Distribution of streptogramin resistance determinants among *Enterococcus faecium* from a poultry production environment of the USA

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**Objectives:** The impact of agricultural use of antimicrobials on the present and future efficacy of therapeutic drugs in human medicine is a growing public concern. Quinupristin/dalfopristin has been approved to treat human disease caused by vancomycin-resistant *Enterococcus faecium* and is related to virginiamycin, a streptogramin complex that has long been used in USA agriculture poultry production.

**Methods:** Streptogramin-resistant isolates of *E. faecium* from poultry production environments on the eastern seaboard were recovered without selection for streptogramin resistance and examined using ribotyping to evaluate clonal bias. Colony PCR screening for the previously described streptogramin resistance determinants *erm*(A), *erm*(B), *msr*(C), *vgb*(A), *vat*(D) and *vat*(E) was performed to determine the prevalence of streptogramin resistance mechanisms from these environments.

**Results:** The collection of *E. faecium* isolates was unevenly distributed among 28 ribogroups and did not cluster geographically. The most prevalent ribogroups was composed of isolates that possessed diverse antimicrobial resistance profiles. Of the 127 isolates examined, 63% were resistant to quinupristin/dalfopristin. The resistance determinants *erm*(A) and *erm*(B) were observed among 6% and 10%, respectively, of streptogramin-resistant isolates. *msr*(C) was detected in a single isolate that was resistant to macrolide and lincosamide antimicrobials. The streptogramin B hydrolase *vgb*(A) and the streptogramin A acetyltransferases genes *vat*(D) and *vat*(E) were not detected in any of the *E. faecium* isolates.

**Conclusions:** These results indicate that there is widespread resistance to streptogramin antimicrobials among *E. faecium* throughout the poultry production region in this study and that the mechanisms of resistance to streptogramin antimicrobials within this population remain largely uncharacterized.

Keywords: enterococci, antibiotic resistance, quinupristin/dalfopristin, MLS

**Introduction**

Enterococci, particularly *Enterococcus faecalis* and *Enterococcus faecium*, present serious challenges to the control of nosocomial infections in intensive care units in the USA. In particular, they are known to be intrinsically resistant to several antibiotics and, perhaps more importantly, are adept at acquiring and transferring elements that confer resistance to antimicrobials. As a result, therapeutic options are becoming increasingly limited for the treatment of enterococcal infections.\(^1\)

In 1999, the Food and Drug Administration approved the use of the streptogramin, quinupristin/dalfopristin (Synercid), to treat infections due to vancomycin-resistant *E. faecium*. The use of analogues of human antimicrobials in food-animal production has given rise to concerns involving the development and transmission of resistance among bacterial pathogens.\(^2\) In the case of

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streptogramin antimicrobials, virginiamycin, an analogue of quinupristin/dalfopristin, has been used in animal production for >20 years.

Whereas genetic determinants can singly confer resistance to macrolide–lincosamide–streptogramin B (MLS_B) antimicrobials, resistance to streptogramin combinations, such as quinupristin/dalfopristin, requires mechanisms that confer resistance to both A and B components. Herein, we describe the diversity and distribution of macrolide–lincosamide–streptogramin resistance, as well as the known genetic determinants that contribute to streptogramin resistance among *E. faecium* from an area of intensive poultry production on the eastern seaboard of the USA.

**Materials and methods**

**Bacterial isolates**

All of the 127 isolates of *E. faecium* came from a previously described collection of enterococci. Briefly, samples were either poultry litter or swabs of poultry-transport containers collected from 82 farms along the Delaware–Maryland–Virginia (Delmarva) Peninsula in 1998. Data on the identity and quantity of antimicrobial use among the poultry production environments were not provided. Susceptibility to antimicrobials was determined using the Sensititre antimicrobial susceptibility testing system (Trek Diagnostic Systems, Inc., Westlake, OH, USA) and interpreted using available NCCLS interpretive criteria.4

**Ribotyping of *E. faecium* isolates**

Automated EcoRI ribotyping of the *E. faecium* isolates was accomplished using the RiboPrinter microbial characterization system (DuPont Qualicon United States, Wilmington, DE, USA) performed under the conditions recommended by the manufacturer. Normalized patterns were then imported into the BioNumerics version 3.0 software (Applied Maths, Austin, TX, USA) using an import script provided by DuPont Qualicon for cluster analysis.

