Prevalence and Antimicrobial Resistance of Enterococci Isolated from Retail Meats in the United States, 2002 to 2014

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ABSTRACT Bacteria of the genus Enterococcus are important human pathogens that are frequently resistant to a number of clinically important antibiotics. They are also used as markers of animal fecal contamination of human foods and are employed as sentinel organisms for tracking trends in resistance to antimicrobials with Gram-positive activity. As part of the National Antimicrobial Resistance Monitoring System (NARMS), we evaluated several retail meat commodities for the presence of enterococci from 2002 to 2014, and we found 92.0% to be contaminated. The majority of isolates were either Enterococcus faecalis (64.0%) or Enterococcus faecium (28.6%), and the antimicrobial resistance of each isolate was assessed by broth microdilution. The resistance prevalences for several drugs, including erythromycin and gentamicin, were significantly higher among poultry isolates, compared to retail beef or pork isolates. None of the isolates was resistant to the clinically important human drug vancomycin, only 1 isolate was resistant to linezolid, and resistance to tigecycline was below 1%. In contrast, a majority of both E. faecalis (67.5%) and E. faecium (53.7%) isolates were resistant to tetracycline. Overall, the robust NARMS testing system employed consistent sampling practices and methods throughout the testing period, with the only significant trend in resistance prevalence being decreased E. faecium resistance to penicillin. These data provide excellent baseline levels of resistance that can be used to measure future changes in resistance prevalence that may result from alterations in the use of antimicrobials in food animal production.

IMPORTANCE Enterococci, including E. faecalis and E. faecium, are present in the guts of food-producing animals and are used as a measure of fecal contamination of meat. We used the large consistent sampling methods of NARMS to assess the prevalence of Enterococcus strains isolated from retail meats, and we found over 90% of meats to be contaminated with enterococci. We also assessed the resistance of the Enterococcus strains, commonly used as a measure of resistance to agents with Gram-positive activity, in foods. Resistance prevalence was over 25% for some antimicrobials and sample sources but was less than 1% for several of the most important therapeutic agents used in human medicine.

KEYWORDS Enterococcus, retail meats, antimicrobial resistance

Enterococci are constituents of the gut microflora in a number of animal species, including humans (1), although some species of Enterococcus cause serious infections in immunocompromised and hospitalized patients (2). The Enterococcus species of greatest importance to human health are Enterococcus faecalis and Enterococcus fae-
cium, each of which causes a variety of infections, including urinary, soft tissue, and bloodstream infections (3). Antimicrobial resistance is one of the primary factors contributing to the morbidity and deaths associated with infections caused by Enterococcus (4, 5).

Enterococci are gut commensals present in both the small and large intestines of...
people (6). In response to antimicrobial therapy, *Enterococcus* populations can increase, relative to other bacteria, because they are resistant to a number of commonly used antimicrobials, such as cephalosporins (7). Treatment with antimicrobials can be a risk factor for the acquisition of vancomycin-resistant *Enterococcus* (VRE), a significant cause of hospital-acquired infections (8). Vancomycin was previously the treatment of choice for enterococcal infections but, with the emergence of vancomycin resistance, the use of more recently developed antimicrobials such as daptomycin and linezolid has increased (9).

Because they are gut commensals in most vertebrates, enterococci are used as markers of fecal contamination of food products (10). Foodborne enterococci are rarely implicated as being directly responsible for foodborne infections, but the acquisition of these bacteria through food may result in intestinal colonization (11). In addition, many *E. faecium* bloodstream infections are of gastrointestinal origin, raising the possibility that food might be a vehicle for such bacteria (3). Food also may be a source of *Enterococcus* causing urinary tract infections (12). In addition, foodborne enterococci can transfer resistance genes to pathogens such as *Campylobacter*, *Listeria*, and *Escherichia coli* (13–15). Furthermore, due to the ubiquity of enterococci in the animal gut, along with their resilience to environmental stress and their ability to acquire antibiotic resistance, the World Health Organization recommends that integrated antimicrobial resistance-monitoring systems use enterococci as sentinel organisms for resistance to agents with Gram-positive activity (16). In the United States, the National Antimicrobial Resistance Monitoring System (NARMS) monitors the antimicrobial resistance of foodborne bacterial pathogens throughout the food supply, including *Enterococcus* spp. isolated from retail meats (17). Randomized retail meat sampling is performed from area grocery stores near NARMS sites in various states, providing a valuable measure of both bacterial prevalence in retail meats and associated resistance prevalence over a long time.

