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NOTES

Characterization of Antimicrobial Resistance in Enterococci of Animal Origin

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Among 97 enterococci cultured from animals, gentamicin MICs were ≥2,000 μg/ml for 9 isolates and between 250 and 1,024 μg/ml for 6 isolates. For two isolates tested (gentamicin MICs, 256 and 512 μg/ml, respectively), there was no in vitro synergy with penicillin plus gentamicin, resistance was transferable, and there was no hybridization with a probe specific for 6′-aminoglycoside acetyltransferase–2″-aminoglycoside phosphotransferase. The results of the study indicate the presence of a unique gentamicin resistance genotype in enterococci of animal origin.

Reservoirs for antibiotic-resistant enterococci have not been completely determined. Animals, human food, and the inanimate environment have been suspected as sources for some resistant clinical isolates (1–4, 19, 23, 24, 32, 38). Evidence for a disseminated erythromycin resistance determinant mediated by Tn*917*-like sequences has been shown in enterococci isolated from pigs, chickens, and humans (32). More recently, glycopeptide-resistant strains (vancomycin-resistant enterococci) have been identified in the feces of animals and chicken carcasses (2, 3, 21, 23–25) as well as in sewage in Barcelona (37) and the United Kingdom (2). In the study described here we surveyed a sample of enterococci of animal origin for penicillin, glycopeptide, and aminoglycoside resistance.

The enterococcal strains used in the study are listed in Table 1. Stool samples for culture were collected from 16 separate horses, six pigs, fecal cow slurry, and 11 separate chickens from four farms in southeastern Michigan. Antibiotics were not used as feed additives at any of the farms. Twenty-eight veterinary enterococcal isolates from 16 horses and 12 birds were from the University of Pennsylvania School of Veterinary Medicine. Food isolates were cultured from 29 whole frozen chicken carcasses (nine different brand names) sold in 17 supermarkets in southeastern Michigan. Other information on the animals was not available. Isolates were initially recovered on Columbia CNA with 5% sheep blood agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.). For each culture, three enterococcal colonies were evaluated when different morphologic types occurred. Conventional biochemical tests were used to identify all isolates (10). DNA probes were used (8) for confirmation of the species of the *Enterococcus faecium* isolates that could not be differentiated by the typing system published by Facklam and Collins (10).

Susceptibilities to ampicillin (Sigma Chemical Co.), gentamicin (Schering Corp., Bloomfield, N.J.), streptomycin (Sigma Chemical Co.), and vancomycin (Eli Lilly & Co., Indianapolis, Ind.) were determined by broth microdilution methods (20, 28). Time-kill experiments, β-lactamase detection, and DNA methods were as described elsewhere (14, 16, 25–27, 35, 36). A probe specific for the bifunctional 6′-aminoglycoside acetyltransferase–2″-aminoglycoside phosphotransferase (AAC6′-APH2″) enzyme in *E. faecalis* was used for localization of the gentamicin resistance determinant (13).

Table 1 shows the results for the enterococcal species isolated and the antibiotic resistances detected in isolates from farm animals and veterinary sources. For isolates from farm animals, ampicillin resistance (MICs, 16 and 32 μg/ml) occurred in 2 (4%), of 51 isolates, and high-level streptomycin resistance occurred in 1 (2%) isolate. For isolates from veterinary sources, ampicillin resistance occurred in 15 (53%) of 28 isolates (MICs 16 to 256 μg/ml), high-level gentamicin resistance in the absence of streptomycin resistance occurred in 2 (7%) isolates, high-level gentamicin resistance with high-level streptomycin resistance occurred in 3 (11%) isolates, and high-level streptomycin resistance in the absence of high-level gentamicin resistance occurred in 3 (11%) isolates.

