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Isolation of streptogramin-resistant *Enterococcus faecium* from human and non-human sources in a rural community

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***Objectives*: To detect quinupristin–dalfopristin and virginiamycin M1 resistance in *Enterococcus faecium* from human, food and environmental sources.**

***Materials and methods*: Enterococcal isolates derived from human faeces and urine, meat and seawater were screened for resistance to quinupristin–dalfopristin and virginiamycin M1 by an agar dilution method. Identification of all *E. faecium* strains and the presence of streptogramin acetyltransferase genes were confirmed using a PCR method.**

***Results*: No high-level quinupristin–dalfopristin-resistant strains were isolated. Two isolates from faeces and five from seawater were confirmed to be high-level virginiamycin M1-resistant *E. faecium* (MIC 32 mg/L); none of these carried the *vat*(D) or *vat*(E) acetyltransferase genes that mediate high-level resistance to streptogramin A compounds.**

***Conclusion*: High-level quinupristin–dalfopristin-resistant strains of *E. faecium* are uncommon in Cornwall. However streptogramin A-resistant strains were detected from human and animal sources.**

Keywords: antibiotic resistance, animal husbandry, epidemiology

Introduction

Quinupristin–dalfopristin is a water-soluble mixture of streptogramin A and B moieties. These two structurally-unrelated molecules bind to bacterial ribosomes, acting synergically to inhibit protein synthesis at the elongation step. This combined action is irreversible.[1] Quinupristin–dalfopristin is used in clinical practice to treat infections due to multi-resistant organisms such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*. *Enterococcus faecalis* is intrinsically resistant, so accurate identification is important if the agent is to be used to treat enterococcal infections.

Although quinupristin–dalfopristin has been in use for only a short time, a similar streptogramin, virginiamycin, was added to animal feeds for many years. This is a mixture of the streptogramin A and B compounds virginiamycin M1 and virginiamycin S. The use of virginiamycin as a growth promoter was prohibited in the European Union as from July 1999 because of fears that high-level streptogramin-resistant *E. faecium* in food animals might compromise the clinical use of quinupristin–dalfopristin. Previous laboratory studies have shown that the use of virginiamycin selects for resistant *E. faecium*, which are cross-resistant to quinupristin–dalfopristin.[2] Moreover, quinupristin–dalfopristin-resistant enterococci have been isolated from animal and human faeces and from meat,[3–5] but it is not clear to what extent human carriage is the result of consumption of contaminated meat.

Resistance to streptogramin B compounds is common in enterococci, mediated by *erm* genes. However, resistance to both the A and B streptogramin components is usually needed to produce high-level resistance to streptogramin combinations such as quinupristin–dalfopristin.[2] Previous workers have identified two transferable acetyltransferase genes, *vat*(D) and *vat*(E), as causes of resistance to streptogramin A compounds in strains of *E. faecium*.[6,7] Low-level resistance has also been demonstrated in the absence of these genes,[2] suggesting the occurrence of other mechanisms. This study examined routine clinical and environmental specimens for streptogramin-resistant *E. faecium* and for *vat*(D) and *vat*(E), to determine where resistance could be found, and how far along the food chain it could be detected 2 years after the ban on virginiamycin use.

Materials and methods

Collection of enterococci

In the first 6 months of 2001, faeces, urine samples, raw meat and seawater were examined for the presence of enterococci by the bacteriology section

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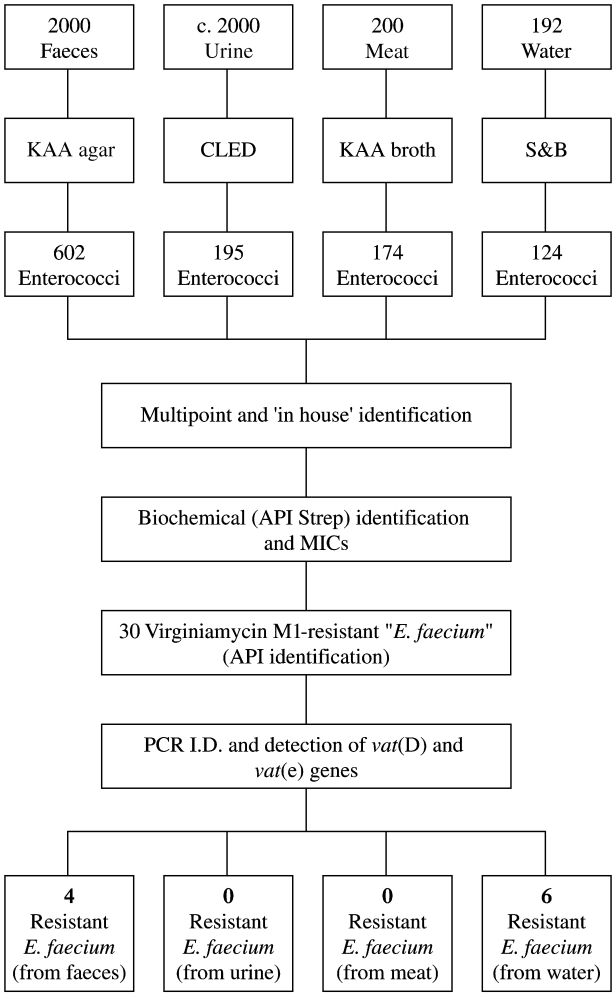