As of 1 January 2017, antimicrobials of therapeutic importance to human medicine are no longer used for growth promotion of food-producing animals in the United States (18). Therefore, prior *Enterococcus* resistance surveillance of retail meats conducted by NARMS can be used as a baseline to measure potential future changes in resistance prevalence among enterococci in the food supply and to better understand selective pressures contributing to resistance in food-producing animals.

**RESULTS AND DISCUSSION**

*Enterococcus* contamination. From 2002 to 2014, NARMS tested a total of 22,922 retail meat samples for the presence of enterococci, with sampling being evenly distributed among retail chicken, ground turkey, ground beef, and pork chop products. *Enterococcus* was consistently isolated from each of these commodities, with 95.0% isolation from retail chicken, 94.4% from ground turkey, 92.7% from ground beef, and 85.8% from pork chops (Fig. 1A), yielding a total of 21,077 *Enterococcus* isolates. *E. faecalis* and *E. faecium* constituted the majority of the *Enterococcus* strains isolated from retail meat (64.0% and 28.6%, respectively), although additional enterococcal species were also isolated, including *Enterococcus hirae* (5.4%), *Enterococcus durans* (1.4%), and *Enterococcus gallinarum* (0.3%).

Although isolation from each of the four retail meat commodities was consistent, the species prevalence varied by source (Fig. 1B). *E. faecium* predominated among retail chicken (55.4% of isolates), whereas *E. faecalis* constituted the majority of isolates from ground turkey, ground beef, and pork chops (78.6%, 59.4%, and 81.3%, respectively). There was a significant increasing trend (*P* < 0.001) in the proportion of *Enterococcus* isolates that were *E. faecalis* and a decreasing trend (*P* = 0.01) in the proportion of *E. faecium* from retail meats from 2004 to 2014. This trend was also significant among each of the retail meat sources (*P* < 0.01) except pork.

Resistance prevalence. *Enterococcus* isolates were tested for resistance to 15 antimicrobials, including many used in both human and veterinary medicine to treat infections caused by *Enterococcus* and other Gram-positive organisms. Only *E. faecalis*...
and *E. faecium* were included in the analysis, because they were of greatest abundance and represent the most medically important *Enterococcus* species.

Vancomycin has long been considered an important treatment for *Enterococcus* infections. Although vancomycin resistance among *E. faecium* in hospitals in the United States can top 80% (19), none of the isolates recovered from retail meats was resistant to vancomycin. Vancomycin and other glycopeptides have never been used in food-producing animals in the United States, potentially accounting for the absence of resistance in retail meats. This suggests that the acquisition and spread of vancomycin resistance determinants in the United States may be largely limited to hospitals and other health care facilities.

Linezolid, an oxazolidinone antimicrobial, is also critically important for the treatment of infections caused by *Enterococcus*. The only retail meat isolate that was resistant was an *E. faecium* strain isolated from retail chicken in 2005. These findings are consistent with the fact that resistance to this antimicrobial is infrequent, even in hospitals (20), and that oxazolidinones are not used in food-producing animals.

Quinupristin-dalfopristin is a clinically important streptogramin combination drug that was approved by the U.S. Food and Drug Administration (FDA) in 1999 to treat vancomycin-resistant *E. faecium* (21). *E. faecalis* is considered to be intrinsically resistant to quinupristin-dalfopristin, while 38.6% of *E. faecium* isolates were found to be resistant (Fig. 2). Resistance was significantly higher among retail chicken and turkey products than among beef or pork products (*P < 0.0001*) (Table 1). The streptogramin combination drug virginiamycin has been used in veterinary medicine for decades, with
its use in animal feed for growth promotion in both chickens and turkeys potentially contributing to the greater prevalence of resistance in those meat commodities (22).