For cultures of food isolates, 29 frozen chickens obtained from 17 southeastern Michigan supermarkets yielded 18 enterococci: 11 *Enterococcus faecalis*, 3 *E. faecium*, 3 *Enterococcus gallinarum*, and 1 *Enterococcus casseliflavus* isolate. Ampicillin resistance occurred in 12 (67%) of 18 isolates, high-level gentamicin resistance in the absence of streptomycin resistance occurred in 2 (11%) isolates, high-level gentamicin resistance with high-level streptomycin resistance occurred in 2 (11%) isolates, and high-level streptomycin resistance in the absence of high-level gentamicin resistance occurred in 4 (22%) iso-

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TABLE 1. Summary of enterococci of animal origin

Animal origins of isolates	No. of animals	No. of isolates	Species identification (no.)	Antibiotic resistance no. (% resistant)[a]
Isolates from farms				
Horses	16	8	*E. casseliflavus* (7) *E. faecium* (1)	
Pigs	6	8	*E. faecium* (5) *E. hirae* (3)	Am, 1 (20) Am + St, 1 (33)
Chickens	11	1	*E. casseliflavus* (1)	
Cows	ND	34	*E. hirae* (22) *E. faecium* (4) *E. casseliflavus* (5) *E. mundtii* (2) *E. gallinarum* (1)	
Isolates from veterinary sources				
Horses	16	16	*E. faecalis* (5) *E. faecium* (4) *E. casseliflavus* (3) *E. gallinarum* (2) *E. hirae* (1) *E. mundtii* (1)	Am, 1 (20); Gm, 1 (20); Am + Gm, 1 (20) Am + St, 1 (25); Am + Gm + St, 3 (75) Am, 1 (33) Am + Gm + St + Van,[c] *1 (50)*
Birds[d]	12	12	*E. faecalis* (8) *E. faecium* (2) *E. gallinarum* (1) *E. mundtii* (1)	Am, 3 (37); Am + St, 2 (25); Gm + St, 1 (12) Am, 1 (50); Am + Gm + St, 1 (50) Van, 1 (100)[c]

[a] Abbreviations: Am, ampicillin; Gm, gentamicin; St, streptomycin; Van, vancomycin, Van. Gentamicin resistance is defined as an MIC of ≥256 μg/ml, streptomycin resistance is defined as an MIC of >2,000 μg/ml, ampicillin resistance is defined as an MIC of ≥16 μg/ml.
[b] ND, not determined.
[c] Vancomycin MIC, 8 μg/ml.
[d] One cockatoo, one cockateil, one emu, one goose, one turkey, and seven poultry.

lates. For three isolates gentamicin MICs were 1,024 μg/ml. Vancomycin MICs were <8.0 μg/ml for all food isolates.

Table 2 summarizes the in vitro susceptibility and transferability of resistance for strains for which gentamicin MICs were ≥256 μg/ml. Isolates for which gentamicin MICs were <256 μg/ml were not evaluated by time-kill experiments or in transferability or DNA hybridization studies. The combination of ampicillin with gentamicin at a gentamicin concentration of 8.0 μg/ml did not have a synergistic bactericidal killing effect. Two isolates, *E. faecium* SF9583, for which the ampicillin MIC was 128 μg/ml and the gentamicin MIC was 512 μg/ml, and *E. gallinarum* SF9117, for which the ampicillin MIC was 16 μg/ml and the gentamicin was MIC 256 μg/ml, had resistance to gentamicin that was transferable, and the DNAs from these

TABLE 2. Summary of gentamicin-resistant enterococcal isolates of animal and food origin[a]

Isolate	Species	Source	Gentamicin MIC (μg/ml)	Transfer frequency	
				FA2-2[b]	GE1[c]
SF9117[d]	*E. gallinarum*	Horse	256	10^{-4}	10^{-3}
SF9119	*E. faecalis*	Poultry	1,024	No transfer	No transfer
SF9583[d]	*E. faecium*	Goose	512	No transfer	10^{-3}
SF9607	*E. faecalis*	Food	≥2,000	10^{-3}	No transfer
SF9679	*E. gallinarum*	Food	1,024	No transfer	10^{-9}
SF10,008	*E. faecium*	Horse	≥2,000	10^{-6}	10^{-5}
SF10,009	*E. faecium*	Horse	≥2,000	10^{-5}	10^{-3}
SF10,012	*E. faecium*	Horse	≥2,000	10^{-6}	10^{-4}
SF10,236	*E. faecalis*	Horse	≥2,000	10^{-7}	10^{-9}
SF10824	*E. faecalis*	Horse	≥2,000	10^{-8}	No transfer
SF11131	*E. faecium*	Food	1,024	No transfer	No transfer
SF11134	*E. faecalis*	Food	≥2,000	10^{-4}	10^{-8}
SF11135	*E. gallinarum*	Food	1,024	No transfer	No transfer
SF11136	*E. casseliflavus*	Food	≥2,000	No transfer	No transfer
SF11137	*E. faecalis*	Food	≥2,000	10^{-7}	No transfer

