Incidence of High-Level Evernimicin Resistance in *Enterococcus faecium* among Food Animals and Humans

Frank Møller Aarestrup* and Paul M. McNicholas

Danish Veterinary Institute, DK-1790 Copenhagen V, Denmark, and Schering-Plough Research Institute, Kenilworth, New Jersey 07033

Received 28 March 2002/Returned for modification 21 May 2002/Accepted 12 June 2002

Six high-level evernimicin-resistant *Enterococcus faecium* isolates were identified among 304 avilamycin-resistant *E. faecium* isolates from animals and 404 stool samples from humans with diarrhea. All four animal isolates, and one of the human isolates, were able to transfer resistance to a susceptible *E. faecium* strain. The resulting transconjugants all tested positive for the presence of *emtA*, a gene encoding a methyltransferase previously linked with high-level evernimicin resistance. The four transconjugants derived from animal isolates all carried the same plasmid, while a differently sized plasmid was found in the isolate from humans. This study demonstrated a low incidence of high-level evernimicin resistance mediated by the *emtA* gene in different *E. faecium* isolates of animal and human origin.

There is a growing concern regarding the emergence of multiply antimicrobial-resistant enterococci as important nosocomial pathogens (7). This has increased the interest in either developing new antibiotics or modifying older antibiotics with activity against multiply resistant staphylococci and enterococci. One of these agents is evernimicin, an oligosaccharide antibiotic with activity against a broad range of gram-positive pathogenic bacteria including glycopeptide-resistant enterococci, methicillin-resistant staphylococci, and penicillin-resistant streptococci (9, 12, 14, 15, 17). Evernimicin inhibits protein synthesis in *Staphylococcus aureus* by binding with high affinity to a single site on the 50S subunit (13). The evernimicin binding site overlaps the binding site of another oligosaccharide antibiotic, avilamycin. Avilamycin has been used as a growth promoter for food animals in the European Union for several years, and as a consequence avilamycin resistance has been observed among *Enterococcus faecium* isolates from broilers in Denmark and other countries (4). High-level (MIC >4 μg/ml) avilamycin resistance is mediated by mutations in the gene encoding ribosomal protein L16; these strains also exhibit low-level (MIC, 2 to 12 μg/ml) cross-resistance to evernimicin (2). Recently, a new methyltransferase, which confers high-level (MIC, >64 μg/ml) evernimicin resistance through methylation of 23S rRNA, was cloned from an avilamycin-resistant *E. faecium* strain isolated from a broiler in Denmark (11). The present study was conducted to determine the occurrence of high-level evernimicin resistance among avilamycin-resistant enterococcal isolates from food animals and humans in Denmark.

A total of 304 avilamycin-resistant (MIC, ≥16 μg/ml) *E. faecium* and 14 *Enterococcus faecalis* isolates were obtained from the continuous monitoring for antimicrobial resistance among broilers and pigs in Denmark (4). These isolates were screened for high-level (MIC, >125 μg/ml) evernimicin resistance by the E-test according to the manufacturer’s guidelines (AB Biodisk, Solna, Sweden); four positive isolates were identified. In March 1998 a total of 254 human stool samples, submitted for examination of diarrheal pathogens, were examined for the presence of avilamycin-resistant enterococci by plating a loopful of fecal material on Mueller-Hinton II agar plates containing 20 μg of avilamycin/ml. All patients sampled had a history of diarrhea but no history of either a recent hospital stay or antimicrobial treatment. One avilamycin-resistant *E. faecium* isolate was identified; this isolate also exhibited high-level evernimicin resistance. In June 2001 an additional 150 fecal samples from humans were, in relation to another study, screened for the presence of enterococcal isolates resistant to erythromycin and tetracycline with Slanetz and Bartley agar plates containing erythromycin (20 μg/ml) and tetracycline (10 μg/ml). Isolates obtained from these samples were also screened for avilamycin resistance; one isolate was recovered which also tested positive for high-level evernimicin resistance. All six isolates tested positive in PCR for the *emtA* gene with the following oligonucleotides as primers: 5′-GGTACGACGATCCAGTTTT-3′ and 5′-GAAAATATCTAAGTTCCTCG-3′.

The six evernimicin-resistant isolates were subjected to pulsed-field gel electrophoresis (PFGE) typing with the restriction enzyme *Smal*. DNA purification and enzyme digestion were performed as previously described (8). Four different PFGE types were identified: three of the four broiler isolates belonged to the same PFGE type, and the two isolates from humans gave different PFGE patterns (Fig. 1). We screened all six isolates for the ability to transfer evernimicin resistance to *E. faecium* BM4105 (resistant to rifampin and fusidic acid) by the filter mating procedure described previously (6). Transconjugants were isolated from within the inhibition zone of an E-test strip laid down on Mueller-Hinton II agar plates containing rifampin (50 μg/ml) and fusidic acid (10 μg/ml). All four animal isolates and one of the human isolates yielded transconjugants. Transconjugants were screened for susceptibility to avilamycin, bacitracin, chloramphenicol, erythromycin, gentamicin, kanamycin, penicillin, streptomycin, quinupristin-dalfopristin, tetracycline, and vancomycin as previously described (3). Resistance to avilamycin was cotransferred, but no
other drug resistance markers appeared to be cotransferred. Plasmid DNA was extracted from the five transconjugants with the Qiagen Plasmid Midi kit (Qiagen, Valencia, Calif.) and restricted with either EcoRI or PvuII. EcoRI digestion of the plasmids from the four broiler isolates yielded three different bands, while PvuII digestion yielded five bands. The plasmids from the four broiler isolates were approximately 36 kb and indistinguishable even though the four isolates fell into two different PFGE patterns. In contrast, the human isolate harbored a plasmid of >100 kb. EcoRI digestion yielded 22 bands, and PvuII digestion yielded 18 bands. To determine if high-level evernimicin resistance was linked to the presence of the \textit{emtA} gene (11), we performed a Southern blot analysis on the restricted plasmids (8). A digoxigenin-labeled \textit{emtA} fragment was prepared by PCR with the oligonucleotides described above. The \textit{emtA} probe hybridized to DNA fragments of approximately 6.9 and 12.5 kb in the EcoRI and PvuII restriction digests, respectively, of the transconjugants from the four broiler isolates (data not shown). In contrast, the \textit{emtA} probe hybridized to fragments of 3.3 and 4.5 kb in the EcoRI and PvuII digests of the transconjugant of the human isolate.

The present study demonstrated that high-level evernimicin resistance has not been widely disseminated among \textit{E. faecium} strains isolated from the animal or human population in Denmark. In the four isolates from broilers, three of which appeared clonal, the \textit{emtA} gene was carried by a similarly sized plasmid in each case. In contrast, a plasmid of different size was observed in the single human isolate capable of transferring evernimicin resistance. Since the \textit{emtA} gene is part of a transposon (11), it is possible that it was transposed from one plasmid to a different plasmid. Several studies have indicated that bacteria from animals and humans share the same resistance genes and that exchange probably occurs fairly frequently (10, 16, 19). This includes resistance genes in enterococci where the genes encoding resistance to important human antibiotics such as vancomycin (\textit{vanA}) and quinupristin-dalfopristin (\textit{var(E)} and \textit{erm(B)}) probably have spread from the animal reservoir to humans and thereby perhaps shortened the life span of these antibiotics (1, 5, 18).

We are grateful to Berith Kummerfelt, René Hendriksen, Betina Elemark, Dorte Nielsen, and Christina Aaby Svendsen for technical assistance.

The E-test was supplied by Schering-Plough Research Institute, Kenilworth, N.J.

REFERENCES