Daptomycin is also critically important for the treatment of Enterococcus and is an approved antimicrobial therapy for VRE (23), although it is not used in food-producing animals. Since daptomycin was added to the NARMS testing panel in 2004, only 4 isolates of E. faecalis have been resistant, while a significantly greater proportion of E. faecium isolates have been found to be resistant ($P < 0.0001$) (Fig. 2). The median MIC of E. faecalis isolates was 1 g/ml, whereas that of E. faecium was 4 g/ml (see Table S1 in the supplemental material), which may explain the greater prevalence of resistance in the latter species.

Nitrofurantoin is used in people to treat urinary tract infections caused by Enterococcus, including VRE (24). Only 0.3% of E. faecalis isolates were resistant to nitrofurantoin.

**TABLE 1 Enterococcus resistance according to species, source, and drug**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Resistance prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. faecalis</td>
</tr>
<tr>
<td></td>
<td>RC</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>6.6</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30.3</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>17.9</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.0</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.0</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>0.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>37.2</td>
</tr>
<tr>
<td>Tylosin</td>
<td>37.4</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>0.6</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.0</td>
</tr>
<tr>
<td>Linezolid</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.8</td>
</tr>
<tr>
<td>Quinupristin-dalfopristin</td>
<td>—b</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>67.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>RC, retail chicken; GT, ground turkey; GB, ground beef; PC, pork chops.

<sup>b</sup>Quinupristin-dalfopristin resistance is not reported for E. faecalis because E. faecalis is considered intrinsically resistant.
toin, with the prevalence of resistance being significantly higher for *E. faecium* (*P* < 0.0001) (Fig. 2). Although nitrofurantoin is not used in food-producing animals, it is likely that the high resistance prevalence for *E. faecium* is due to isolates having a median MIC of 64 μg/ml, compared to 32 μg/ml among *E. faecalis*, with the resistance breakpoint being 128 μg/ml. This indicates that many resistant *E. faecium* isolates may be part of the wild-type MIC distribution without having acquired resistance mechanisms.

Aminoglycosides can be used to treat *Enterococcus* infections in people, particularly in combination therapy with penicillins (25). Resistance prevalences for gentamicin and kanamycin were significantly higher in *E. faecalis* than in *E. faecium* (*P* < 0.0001), but prevalences for streptomycin did not differ significantly (Fig. 2). For both enterococcal species, prevalences of resistance to gentamicin and kanamycin were significantly higher among chicken and turkey products than among pork and beef products (*P* < 0.0001) (Table 1). Gentamicin is approved for use in 1-day-old chickens and 1- to 3-day-old turkeys for disease prevention purposes, although specific sales and use data for these indications are not available and their contributions to resistance selection are not known. Gentamicin resistance in enterococci is most commonly conferred by the resistance gene *aac(6’)-aph(2’)* (26), which also confers resistance to kanamycin; therefore, greater prevalence of this gene in poultry isolates could account for the greater resistance to both aminoglycosides.

Resistance to ciprofloxacin, a fluoroquinolone drug, for *E. faecium* was significantly higher than that for *E. faecalis* (*P* < 0.0001) (Fig. 2). The greater relative MIC distribution of *E. faecium* might have accounted for this, as the median MIC was 1 dilution higher than that for *E. faecalis* (Table S1). Quinolones are not typically used to treat *Enterococcus*, although they are used in both human and animal medicine for the treatment of infections caused by other important pathogens. The use of fluoroquinolones in poultry was banned in the United States in 2005, due to concerns about contributing to fluoroquinolone-resistant *Campylobacter* infections in people (27). Resistance is typically derived from mutations in core chromosomal genes, as opposed to plasmids that may be lost in the absence of selection pressure, potentially accounting for the continued resistance among *E. faecium* isolates from poultry sources (Table 1).