[a] There was no bactericidal killing of any of the isolates with the combination of ampicillin plus gentamicin.
[b] FA2-2, plasmid-free *E. faecalis*.
[c] GE1, plasmid-free *E. faecium*.
[d] No hybridization with a probe specific for AAC6′-APH2″ to DNAs from these isolates.

isolates did not hybridize to the AAC6′-APH2″ probe. For these two isolates, the combination of ampicillin and gentamicin at gentamicin concentrations of 8.0 and 64 μg/ml did not have a synergistic bactericidal killing effect.

Nonhuman sources have been suspected as reservoirs for some antibiotic-resistant bacteria (2–5, 17, 18, 21, 23–25, 29–34). In recent years, a great deal about the epidemiology for the nosocomial acquisition of antibiotic-resistant enterococci has been learned (4, 11, 27, 30). Little is known about the prevalence, risk factors, and reservoirs for resistant enterococci outside of the hospital. Use of antimicrobial agents in animal feed is common and has been suspected as an important risk factor for resistant strains in animals (9, 17). It has been suggested that antibiotic use in food animals has resulted in new resistance genes, and multiresistant pathogens have emerged in these animals as a consequence of antibiotic exposure (5, 9, 16–18, 29, 30, 33, 34, 36, 37).

In the present study we not only evaluated antibiotic resistance rates in some animal isolates but also evaluated the identities of the enterococcal species isolated. Enterococci present in stool in small numbers or some strains exhibiting resistance may have been below the limits of our detection methods and may have been missed. In an earlier study (6), the recovery rate of enterococci exhibiting high-level resistance to streptomycin was higher when a method of direct plating of the stool sample to antibiotic-containing medium was used than when single colonies were plated onto agar without aminoglycosides. The recovery rate of strains exhibiting high-level resistance to gentamicin was similar by either method (6). In prior surveys of human fecal carriage of enterococci, *E. faecalis* was found in the stools of about 50% of healthy adults and *E. faecium* was found in the stools of 25% (22). Other enterococcal species were uncommon. In the present study, *Enterococcus hirae*, *E. casseliflavus*, *E. gallinarum*, and *Enterococcus mundtii* were more common among isolates from animals than the reported frequencies of clinical isolates of these enterococci from humans. The isolates recovered from chickens sold in supermarkets were more similar to the species types isolated from humans. These findings are explained, in part, by the known differences in the compositions of enterococci in the intestinal flora of poultry (1, 7). In the farm animals that we studied that did not receive antibiotics, antibiotic-resistant enterococci were uncommon. No gentamicin-resistant enterococci were isolated from these animals, and ampicillin resistance was rare. In isolates from veterinary sources and food, gentamicin resistance at levels of ≥256 μg/ml was not uncommon and was identified in 50% of *E. gallinarum* isolates, 55% of *E. faecium* isolates, 25% of *E. faecalis* isolates, and 25% of *E. casseliflavus* isolates. In the United States, aminoglycosides (apramycin, hygromycin, and neomycin) and penicillin are approved by the U.S. Food and Drug Administration for use as feed additives and are used in veterinary medicine (9, 12, 15). In the present study it was not possible to evaluate a potential link between the use of antibiotics in animals and the presence of antibiotic-resistant bacteria. It was also not possible to determine the source or route of acquisition of resistant isolates by animals.

Gentamicin resistance and the loss of synergy of gentamicin and antibiotics that act against the cell wall in these isolates would not be detected by current standard clinical laboratory testing methods. The results of the study indicate a unique gentamicin resistance genotype in these enterococci of animal origin. The genetic and biochemical mechanisms of resistance were not determined. A comparison of the resistance determinants in isolates from animals with the genes responsible for resistance in isolates from humans also requires further study.

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