**PCR screening for streptogramin resistance determinants**

DNA template was prepared by boiling four to six colonies in 200 μL of sterile water for 10 min in MicroAmp 96-well plates (Applied Biosystems, Foster City, CA, USA). Plates were centrifuged at 2500 rpm for 3 min in a Centra-CL3 centrifuge (Thermo IEC, Needham Heights, MA, USA) and the supernatant was transferred to another plate for storage at −20°C. Ten microliters of template DNA was used in all PCR reactions, which were carried out using a 96-well GeneAmp PCR System 9700 thermocycler (Applied Biosystems) in a 50 μL volume. Fifty pmol of each primer was used in resistance screening.

PCR reactions for each of the previously described streptogramin resistance determinants [erm(A), erm(B), msr(C), vgb(A), vat(D) and vat(E)] as well as the *E. faecium*-specific gene D-alanine:D-alanine ligase (ddl) were performed using AmpliTaq Gold (Applied Biosystems) according to the manufacturer’s suggestions. PCR primers and reaction conditions were as described previously.5,6 Primer sequences for msr(C) (5’-TATAAACAACCTGCAAGTTC-3’, 5’-CTTCAATTAGTCGATCCATA-3’), and vgb(A) (5’-ACCATTATGGTTATACGTTT-3’, 5’-GTATTCACGAATTTTACCGT-3’) were determined using the Vector NTI software package (Informax, Frederick, MD, USA).

Positive controls included: *E. faecium* CVM3480 [erm(A)], *E. faecium* CVM3002 [erm(B), vat(D)], *E. faecium* CVM3001 [vat(E)], *E. faecium* TX1330 SE34 [msr(C)] (kindly provided by J. R. Hayes et al. 124

![Figure 1. Ribogroups of *E. faecium* from the poultry production environment and occurrence of MLS phenotypes.](image-url)
Streptogramin-resistant *E. faecium* from poultry

B. Murray, University of Texas, Houston, TX, USA), and *Staphylococcus aureus* BM3093 ([vgb(A)](kindly provided by N. El Solh, Institut Pasteur, Paris, France)).

PCR reactions were visualized using the 2% agarose E-Gel 96 High-Throughput Agarose Electrophoresis System (Invitrogen, Carlsbad, CA, USA). PCR screening was performed in triplicate among all isolates that produced an amplicon and among an equal number of PCR-negative isolates.

Results

Diversity and dispersion of *E. faecium* isolates

The collection of 127 *E. faecium* isolated from poultry farms on the Delmarva Peninsula were distributed among 28 distinct ribogroups (Figure 1). Five of the isolates did not generate a ribotype pattern despite repeated testing. Sixteen ribotypes were rarely encountered and were represented by single isolates of *E. faecium*. Interestingly, a single ribogroup (5) was observed among 33% of the isolates, whereas the four most prevalent ribogroups (5, 1, 14 and 10) constituted 82/127 (65%) of the observed population, but displayed inconsonant antibiograms within each ribogroup. The four most prevalent ribogroups were observed to be widely distributed across the sampling region with no obvious clustering.

Geographic dispersion of MLS resistance phenotypes of *E. faecium* from the Delmarva Peninsula

Although phenotypic resistance to streptogramin antimicrobials was a primary goal, we were also interested in the presentation of resistance to other MLS antimicrobials that might demonstrate a stepwise progression to streptogramin resistance. As such, phenotypic resistance to MLS antimicrobials was observed to be heterogeneous among the *E. faecium* isolated. The most prevalent phenotype observed was resistance to lincosamide and streptogramin antimicrobials (47%) (Table 1). Macrolide–lincosamide–streptogramin resistance was observed among 17% of isolates in addition to macrolide–lincosamide resistance and isolated lincosamide resistance. Four isolates (3%) were observed to possess resistance to macrolide antimicrobials and a single isolate (0.8%) was susceptible to MLS antimicrobials.

