of the prevalence of resistance due to the use of growth-promoting antimicrobial agents.[1–3] *Enterococcus faecalis* and *Enterococcus faecium* are found commonly in animals and humans, but the latter is the more useful indicator since it is considered a more significant resistance gene reservoir for the anti-Gram-positive antibiotic classes used as growth promoters in animals and as therapeutic agents in humans.

Data have been published from individual countries such as Denmark,[1] Sweden[2] and The Netherlands[3] showing the susceptibility of *E. faecium* isolated from farm animals, but an international centralized study comparing the susceptibility patterns in different countries has not hitherto been attempted. This study was set up to establish the susceptibility, over a 2 year period, of *E. faecium* to seven growth-promoting antimicrobial agents widely used in the early 1990s in Europe. Identical sampling protocols were used in six different EU countries and in two host species, and a single laboratory carried out susceptibility testing, to overcome the technical variations that might occur between

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Table 1. Availability of antimicrobial growth promoters in year 1 and year 2

Antimicrobial growth promoters	Year 1	Year 2
Avilamycin	+	+
Avoparcin	–	–
Bacitracin	+	–
Flavophospholipol	+	+
Spiramycin	+	–
Tylosin	+	–
Virginiamycin	+*	–

+, Available throughout the EU except Sweden; –, withdrawn in the EU; *unilaterally withdrawn in Denmark 6 months before the study began.

laboratories. Seven growth-promoting antibiotics were tested in this study (Table 1). Six of them were available for use in chickens and swine throughout the EU in 1998 (with the exception of certain Scandinavian countries) although one compound, avoparcin (a glycopeptide), had already been withdrawn within the EU. Unexpectedly, after the first year's samples had been collected, but prior to the second year of the study, four others, bacitracin (a polypeptide), spiramycin, tylosin (macrolides) and virginiamycin (a streptogramin), were banned as of July 1999. The removal of the four compounds provided an opportunity to follow the early effects of such removal on the antimicrobial susceptibility of *E. faecium* in the two host species. The other two compounds tested were avilamycin (an orthosomycin) and flavophospholipol (a glycophospholipid). These latter two compounds were available through both years of the study (but are to be withdrawn in the EU from 1 January 2006).

Methods

During successive 1 year periods (July 1998–June 1999: year 1; and September 1999–August 2000: year 2) samples were taken from chickens and pigs in four EU countries for each species. Denmark, The Netherlands, Spain, and Sweden were chosen for pigs, and France, The Netherlands, Sweden and the UK for poultry. Sampling coordinators in each country selected at least four abattoirs and four poultry-processing plants (excluding those that serviced only 'organic' systems). The sites chosen were geographically spread to be representative of the main animal-producing areas. Samples of colonic contents from pigs and caeca from chickens were collected randomly at the time of slaughter, with only one sample from each flock or herd, and sent to selected national microbiology laboratories in the country in which they were taken. There samples were homogenized, diluted and inoculated on Slanetz and Bartley agar, a selective, differential medium for enterococci, and incubated at 42°C for 48 h. Up to three colonies with the distinctive morphology of *E. faecium* were subcultured and their identity confirmed by a simplified scheme[4] based on the work of Devriese *et al*.,[5] modified by the addition of a test for methyl-α-D-glucopyranosidase.[6] The tests and the expected results for *E. faecium* were as follows: tetrazolium chloride reduction (–), acid from ribose and mannitol (+) and aesculin hydrolysis (+), and for confirmation in cases of doubt, motility (–), pigmentation (–) and acid from methyl-α-D-glucopyranoside (–). One isolate of *E. faecium* per specimen was stored at –70°C (or as a default, as a slope or stab culture on tryptone soya agar). For each country, the target number of isolates was 300 per host animal—in total 1200 isolates per host species per year.

