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Draft Genome Sequences of Eight Streptogramin-Resistant *Enterococcus* Species Isolated from Animal and Environmental Sources in the United States

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ABSTRACT Here, we present the draft genome sequences of eight streptogramin-resistant *Enterococcus* species isolated from animals and an environmental source in the United States from 2001 to 2004. Antimicrobial resistance genes were identified conferring resistance to the macrolide-lincosamide-streptogramins, aminoglycosides, tetracyclines, beta-lactams, and glycopeptides.

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Enterococci are primarily described as commensals, but they are also opportunistic pathogens and one of the leading causes of nosocomial infections in the United States (1). Treatment of enterococcal infections can be hampered by resistance in the bacterium. They potentially harbor many antimicrobial resistance genes, some of which confer cross-resistance to antimicrobials used in both humans and animals (2), as observed in the streptogramin class of antibiotics. Virginiamycin, a streptogramin antibiotic, was used extensively as a growth promoter in poultry and animal production worldwide for several decades. In 1999, Synercid (quinupristin-dalfopristin [Q-D]), also a streptogramin antibiotic, was approved for treatment of vancomycin-resistant *Enterococcus faecium* in humans. Resistance to streptogramins was first discovered in staphylococci in 1975 (3), and many mechanisms of streptogramin resistance have been described in staphylococci from both humans and animals since then, including mechanisms in other Gram-positive bacteria (4–6). Horizontal transfer of streptogramin A and B resistance elements has contributed to the spread of Q-D-resistant bacteria between animals and humans (7).

Here, we report the draft genome sequences of streptogramin-resistant *E. faecium* ($n = 5$), *E. hirae* ($n = 2$), and *E. gallinarum* ($n = 1$) isolated from different animal and environmental sources in the United States from 2001 to 2004, as shown in Table 1.

Genomic DNA from *Enterococcus* species was extracted using the blood and tissue genomic DNA extraction kit (Qiagen, USA). Extracted DNA was quantified using the Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit according to the manufacturer's instructions (Life Technologies, Inc., USA). The Illumina libraries were prepared using the Nextera XT DNA library preparation kit and Nextera XT index primers (Illumina, USA). The library fragment size distribution was checked using the Bioanalyzer 2100 with an Agilent HS DNA kit (Agilent Technologies, USA) and quantified using a Qubit DNA HS assay kit in a Qubit fluorometer (Thermo, Fisher Scientific, USA). The generated libraries were then sequenced using a MiSeq version 3 reagent kit with 600 cycles and a paired-end read length of 2 × 300 bp on the Illumina MiSeq platform. The quality metrics of the reads were performed by FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). The sequence data were assembled using the A5-miseq assembler (8), and the genome sequence was annotated

TABLE 1 Statistics of the genome assembly

Species	Isolate	Isolation source	Genome size (bp)	N_{50} (bp)	No. of contigs	GC content (%)	No. of tRNAs	Total no. of genes	Antibiotic resistance genes	Accession no.
E. faecium	825	Dairy cattle feces	2,983,742	120,949	90	38.12	67	3,165	*vatE*, *ermB*, *msrC*, *aac6*, *ant6*, *tetM*, *tetS*, *tetL*	NSDF00000000
E. faecium	615	Playground slide	2,640,693	41,131	161	37.78	67	2,937	*vatE*, *ermB*, *msrC*, *mphD*, *aac6*, *tetM*, *tetS*, *tetL*	NSDE00000000
E. hirae	1725	Chicken carcass rinse	3,146,929	62,861	137	36.53	67	3,172	*vatE*, *tetM*, *tetS*	NSDD00000000
E. hirae	1013	Chicken carcass rinse	3,061,562	180,913	67	36.61	65	2,971	*vatE*, *ermB*, *aph3*, *tetM*, *tetS*	NSDC00000000
E. faecium	7527	Chicken carcass rinse	2,775,928	31,298	220	37.73	68	3,124	*vatE*, *ermB*, *mphD*, *aac6*, *tetM*, *tetS*, *tetL*	NSDB00000000
E. faecium	5209	Chicken carcass rinse	2,960,238	1,86017	82	37.61	66	3,114	*vatD*, *msrC*, *mphD*, *lnuB*, *linB*, *aac6*, *ant6*, *tetM*, *tetS*, *tetL*	NSDA00000000
E. faecium	6605	Chicken carcass rinse	2,925,634	186,017	65	37.64	67	3,069	*vatD*, *msrC*, *mphD*, *aac6*	NSCZ00000000
E. gallinarum	9402	Chicken carcass rinse	3,313,546	333,154	54	40.38	53	3,275	*vgaB*, *vatB*, *vanT*, *vanTc*, *vanS*, *vanC*, *vanC1*, *vanR-C*, *vanX-Yc*, *vanRc4*, *vanRc3*, *vanSc3*, *ermB*, *tetM*, *tetS*	NSCY00000000

via the NCBI Prokaryotic Genome Annotation Pipeline (9). Genome statistics are shown in Table 1.