Tylosin is a veterinary antimicrobial used in food-producing animals (28), and its administration can lead to macrolide resistance in *Enterococcus* and other bacteria (29). Resistance to macrolides in *Enterococcus* is most frequently attributed to the presence of *erm* genes such as *ermB*, which confer resistance by encoding macrolide phosphotransferases. The *erm* gene products are able to modify both erythromycin and tylosin, which accounts for the finding that resistance was typically in tandem (Table S1). It is unclear why >5% of *E. faecium* isolates were resistant to erythromycin but not tylosin (Fig. 2), but the finding indicates the likely presence of resistance mechanisms other than known *erm* genes in those isolates. Prevalences of resistance to both macrolide drugs were significantly higher in poultry products (*P* < 0.0001), although the underlying reason for this is not clear.

Penicillins still have utility in combination treatments for human infections caused by enterococci (30). Penicillins also account for the third-highest level of sales of any drug class for use in food-producing animals (31), although some of this use is via injectable form. Most *E. faecalis* isolates from retail meats have remained susceptible, with only 0.1% of all strains being resistant (Fig. 2); significantly greater resistance prevalence was observed in *E. faecium* (*P* < 0.0001). Resistance is likely higher in *E. faecium* at least partly due to a higher MIC distribution, relative to that for *E. faecalis*, as described previously (32).

Tetracycline is not typically used to treat *Enterococcus* infections, although tetracyclines represent the drug class with the highest level of sales for use in food-producing animals (31); a potential consequence of this is that a majority of both *E. faecium* and *E. faecalis* isolates were resistant (Fig. 2). Tigecycline is an expanded-spectrum tetracycline derivative in the glycyclline class of drugs. It is effective in treating infections caused by *Enterococcus*, including vancomycin-resistant strains (33). Only 8 resistant
isolates each of *E. faecium* and *E. faecalis* were identified during the testing period. Tetracycline is not used in food-producing animals in the United States, which may account for the low resistance prevalence among enterococci from retail meats.

Chloramphenicol is rarely used in the treatment of *Enterococcus* but may be employed in situations where treatment options are limited (34). Prevalences of resistance among retail meat enterococci were only 0.5% in *E. faecalis* and 0.2% in *E. faecium*. The related drug florfenicol is used in animal medicine but accounts for less than 1% of all antimicrobials sold for use in food-producing animals (31).

### Resistance trends

In general, prevalences of resistance for antimicrobials did not change significantly from 2002 to 2014 for *E. faecalis* (Fig. 3A and Table S2) or *E. faecium* (Table S3). However, penicillin resistance decreased significantly for *E. faecium* (Fig. 3B), from 37.2% in 2003 to 13.9% by 2014 (*P* = 0.003), while the *E. faecalis* resistance to penicillin remained below 1% throughout the testing period. It is unclear what is responsible for the decline in penicillin resistance, because the sale of penicillins for use in food-producing animals in the United States did not significantly change from 2009 to 2015 (31). It is possible that population-level changes in the types of *Enterococcus* present are responsible for changes in resistance prevalence, because strains were not subtyped beyond the species level.

*E. faecium* resistance prevalences were higher for nitrofurantoin, penicillin, daptomycin, and ciprofloxacin, whereas *E. faecalis* had greater resistance to gentamicin,
**Enterococcus** faecalis, during the study period (Fig. 1B), these drugs. Interestingly, while the relative proportion of acquired resistance mechanisms may not significantly differ between the species for drugs such as nitrofurantoin and ciprofloxacin, indicating that the presence of acquired resistance mechanisms may not significantly differ between the species for these drugs. Interestingly, while the relative proportion of **E. faecalis** fell, relative to **E. faecium**, during the study period (Fig. 1B), **E. faecium** infections in people have been increasing (35).

The different resistance prevalences for various drugs appear to be partially explained by their varied uses in animal medicine. For instance, vancomycin, linezolid, and tigecycline are not used in food-producing animals, and resistance prevalences were less than 1% for these drugs with any species or meat source. In contrast, tetracyclines are the antibiotic class with the greatest use in these animals, and they had the greatest resistance prevalence (Fig. 2).

Poultry meats were associated with the greatest resistance prevalences for many of the drugs, particularly for the aminoglycosides gentamicin and kanamycin and the macrolides erythromycin and tylosin; this was statistically significant in both **E. faecium** and **E. faecalis** (P < 0.0001). This finding mirrors data from NARMS for **E. coli**, which also has demonstrated greater resistance to aminoglycosides for isolates derived from retail chicken or turkey (36). Although data on specific antimicrobial use in the United States are not available, it is possible that use practices for poultry, including the administration of antimicrobials in feed and water, contributed to greater resistance in enterococci from those sources.