Whereas increased tolerance to lincomycin was observed independently of resistance to other antimicrobials, only a small percentage of isolates was resistant to macrolide antimicrobials and no isolates were observed to be resistant to streptogramin antimicrobials in the absence of macrolide or lincosamide resistance.

Many diverse MLS phenotypes were observed across the sampled region. Lincosamide resistance was observed to be a nearly ubiquitous trait among the isolates, as well as geographically over the sampled region, especially in concert with streptogramin resistance.

Distribution of MLS resistance elements among *E. faecium*

PCR screening for MLS resistance determinants revealed the presence of the rRNA methylase genes *erm*(A) and *erm*(B) in 6.3 and 14.2% of all isolates (Table 1). The prevalences of the individual genes were predictably highest among macrolide-resistant isolates, with 15% possessing *erm*(A) and 30% having *erm*(B). The *msr*(C) gene was detected among 1% of all isolates.

Of particular interest was the detection of *erm*(A) and *erm*(B) among isolates that were not phenotypically resistant to macrolide antimicrobials, ostensibly indicating that the genes are inactive. Additionally, neither *erm*(A) nor *erm*(B) was detected among 25/46 isolates that were phenotypically resistant to macrolides, suggesting the presence of other resistance mechanisms.

Resistance to streptogramin antimicrobials was largely unaccounted for by PCR screening for specific resistance determinants. The genes *vgb*(A), *vat*(D) and *vat*(E) were not detected in any of the *E. faecium* isolates. The genes *erm*(A) and *erm*(B) were found in only 6% and 10%, respectively, of isolates that were phenotypically resistant to streptogramins.

Discussion

Our findings suggest that common lineages of *E. faecium* can be isolated from poultry production environments that are geographically widely distributed. Among this population, less...
common subpopulations could also be observed. The heterogeneous nature of MLS resistance phenotypes observed among *E. faecium* belonging to distinct ribogroups suggests that acquisition of resistance to these antimicrobials is dynamic. In addition, the absence of known resistance determinants among resistant isolates suggests that other mechanisms are prevalent in this environment. Despite the long-term approval and presumed industrial usage of MLS antimicrobials in this region, the diversity of resistance noted in this study is striking.

The absence of uniform resistance to all MLS antimicrobials may be due, in part, to the regular entry of a sizeable population of susceptible *E. faecium* into the poultry production environment from sources such as incoming flocks. Resistant populations may be masked by the comparatively larger density of susceptible populations, in which a selective cultural method has not been used. In this situation, a small, resistant population of *E. faecium* may persist in the production environment until such time as it is selectively amplified by the use of MLS or other antimicrobials, whereupon the prevalence of resistant *E. faecium* could rapidly become predominant.

The European experience of the use of the vancomycin analogue, avoparcin, in food-animal production clearly sets a precedent for the establishment of a base of resistance to production drugs among healthy members of the community. Curiously, the long-term use of streptogramin antimicrobials in USA food-animal production environments has not resulted in established resistance of similar magnitude. Current estimates of the prevalence of streptogramin-resistant *E. faecium* range from 0–1% among healthy humans in the USA. This small population of resistant isolates in ordinarily healthy individuals, however, could be amplified as a result of an increase in selective pressure in the clinical environment and present a challenge to the control of nosocomial infections.

Implications that enterococci of food-borne origin should be regarded as human pathogens remain contentious. Minimally, they could serve as potential reservoirs of antimicrobial resistance genes to host-adapted strains. The observations of this study suggest that resistant isolates of *E. faecium* are commonly found within poultry production environments, and ostensibly can contaminate retail meat products. In the face of an increased frequency of resistant enterococci responsible for human disease, particularly in hospital environments, control strategies may need to be implemented in food-animal production to reduce the size of the resistant population to which consumers are exposed.

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References