Antibiotic resistance genes were identified using ARG-ANNOT (10). Five isolates had at least two genes conferring resistance to the macrolide, lincosamide, and streptogramin B classes of antibiotics (*ermB*, *linB*, *lnuB*, *mphD*, and *msrC*). Resistance to streptogramin A antibiotics was also identified (*vatD* and *vatE*). In comparison to the other isolates, isolate 9402 contained streptogramin A resistance genes (*vgaB* and *vatB*) rarely found in enterococci, suggesting a novel mechanism of resistance in this bacterial genus and probable transfer of a mobile genetic element (11). These resistance genes identified in the isolates were consistent with their reported phenotypes (11, 12).

The objective of this study was to further analyze genetic determinants of streptogramin resistance in enterococci isolated from animals in the United States. The routine collection and analysis of animal- and environment-associated bacteria will help improve the monitoring and surveillance of antimicrobial resistance and will identify novel mechanisms of resistance.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under BioProject number PRJNA398373, and the accession numbers are given in Table 1. The versions described in this paper are the first versions.

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REFERENCES

1. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK, National Healthcare Safety Network Team, Participating National Healthcare Safety Network Facilities. 2008. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infect Control Hosp Epidemiol 29:996–1011. https://doi.org/10.1086/591861.
2. Huycke MM, Sahm DF, Gilmore MS. 1998. Multiple-drug resistant enterococci: the nature of the problem and an agenda for the future. Emerg Infect Dis 4:239–249. https://doi.org/10.3201/eid0402.980211.
3. El Solh N, Allignet J. 1998. Staphylococcal resistance to streptogramins and related antibiotics. Drug Resist Updates 1:169–175. https://doi.org/10.1016/S1368-7646(98)80036-8.
4. Hershberger E, Donabedian S, Konstantinou K, Zervos MJ. 2004. Quinupristin-dalfopristin resistance in gram-positive bacteria: mechanism of resistance and epidemiology. Clin Infect Dis 38:92–98. https://doi.org/10.1086/380125.
5. El Solh N, Fouace JM, Shalita Z, Bouanchaud DH, Novick RP, Chabbert YA. 1980. Epidemiological and structural studies of *Staphylococcus aureus* R plasmids mediating resistance to tobramycin and streptogramin. Plasmid 4:117–120. https://doi.org/10.1016/0147-619X(80)90087-6.
6. Soltani M, Beighton D, Philpott-Howard J, Woodford N. 2000. Mechanisms of resistance to quinupristin-dalfopristin among isolates of *Enterococcus faecium* from animals, raw meat, and hospital patients in Western Europe. Antimicrob Agents Chemother 44:433–436. https://doi.org/10.1128/AAC.44.2.433-436.2000.
7. Donabedian SM, Perri MB, Vager D, Hershberger E, Malani P, Simjee S, Chow J, Vergis EN, Muder RR, Gay K, Angulo FJ, Bartlett P, Zervos MJ. 2006. Quinupristin-dalfopristin resistance in *Enterococcus faecium* isolates from humans, farm animals, and grocery store meat in the United States. J Clin Microbiol 44:3361–3365. https://doi.org/10.1128/JCM.02412-05.
8. Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. https://doi.org/10.1093/bioinformatics/btu661.
9. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI

prokaryotic genome annotation pipeline. Nucleic Acids Res 44: 6614–6624. https://doi.org/10.1093/nar/gkw569.
10. Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, Rolain JM. 2014. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 58:212–220. https://doi.org/10.1128/AAC.01310-13.
11. Jackson CR, Fedorka-Cray PJ, Barrett JB, Hiott LM, Woodley TA. 2008. First report of *vatB* and *vgaB* from *Enterococcus gallinarum* in the USA. Int J Antimicrob Agents 31:175–176. https://doi.org/10.1016/j.ijantimicag.2007.08.020.
12. Jackson CR, Fedorka-Cray PJ, Barrett JB, Hiott LM, Woodley TA. 2007. Prevalence of streptogramin resistance in enterococci from animals: identification of *vatD* from animal sources in the USA. Int J Antimicrob Agents 30:60–66. https://doi.org/10.1016/j.ijantimicag.2007.03.010.