Figure 1. Detection and identification of virginiamycin M1-resistant *E. faecium*. KAA agar, kanamycin aesculin azide agar; CLED, cysteine-lactose-electrolyte deficient medium; KAA broth, kanamycin aesculin azide broth; MIC, minimum inhibitory concentration; PCR, polymerase chain reaction; S & B, Slanetz and Bartley medium.

of the Truro Public Health Laboratory in Cornwall, UK. The faeces and urine samples were derived from consecutive clinical samples received routinely from patients in local hospitals and in the community; water samples were collected as part of a programme of environmental monitoring of bathing waters; meat samples were submitted to the laboratory for quality control testing before distribution to retail outlets. The area served by the laboratory is largely rural with an extensive coastline. Local livestock farming is mostly concerned with cattle and sheep.

In the course of the study, 2000 faecal samples were inoculated on to kanamycin aesculin azide (KAA) agar (LabM IDG UK Ltd., Bury, UK). Enterococci were isolated on CLED agar (Oxoid, Basingstoke, UK) from urine samples sent into the laboratory for investigation of urinary tract infection. Bathing beach waters (192 samples) collected from eight sites around the north and south coasts of Cornwall were analysed by membrane filtration using Slanetz and Bartley agar (Oxoid) as the primary isolation medium. Two hundred raw meat samples consisting of 49 pork, 61 beef, 60 lamb, 20 poultry and 10 venison, were screened using KAA broth (LabM). Isolates were stored on nutrient agar slopes at 4°C until further identification. Further details are given in Figure 1.

Identification and susceptibility testing

All enterococci isolated on the primary media were then tested with an 'in-house' multipoint agar-based biochemical identification scheme. Isolates of *E. faecalis* were identified on the basis of fermentation of pyruvate, but not arabinose, reduction in tellurite and production of formazan from tetrazolium.[8] Isolates with this profile were excluded from further study. Non-*E. faecalis* enterococci were screened for streptogramin resistance by an agar breakpoint method using a an inoculum of 10^4 cfu/spot on DST (Direct Sensitivity Agar, Oxoid, Basingstoke, UK) containing 2 mg/L of either virginiamycin M1 (Sigma) or quinupristin–dalfopristin (Aventis), with incubation at 37°C for 24 h. Their susceptibility to vancomycin, teicoplanin and linezolid was determined by disc diffusion on DST agar. Strains of *E. faecium* with quinupristin–dalfopristin MICs of 32 mg/L [resistant; containing the *vat*(E) gene] and 0.25 mg/L (susceptible), and *E. faecalis* strain NCTC 775 were used as controls.

MICs of virginiamycin M1 and quinupristin–dalfopristin were determined by agar dilution for streptogramin-resistant isolates, using a dilution range of 0.5–128 mg/L on DST agar. The MIC was defined as the lowest concentration of antimicrobial that inhibited bacterial growth after 24 h of incubation. All isolates with a virginiamycin M1 or quinupristin–dalfopristin MIC ≥ 8 mg/L were identified using the API Strep kit (BioMérieux), and those provisionally identified as *E. faecium* were confirmed as such by amplification of the *E. faecium*-specific gene, $ddl_{E.\,faecium}$, encoding D-alanyl-D-alanine ligase as previously described.[5]

Detection of resistance genes

Virginiamycin M1-resistant isolates were screened for genes likely to encode streptogramin A acetyltransferases [*vat*(D), *vat*(E) or novel genes] using a pair of degenerate primers (M and N) and cycling conditions described previously.[2]

Results

Six hundred and two presumptive *Enterococcus* spp. were recovered from human faeces, 195 from urine samples, 174 from raw meat and 124 from seawater samples. Of the non-*E. faecalis* isolates recovered, 33 virginiamycin M1-resistant isolates were tentatively identified as *E. faecium* by API strep, of which 30 were available for further study. Only 10 of these (four from human faeces and six from seawater samples) were confirmed to be *E. faecium* by species-specific PCR (Table 1); nine were *Enterococcus gallinarum*; two were *Enterococcus casseliflavus*; and nine were not identified by the PCR assay used.