Isolates were transported in batches to the laboratories of Inveresk Research, Tranent, Scotland. There, after isolates with yellow colonies had been rejected, the identity of a proportion of the rest was reconfirmed either by the use of commercial identification kits [API 20 Strep or rapid ID 32 Strep, (bioMérieux, Basingstoke, UK)] or by a PCR method[7] as later corrected.[8] The primers for the PCR, EfeddlF1 (5′+GCAAGGCTTCTTAGAGA 3′) and EfeddlF2 (3′-CATCGT-GTAAGCTAACTTC 5′) were supplied by PE-Applied Biosystems (Beaconsfield, UK) at a concentration of 1 mg/mL. Briefly, the conditions for PCR were as follows. Samples were prepared by the addition of a large loopful of pure overnight culture to 500 μL distilled water in an Eppendorf tube. This was then placed in a thermocycler at 100°C for 10 min and then held at –70°C. Before use, the tube was spun at 9000**g** for 2 min and the supernatant was added to 50 μL of reaction mix, which consisted of 37.4 μL of dH_2O, 10 μL of 10 × PCR buffer (200 mM Tris pH 9.0, 1 M KCl 0.2 mg/mL gelatine), 0.7 μL of Efeddl F1, 0.7 μL of Efeddl F2, 0.8 μL of dNTP mixture (Pharmacia, Milton Keynes, UK) and 0.4 μL of *Taq* polymerase 250 U (Boehringer Mannheim GmbH, Germany). Tubes were placed in a Hybaid Omnigene Thermocycler and heated as follows: one cycle of 2 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 54°C and 1 min at 72°C, and one cycle of 10 min at 72°C. Samples were stored at –20°C pending analysis. PCR product (10 μL) + 2 μL of 5 × loading dye was added to a 3% agarose gel with ϕX174 *Hae*III markers and run at 150 mA for 1 h. The presence of a band of 550 bp in length (subject to the correct performance of negative and positive controls) indicated that the organism was *E. faecium*.

Minimum inhibitory concentrations of the following antibiotics obtained from their manufacturers were determined by agar dilution as described by the National Committee for Clinical Laboratory Standards (NCCLS, now re-named Clinical and Laboratory Standards Institute CLSI) document M7-A4:[9] ampicillin (for quality control purposes), avilamycin (Eli Lilly), avoparcin (Roche), bacitracin (Alpharma), flavophospholipol (Hoechst Roussel Vet), spiramycin (Rhone Poulenc Rorer), tylosin (Eli Lilly) and virginiamycin (Pfizer). The following bacteria were used for quality control: *E. faecium* ATCC6569, *E. faecalis* ATCC29212 and *Staphylococcus aureus* ATCC29213. Although QC values were not available for any of the growth-promoting agents tested, these were generated in the course of the investigation as study-specific quality control ranges. Ampicillin, for which QC values were available, was used to ensure general test validity.

MIC data for each antimicrobial were then tabulated by year, by host animal and by country. Distributions of MICs were determined for each antibiotic and for QC strains, and on this basis the range of susceptibility of the wild-type population was defined for each antibiotic. This wild-type population was labelled 'susceptible' while other populations were defined as 'resistant', having low-level or high-level microbiological resistance as defined by the European Committee for Antimicrobial Susceptibility Testing (EUCAST, http://www.eucast.org) of the European Society for Clinical Microbiology and Infectious Diseases (ESCMID).[10,11] It is emphasized that assigned breakpoints were microbiological, and differed in principle from those used for animal or human therapy, which must also take into account pharmacological parameters and clinical and microbiological results of treatment.[9] 'Associated resistance' was identified when bacterial isolates resistant to one antibiotic were significantly more often resistant to a second antibiotic than were susceptible isolates.

The statistical significances of differences in the prevalence of resistance rates were assessed by Fisher's exact test (two-tailed) by

the method of summing of small values (http://www.graphpad.com/quickcalcs/CatMenu.cfm).

Results

Submitted isolates of E. faecium

During year 1 and year 2, respectively, 2353 and 2137 isolates of *E. faecium* were submitted to the central susceptibility testing laboratory. Of this total, 181 (4.1%) were rejected on the basis of phenotypic or PCR results. Of the remaining 4309 isolates, a further 635 selected at random were confirmed as *E. faecium* by PCR. The antimicrobial susceptibility of the remaining 4309 isolates was determined.

Antimicrobial susceptibility

Quality control strains. The quality control ranges established during the course of the investigation are shown in Table 2. Although the NCCLS Antimicrobial Susceptibility Testing Subcommittee[12] has established procedures to derive multi-laboratory quality control ranges for therapeutic antibiotics, there were no values available to use for these antibiotics during the study. However, study-specific ranges were defined. The tylosin quality control range subsequently established by the NCCLS for ATCC 29212 was 0.5–4 mg/L,[13] with which the range determined by the study-specific calculations conformed, thus supporting the validity of the method.