Measuring the prevalence and resistance of enterococci from retail meats provides useful understanding of the level of resistance of Gram-positive organisms derived from animal food production. Overall, our robust data on over 20,000 isolates and 300,000 MICs provide context for understanding existing levels of resistance, particularly as NARMS sampling of **Enterococcus** continues to expand. This study also provides baseline data to measure potential changes in resistance prevalence as medically important antimicrobials cease to be used for growth promotion of food-producing animals in the United States, demonstrating the power of One Health surveillance in driving policy designed to improve public health.

**MATERIALS AND METHODS**

**Enterococcus isolation and culture.** Strains tested in this study were recovered as part of the NARMS retail meat testing program (17). Between 2002 and 2014, retail meat samples were obtained from Georgia, Maryland, Oregon, and Tennessee, with samples being collected from randomized lists of area grocery stores near state public health laboratories. The retail meats tested included 5,751 ground beef products, 5,684 ground turkey products, 5,745 pork chop products, and 5,742 retail chicken products. **Enterococcus** isolates were obtained from retail meat rinse as described previously (37). Briefly, 50 ml of Enterococcus broth (Becton Dickinson, Franklin Lakes, NJ) was added to 50 ml of retail meat rinse and incubated at 45°C for 18 to 24 h. A loopful was streaked onto an Enterococcus agar plate for isolation, and the plate was incubated at 45°C for 18 to 24 h. One well-isolated colony per sample was subcultured and sent to the FDA Center for Veterinary Medicine for subsequent identification to the species level and antimicrobial susceptibility testing.

**Species identification and antimicrobial susceptibility testing.** For **Enterococcus** strains grown on blood agar plates, identification to the species level was performed with the Vitek 2 compact microbial identification system (bioMérieux, Hazelwood, MO). Antimicrobial susceptibility testing was performed by broth microdilution in 96-well plates, to determine antimicrobial MICs. Clinical and Laboratory Standards Institute (CLSI) methods and standards were used (38), with the quality control organisms **Escherichia coli** ATCC 25922, **Staphylococcus aureus** ATCC 29213, **Enterococcus faecalis** ATCC 29212, and **Enterococcus faecalis** ATCC 51299. Resistance breakpoints followed CLSI standards for gentamicin (≥12 μg/ml), streptomycin (≥0.125 μg/ml), vancomycin (≥32 μg/ml), erythromycin (≥1 μg/ml), nitrofurantoin (≥128 μg/ml), linezolid (≥4 μg/ml), penicillin (≥0.06 μg/ml), chloramphenicol (≥32 μg/ml), ciprofloxacin (≥0.06 μg/ml), quinupristin-dalfopristin (≥4 μg/ml, reported only for **E. faecium**), and tetracycline (≥16 μg/ml). NARMS provisional breakpoints were used for antimicrobials lacking CLSI standards, i.e., kanamycin (≥1024 μg/ml), tigecycline (≥0.5 μg/ml), daptomycin (≥0.0001 μg/ml), and tylosin (≥32 μg/ml).

**Statistical analyses.** The proportions of resistant **E. faecalis** and **E. faecium** isolates and 95% Clopper-Pearson confidence intervals were estimated. With an α value of 0.05, we compared the proportions of resistant **E. faecalis** and **E. faecium** isolates using a two-tailed chi-square test. A one-tailed test was used to examine whether decreased MICs from the previous year were significant.
chi-square test with Bonferroni correction was used to test whether overall proportions of resistance in chicken products and ground turkey were greater than the proportions of resistance in ground beef and pork chops. We used the Mann-Kendal trend test to assess the significance of resistance trends over time. All statistical analyses were performed in R version 3.3.3.

SUPPLEMENTAL MATERIAL
Supplemental material for this article may be found at https://doi.org/10.1128/AEM.01902-17.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.
SUPPLEMENTAL FILE 2, XLSX file, 5.9 MB.

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REFERENCES


