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GENOME SEQUENCES

Complete Genome Sequences of Two Avilamycin-Resistant *Enterococcus faecium* Strains Isolated from Chicken in the United States

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ABSTRACT Avilamycin-resistant *Enterococcus* spp. have never been reported in the United States. Here, we report the complete genome sequences of two avilamycin-resistant (Avi^r) *Enterococcus faecium* strains isolated from a retail chicken and a cecal sample from a young chicken. Both isolates are multidrug resistant (MDR) and carry *emtA* on MDR plasmids.

Enterococcus species are a leading cause of hospital-acquired infections in the United States and have been commonly used as sentinel organisms for antimicrobial resistance monitoring programs (1, 2). Avilamycin is an antibiotic with activity primarily against Gram-positive bacteria. It was used in animal feeds as a growth promoter in Denmark before production uses were halted in 2000 (3). In the United States, avilamycin was recently approved to use for the reduction in incidence and overall severity of diarrhea in the presence of pathogenic *Escherichia coli* in groups of weaned pigs (2015) and for the prevention of mortality caused by necrotic enteritis associated with *Clostridium perfringens* in broiler chickens (2016). Since these approvals represent the first uses of avilamycin in the United States, there are no data available to assess the potential impact of avilamycin on the emergence of antimicrobial resistance among pathogens from food-producing animals. Therefore, we performed avilamycin susceptibility testing on more than 1,000 *Enterococcus* isolates from chickens and swine from the National Antimicrobial Resistance Monitoring System (NARMS) using broth microdilution (4). Two avilamycin-resistant (Avi^r) *Enterococcus faecium* isolates were identified, with one (strain N56454) from a retail chicken sample (2015) and another (strain F17E0263) from a chicken cecal sample (2018). Both isolates were multidrug resistant (MDR) and exhibited MIC(s) to avilamycin of $\geq$64 μg/ml, with a breakpoint of 16 μg/ml (5).

N56454 showed resistance to 9 of the 17 antimicrobial drugs tested, including avilamycin, erythromycin, kanamycin, lincomycin, penicillin, tetracycline, tylosin, streptomycin, and quinupristin-dalfopristin. Strain F17E0263 showed resistance to 5 antimicrobials, avilamycin, gentamicin, kanamycin, lincomycin, and streptomycin. To identify all resistance genes and their locations, we closed both Avir^r *Enterococcus* genomes using the PacBio Sequel platform (Pacific Biosciences, Menlo Park, CA). The isolates were grown on sheep blood agar plates at 37°C for ~16 h. DNA isolation was accomplished with a DNeasy blood and tissue kit (Qiagen, Valencia, CA) The quantity and quality were measured on a Qubit fluorometer (Invitrogen, Carlsbad, CA) and Femto Pulse system (Agilent, Folsom, CA). The DNA was fragmented to ~10 kb using a g-TUBE (Covaris, Woburn, MA). The library was prepared following a 10-kb template preparation protocol with the SMRTbell template prep kit v1.0. Sequencing was performed on a PacBio Sequel system with sequencing kit v3.0. The sequencing was collected after 120 min of preextension (600-min movie length).

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TABLE 1 Metadata, genome sequence characteristics, resistance genotypes, and accession numbers

Strain identifier	Source	Yr of isolation	State of origin	Resistance genes	SRA accession no.	Size (bp) of:		Coverage (×)	G+C content (%)	GenBank accession no.
						Chromosome	Plasmid			
N5645	Retail chicken	2015	Maryland	*emt*(A) (2 copies), *tet*(L), *tet*(M) (2 copies), *erm*(B), *aph(3′)-IIIa*, *sat-4* (truncated), *ant(6)-Ia*, *lnu*(B), *lsaE*, *spw*, *dfrG*, *eat*(A), *aac(6′)*, *msr*(C)	SRR9668445	2,495,617	198,480	372 (chromosome), 369 (plasmid)	38.24 (chromosome), 36.18 (plasmid)	CP040904 (chromosome), CP040905 (plasmid)
F17E0263	Young chicken	2018	Delaware	*emt*(A), *aac(6″)-Ie*, *aph(2″)-Ia*, *lsa*(E), *lnu*(B), *ant(6)-Ia*, *spw*, *eat*(A), *aac(6′)*, *msr*(C)	SRR9668446	2,659,111	63,632 (plasmid 1), 207,651 (plasmid 2)	176 (chromosome), 168 (plasmid 1), 287 (plasmid 2)	38.13 (chromosome), 35.91 (plasmid 1), 35.62 (plasmid 2)	CP040849 (chromosome), CP040850 (plasmid 1), CP040851 (plasmid 2)

There were 24,000 to 26,000 polymerase reads with a mean read length of 36,000 to 42,000 bp, 24,000 to 26,000 subreads filtered with a high-quality region filter (HQRF), and an N_{50} value of 8,000 bp. The reads were *de novo* assembled using the Hierarchical Genome Assembly Process v4.0, with default settings. Each assembled contig had a mean confidence quality value (QV) score of $>$90 (1 error in 1 Gb). Circularization of the chromosomes and plasmids was performed with Circulator (https://github.com/sanger-pathogens/circlator/tree/master/circlator) using the "all" option. Resistance genes with at least 50% length and 90% sequence identity to known resistance genes were identified using ResFinder (https://github.com/resfinder/resfinder).

Metadata, genome sequence characteristics, resistance genotypes, and accession numbers of these two isolates are summarized in Table 1. The *emtA* gene, linked to avilamycin resistance (5, 6), was found in both strains. Two copies were present in one of the isolates, with one on the chromosome and another on the plasmid.

Data availability. The complete genome sequences of the two Avi^r *E. faecium* bacterial chromosomes and associated plasmids were deposited in GenBank (Table 1).

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