Field strains. The distribution of MICs for field strains isolated during the first and second year of study for each of the growth promoters is shown in Table 3, in which data from each country were combined for each of the seven antibiotics. The modes in these distributions were mostly apparent, and the breakpoints (mg/L) based on them were: avilamycin: >16; avoparcin: >8; bacitracin: >8; flavophospholipol: >32; spiramycin: >8; tylosin: >8; virginiamycin: >8 mg/L. The distribution of bacitracin MICs had three separate modes: the chosen breakpoint related to the first of these. A small proportion of isolates had flavophospholipol MICs of ≤32 mg/L, and these were considered to represent the wild-type susceptible population. Strains were commonly resistant to bacitracin and flavophospholipol but susceptible to avoparcin.

There were differences in resistance between samples taken in year 1 and year 2 following the withdrawal of bacitracin, virginiamycin, tylosin and spiramycin. With the exception of bacitracin, the prevalence of resistance decreased significantly by the second sample point. The prevalence of resistance to avilamycin increased while that of flavophospholipol was unchanged.

The prevalence of resistance for chicken and pig isolates for year 1 and year 2 for each of the countries is shown in Table 4. Results were similar in isolates from pigs and chickens for compounds other than avilamycin, to which isolates from pigs were less often resistant. Comparison of the results by country showed that Swedish isolates had a lower incidence of resistance to the compounds tested except for flavophospholipol and bacitracin, and that avoparcin resistance was uncommon in all countries sampled.

Associated resistance. The incidence of associated resistance between the agents is shown in Table 5, which includes results for tylosin but not spiramycin, since these were virtually identical. Resistance was very common throughout for flavophospholipol and bacitracin, so statistical analysis was not included for these compounds. The clearest correlations were between virginiamycin and macrolides: virginiamycin-resistant isolates were significantly more likely to be resistant to macrolides than were virginiamycin-susceptible isolates, and macrolide-resistant isolates were more often resistant to virginiamycin than were macrolide-susceptible isolates. Avilamycin-resistant isolates were more often resistant to avoparcin, tylosin and virginiamycin. Only 63 isolates (1.5%) were resistant to virginiamycin, macrolides and avoparcin.

Table 2. Susceptibility testing quality control results, based on 59 experiments

	E. faecalis ATCC 29212		*E. faecium* ATCC 6569	
Antimicrobial growth promoters	QC range mg/L	% out of range	QC range mg/L	% out of range
Avilamycin	1–4	2.9	1–8	6
Avoparcin	1–8	0.7	0.5–4	0
Bacitracin	16–64	0	16–64	2.8
Flavophospholipol	0.25–2	0.7	≥128	0
Spiramycin	0.25–2	0	1–4	0
Tylosin	0.5–2	0	2–16	0
Virginiamycin	2–16	0	1–4	0
Ampicillin	0.5–2	0	1–8	0

Discussion

Despite the unexpected withdrawal of EU authorization for four of the growth promoters, we were able to reach valid conclusions on the distribution of MICs of growth-promoting antibiotics for *E. faecium* isolated from food animals in Europe. Since Council Regulation 2821/98 (17.12.98) requiring the removal came into effect from 1 July 1999 (between the two sample points), the results also gave interesting information on the early effects of such a change on the susceptibility of these organisms.

By plotting the MIC distributions of each compound and combining the data from isolates obtained in the different countries in the way currently used by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), it was in each case possible to identify apparent wild-type populations—that is, a group of organisms without acquired resistance mechanisms. Interestingly, the modal MIC for each antibiotic for such populations was around 1–2 mg/L, although for flavophospholipol it seems possible that such low MICs for a very few isolates may have been aberrant, or that the results were correct but very unusual for the species. In fact, it has been shown that *E. faecium* is seldom susceptible to the substance, although *E. faecalis* is susceptible.[14] For avoparcin, whose use had been discontinued in all of the countries approximately a year before our study started, the prevalence of resistance was low and about 95% of isolates were in this wild-type group. Indeed, in Sweden, where avoparcin had not been used since 1986, all isolates had wild-type susceptibility, as did all isolates from pigs in Spain. Finally, the very large majority of isolates from pigs were of wild-type susceptibility to avilamycin, perhaps because the compound is not widely used in this species.