Three of the 10 *E. faecium* isolates had low-level virginiamycin M1 resistance (MIC 8 mg/L). Two of these were also low-level quinupristin–dalfopristin-resistant (MIC 4 mg/L) (Table 1). The remaining seven isolates were highly resistant to virginiamycin M1 (MIC 32 mg/L). None of these 10 isolates carried *vat*(D) or *vat*(E) and, as no amplicons were obtained using degenerate primers, it is unlikely that they contained genes encoding novel acetyltransferases. All *E. faecium* isolates were susceptible to vancomycin, teicoplanin and linezolid.

Discussion

Definitive identification of the enterococci proved problematic with the routine methods used in this study. Similar problems have been encountered in previous surveys of antibiotic resistance in enterococci,[9] and are a significant limitation to studies that do not include molecular identification. We used a PCR assay designed to identify

Table 1. Characteristics of virginiamycin M1-resistant *E. faecium*

Source	PCR ID	MIC (mg/L)		VAN	TEC	LNZ	Degenerate *vat* PCR
		VIR	Q–D				
Faeces	*E. faecium*	32	2	S	S	S	–
Faeces	*E. faecium*	32	2	S	S	S	–
Faeces	*E. faecium*	8	4	S	S	S	–
Faeces	*E. faecium*	8	4	S	S	S	–
Seawater	*E. faecium*	32	2	S	S	S	–
Seawater	*E. faecium*	32	2	S	S	S	–
Seawater	*E. faecium*	32	2	S	S	S	–
Seawater	*E. faecium*	32	1	S	S	S	–
Seawater	*E. faecium*	32	0.5	S	S	S	–
Seawater	*E. faecium*	8	1	S	S	S	–
Control 1	*E. faecium* [AO3(Sat G)]	32	32	S	S	S	
Control 2	*E. faecium* [A47(GE-1)]	0.5	0.5	S	S	S	

VIR, virginiamycin M1; Q-D, quinupristin–dalfopristin; VAN, vancomycin; TEC, teicoplanin; LNZ, linezolid; S, susceptible.

major clinically-relevant *Enterococcus* spp., but even so, nine quinupristin–dalfopristin-resistant isolates were not identified.

The use of virginiamycin as a growth promoter in the European Union ceased in 1999. Other studies have shown a decline in resistance to streptogramins within 12 months of this ban. In Denmark for instance, streptogramin resistance in *E. faecium* fell from 66% in broiler fowl and pigs to 34% between 1998 and 2000[10] following withdrawal. The prevalence in UK farm animals is unknown, but our study suggests that it is now uncommon.

The presence of virginiamycin M1-resistant *E. faecium* in human specimens was unexpected because virginiamycin has never been licensed for human use. Their source is a matter for speculation. The only environmental isolates we detected were found in seawater. These organisms probably derive from contamination of the marine environment with human or animal faeces and could represent an environmental source of human colonization and infection. Seawater bathing and water sports are popular in Cornwall and have been associated with transmission of other faecal organisms. We found no evidence of virginiamycin M1-resistant *E. faecium* in meat, however the small sample size does not exclude this possibility and we studied only UK-produced meat. Finally, we cannot exclude the possibility that human carriage is the result of direct transmission from farm animals in a rural area. A further epidemiological study of human carriage of virginiamycin-resistant *E. faecium* would address these questions.

The high-level virginiamycin M1-resistance observed in seven isolates was not the result of the acetyltransferases encoded by the transferable *vat*(D) and *vat*(E) genes which code for high-level streptogramin A resistance. These genes also usually confer high-level quinupristin–dalfopristin resistance, which was not seen in our isolates. Some other mechanism or mechanisms must underlie the high-level streptogramin A resistance among the strains detected by the study. We are investigating the nature and transferability of this resistance. This resistance is associated with quinupristin–dalfopristin MICs (range 0.5–4 mg/L), which lie close to the breakpoint (2 mg/L) for this agent. The significance of strains with such low-level resistance on therapy with quinupristin–dalfopristin is uncertain, but it is possible that they are a population from which fully quinupristin–dalfopristin-resistant strains may more readily emerge.

Our study screened large numbers of samples from human, meat and environmental sources for the presence of streptogramin-resistant *E. faecium*. We were able to detect a few streptogramin A-resistant isolates. Further research is indicated to elucidate the nature of this resistance and to assess its potential as a source of fully quinupristin–dalfopristin-resistant strains. It appears, in Cornwall at least, that there is not a significant reservoir of high-level quinupristin–dalfopristin-resistant *E. faecium* among the human population, in raw meat or in seawater. These data will provide a useful baseline should quinupristin–dalfopristin resistance emerge in the future if its use increases in clinical practice.

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