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.

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.

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via the NCBI Prokaryotic Genome Annotation Pipeline (9). Genotype 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, linU, mfrD, 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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| Table 1: Statistics of the genome assembly |

| Species       | Isolate | Isolation source | Genome size (bp) | Ncontigs (bp) | Nsize (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 | NSDF000000000 |               |
| E. faecium    | 615     | Playground slide  | 2,640,693        | 41,131       | 161       | 37.78         | 67             | 2,937       | vatE, ermB, msrC, mphD, aac6, tetM, tetS, tetL | NSDE000000000 |               |
| E. hirae      | 1725    | Chicken carcass rinse | 3,146,929       | 62,861       | 137       | 36.53         | 67             | 3,172       | vatE, ermB, aap3, tetM, tetS | NSDD000000000 |               |
| E. hirae      | 1013    | Chicken carcass rinse | 3,061,562       | 180,913      | 67        | 36.61         | 65             | 2,971       | vatE, ermB, aap3, tetM, tetS | NSDC000000000 |               |
| E. faecium    | 7527    | Chicken carcass rinse | 2,775,928       | 31,298       | 220       | 37.73         | 68             | 3,124       | vatE, ermB, mphD, aac6, tetM, tetS, tetL | NSDB000000000 |               |
| E. faecium    | 5209    | Chicken carcass rinse | 2,960,238       | 1,860,17     | 82        | 37.61         | 66             | 3,114       | vatD, msrC, mphD, lnuB, lnuB, aac6, ant6, tetM, tetS, tetL | NSDA000000000 |               |
| E. faecium    | 6605    | Chicken carcass rinse | 2,925,634       | 186,017      | 65        | 37.64         | 67             | 3,069       | vatD, msrC, mphD, aac6 | NSCZ000000000 |               |
| E. gallinarum | 9402    | Chicken carcass rinse | 3,313,546       | 333,154      | 54        | 40.38         | 53             | 3,275       | vgaB, vatB, vanTc, vanS, vanC, vanC, vanC, vanX-Yc, vanK-C, vanK-C, vanK-C, vanK-C, vanS, vanS,vanS3, vanS3 | NSCY000000000 |               |
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