For most of the compounds the distribution of MICs was clearly bimodal or, in the case of bacitracin, trimodal. It was therefore relatively easy in each case to allocate microbiological MIC

Table 3. Distribution of MICs of all compounds for *E. faecium* isolates from chickens and pigs, in years 1 (2231 isolates) and 2 (2078 isolates) and both (4309 isolates); modal MICs for wild-type population, breakpoints used and percentage resistant

	MIC (mg/L)												
Antimicrobial growth promoters	≤0.25	0.5	1	2	4	8	16	32	64	≥128	Modal MIC	Breakpoint[a] (mg/L)	Percentage resistant
Avilamycin													
year 1		12	104	789	622	185	76	20	188	235			20[b]
year 2		1	42	849	491	14	29	21	310	321			31[c]
total		13	146	1638	1113	199	105	41	498	556	2	>16	
Avoparcin													
year 1		5	1137	874	81	24	5	8	40	57			5[b]
year 2		33	1503	416	30	1	5	23	38	39			5[b]
total		38	2640	1290	111	25	10	31	78	86	1	>8	
Bacitracin													
year 1		6	27	164	91	77	377	311	47	1131			87[b]
year 2		1	8	112	114	40	165	581	134	823			89[b]
total		7	35	276	205	117	542	892	181	1954	2	>4	
Flavophospholipol													
year 1	0	3	13	9	14	30	24	17	23	2098			95[b]
year 2	2	2	7	11	25	25	23	27	39	1917			94[b]
total	2	5	20	20	39	55	47	44	62	4015	8	>32	
Spiramycin													
year 1	25	354	441	188	25	4	4	0	3	1213			54[b]
year 2	10	283	488	330	59	7	0	2	1	896			43[c]
total	35	637	929	518	84	11	4	2	4	2099	1	>8	
Tylosin													
year 1		58	356	347	243	33	2	2	3	1187			54[b]
year 2		60	431	373	273	39	1	2	1	898			43[c]
total		118	787	720	516	72	3	4	4	2085	1	>8	
Virginiamycin													
year 1		228	198	524	271	279	147	127	315	42			28[b]
year 2		326	430	493	249	252	71	126	131	0			16[c]
total		554	628	1017	520	531	218	253	446	42	2	>8	

[a]The breakpoint was set in troughs of MIC distribution except in the case of flavophospholipol, for which a breakpoint three concentrations above the mode was used. Vertically paired figures with different superscripts differ significantly ($P < 0.05$), while those with identical superscripts do not.

breakpoints in distribution troughs, and where NCCLS human clinical breakpoints were available (glycopeptide, macrolide, streptogramin) they were similar, except for glycopeptides where our breakpoint for avoparcin (>8 mg/L) was lower that the NCCLS equivalent for vancomycin (≥32 mg/L). The study-specific breakpoints were used to calculate the prevalence of microbiological resistance. In the first year this was of the order of 30–50% for the two macrolides and virginiamycin. However, there were considerable differences between countries. In Sweden, where growth-promoting antibiotics had not been used since 1986, more than 85% of isolates were susceptible to all the agents except flavophospholipol (see above) and bacitracin, for which, however, high-level resistance (MICs ≥ 128 mg/L) was uncommon. Virginiamycin resistance in Denmark was low at both sample points, perhaps as a result of the unilateral removal of this compound 6 months before the study began. Unfortunately, quantitative antimicrobial consumption data for the host species were neither available for the countries nor for the individual farms from which the isolates originated. Such data would have been helpful in interpreting geographic variation.

The removal of virginiamycin, spiramycin, bacitracin and tylosin as growth promoters (1 July 1999)—just 3 months before the start of sampling for year 2—apparently resulted in rapid changes in the prevalence of susceptible strains, although the results must be interpreted with caution, given that sampling was in only two time frames. However, the rapid responses to removal were consistent with those reported by others for Denmark,[1,15] and suggests instability of the resistant *E. faecium* population in the absence of selection. Results for year 2 were generally consistent with the removal, in that resistance rates were significantly lower for the newly discontinued compounds virginiamycin, spiramycin and tylosin (despite the latter's continued availability for therapeutic purposes). For bacitracin, despite an apparent shift from high to intermediate MICs, there was no overall change in the prevalence of resistance. Avoparcin resistance rates were already low and remained so with no statistical evidence of change, in keeping with the fact of its earlier removal. Flavophospholipol resistance rates showed no significant change. Finally, resistance rates for avilamycin increased significantly in chickens (but not in pigs). This decrease in susceptibility to avilamycin (not removed) might

Table 4. Prevalence of resistance among *Enterococcus faecium* isolated from faeces of chickens and pigs in different countries in year 1 (2231 isolates) and year 2 (2078 isolates)

				Percentage of isolates resistant[a]						
		year	no. of isolates	avila	avo	baci	flavo	spira	tylo	virg
Chickens	France	1	312	44	8	99	95	59	59	32
		2	268	58	7	88	89	42	42	12
	Holland	1	318	21	2	96	91	73	73	55
		2	315	64	2	94	91	54	54	36
	Sweden	1	341	0	0	74	92	15	15	6
		2	301	0	0	87	93	8	8	1
	UK	1	359	64	12	90	94	69	69	60
		2	353	83	11	95	93	47	47	37
Pigs	Denmark	1	305	0.6	6	86	99	43	43	6
		2	249	0.4	7	80	96	40	41	5
	Holland	1	311	0	6	75	97	83	83	24
		2	245	0.4	6	66	98	56	56	6
	Spain	1	85	0	0	96	99	89	89	21
		2	168	0	0	85	99	100	100	14
	Sweden	1	200	3	0	90	98	8	8	0.5
		2	179	0.6	0	94	99	4	4	0.6

[a]Antibiotic abbreviations and breakpoints: avila, avilamycin (>16 mg/L); avo, avoparcin (>8 mg/L); baci, bacitracin (>8 mg/L); flavo, flavophospholipol (>32 mg/L); spira, spiramycin (>8 mg/L); tylo, tylosin (>8 mg/L); virg, virginiamycin (>8 mg/L).

Table 5. Associated resistance (%) in *E. faecium* (year 1 + year 2)

		Percentage resistant to:					
Antimicrobial growth promoter	*n* (year 1 + year 2)	avilamycin	avoparcin	bacitracin	flavophospholipol	tylosin	virginiamycin
Avilamycin							
susceptible	3214	0	3.2[a]	86	95	45[a]	15[a]
resistant	1095	100	9.3[b]	94	93	59[b]	43[b]
Avoparcin							
susceptible	4104	24[a]	0	88	95	47[a]	22[a]
resistant	205	49.7[b]	100	73	95	71[b]	18[a]
Bacitracin							
susceptible	523	12.2	10.5	0	74	53	18
resistant	3786	27	3.9	100	95	48	23
Flavophospholipol							
susceptible	232	33	4.3	85	0	50	31
resistant	4077	25	4.8	89	100	48	21.7
Tylosin							
susceptible	2213	20[a]	2.7[a]	89	95	0	2.9[a]
resistant	2096	31[b]	6.9[b]	87	94	100	43[b]
Virginiamycin							
susceptible	3350	18[a]	5.0[a]	87	95	36[a]	0
resistant	959	50[b]	3.7[a]	90	93	93[b]	100

Below each compound listed horizontally are paired figures showing the percentage resistant to those organisms which were, respectively, either susceptible or resistant to the comparator compound listed vertically.
Vertically paired figures with different superscripts differ significantly ($P < 0.05$), while those with identical superscripts do not. Bacitracin and flavophospholipol were not analysed because of the high intrinsic resistance, and spiramycin behaved similarly to tylosin.

have resulted from increase usage in the absence of the proscribed compounds.

We were able to detect significant associated resistance between antibiotic classes. For the macrolides and virginiamycin, this could have been cross-resistance based on the shared MLS_B mechanism. The only example of multiresistance that might have significance for human medicine—associated resistance to streptogramins, macrolides and glycopeptides—was encountered in only 1.5% of the isolates.

The criteria used for the identification of *E. faecium* appeared fully supportable, although a very few isolates of *E. faecalis* may have been included, as suggested by the small number of out-of-range susceptibility test results. These were too few to affect our conclusions. It is notable that whenever surveys of enterococcal antibiotic susceptibility are performed, the problem of misidentification may arise, despite efforts to avoid it.[16]

Although the procedure used for establishment of breakpoints differed in some respects from the protocol recommended by the NCCLS,[12] we believe that we have successfully established provisional parameters which we encourage others to test further. Furthermore, since the MIC results were obtained by the use of the NCCLS method (also now suggested as a reference method by EUCAST),[17] comparisons of our quantitative results with others from studies based on these standards are possible—a goal that has been widely supported.[18]

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Transparency declarations

RJB is a pharmaceutical industry consultant. TRS is an employee of Elanco Animal Health, a division of Eli Lilly & Co.

References

1. *DANMAP 2000. Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark*. Danish Veterinary Laboratory, Copenhagen, Denmark, 2001.

2. *SVARM 2001. Swedish Veterinary Antimicrobial Resistance Monitoring*. Uppsala: National Veterinary Institute, 2002.

3. Mevius DJ, van Pelt W, eds. *MARAN 2002. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in The Netherlands in 2002*. Dutch Food and Consumer Product Safety Authority, The Hague, 2002.

4. Devriese LA, Pot B, Van Damme L *et al*. Identification of *Enterococcus* species isolated from foods of animal origin. *Int J Food Microbiol* 1995; **26**: 187–97.

5. Devriese LA, Pot B, Collins MD. Phenotypic identification of the genus Enterococcus and differentiation of phylogenetically distinct enterococcal species and species groups. *J Appl Bacteriol* 1993; **75**: 399–408.

6. Devriese LA, Pot B, Kersters K *et al*. Acidification of methyl-α-D-glucopyranoside: a useful test to differentiate *Enterococcus casseliflavus* and *Enterococcus gallinarum* from *Enterococcus faecium* Species Group and from *Enterococcus faecalis*. *J Clin Microbiol* 1996; **34**: 2607–8.

7. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J Clin Microbiol* 1995; **33**: 24–7.

8. Dutka-Malen S, Evers S, Courvalin P. Erratum. Detection of glycopeptide resistance genotypes and identification to species level of clinically relevant enterococci by PCR. *J Clin Microbiol* 1995; **33**: 1434.

9. National Committee for Clinical and Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Fourth Edition: Approved Standard M7-A4*. NCCLS, Wayne, PA, USA, 1997.

10. European Committee for Antimicrobial Susceptibility Testing (EUCAST). Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. EUCAST Definitive Document E. Def 1.2. *Clin Microbiol Infect* 2000; **6**: 503–8.

11. European Committee for Antimicrobial Susceptibility Testing (EUCAST). Determination of antimicrobial susceptibility test breakpoints. EUCAST Definitive Document E. Def. 2.1. *Clin Microbiol Infect* 2000; **6**: 570–2.

12. National Committee for Clinical and Laboratory Standards. *Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters—Second Edition: Approved Guideline (2001) M23-A2*. NCCLS, Wayne, PA, USA, 2001.

13. National Committee for Clinical and Laboratory Standards. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Informational Supplement M31-S1*. NCCLS, Wayne, PA, USA, 2004.

14. Butaye P, Devriese L, Haesebrouck F. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on Gram-positive bacteria. *Clin Microbiol Rev* 2003; **16**: 175–88.

15. Bager F, Aarestrup FM, Madsen M *et al*. Glycopeptide resistance in *Enterococcus faecium* from broilers and pigs following discontinued use of avoparcin. *Microbiol Drug Resist* 1999; **5**: 53–6.

16. Jones RN, Ballow CH, Biedenbach DJ *et al*. Antimicrobial activity of quinupristin-dalfopristin (RP 59500, Synercid®) tested against over 28000 recent clinical isolates from 200 medical centers in the United States and Canada. *Diag Microbiol Infect Dis* 1998; **30**: 437–51.

17. European Committee for Antimicrobial Susceptibility Testing (EUCAST). Determination of minimum inhibitory concentrations (MICs) of antibiotics by agar dilution. EUCAST Definitive Document E. Def. 3.1. *Clin Microbiol Infect* 2000; **6**: 509–15.

18. O.I.E. Guidelines for the harmonisation of antimicrobial resistance surveillance and monitoring programmes. [Online.] http://www.oie.int/eng/normes/mcode/en_chapitre_3.9.1.htm (22 July 2005, date last accessed).