A NARMS Survey of Antimicrobial Resistant Foodborne Bacteria Isolated from Retail Veal in the United States

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Abstract

Little is known about the prevalence of antimicrobial resistant (AMR) bacteria in veal meat in the United States. We estimated the prevalence of bacterial contamination and AMR in various veal meats collected during the 2018 U.S. National Antimicrobial Resistance Monitoring System (NARMS) survey of retail outlets in nine states and compared to the frequency of AMR bacteria from other cattle sources sampled for NARMS. Additionally, we identified genes associated with resistance to medically important antimicrobials and gleaned other genetic details about the resistant organisms. The prevalence of *Campylobacter, Salmonella, E. coli* and *Enterococcus* in veal meats collected from grocery stores in nine states was 0% (0/358), 0.6% (2/358), 21.1% (49/232) and 53.5% (121/226) respectively, with ground veal posing the highest risk for contamination. Both *Salmonella* were resistant to at least one antimicrobial as were 65.3% (32/49) of *E. coli* and 73.6% (89/121) of *Enterococcus* isolates. Individual drug and multidrug

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(MDR) resistance levels were significantly higher ($p < 0.05$) in *E. coli* and *Enterococcus* from retail veal compared to 2018 NARMS data from sampling dairy cattle ceca and retail ground beef. Whole genome sequencing (WGS) was conducted on select *E. coli* and *Salmonella* from veal. Cephalosporin resistance genes (bla_{CMY} and bla_{CTX-M}), macrolide resistance genes (*mph*), plasmid-mediated quinolone resistance genes (PMQR, *qnr*), and *gyrA* mutations were found. We also identified heavy metal resistance genes (HMRG) *ter, ars, and mer, fieF, and gol,* and disinfectant resistance genes (DRG) *qac* and *emrE*. An str_{1a}-containing *E. coli* was also found. Sequence types were highly varied among the nine *E. coli* isolates that were sequenced. Several plasmid types were identified in *E. coli* and *Salmonella*, with the majority (9/11) of isolates containing IncF. This study illustrates that veal meat is a carrier of AMR bacteria.

Keywords

antimicrobial resistance; retail veal; Escherichia coli; Salmonella; Enterococcus; NARMS

The ongoing threat of antimicrobial resistance (AMR) is a looming public health concern. Efforts to study the epidemiological connection between the use of medically important antimicrobials in food animals and AMR foodborne infections in humans have resulted in the establishment of a number of integrated surveillance programs worldwide. The U.S. National Antimicrobial Resistance Monitoring System (NARMS) is one such program that tracks the movement of AMR enteric bacteria and antimicrobial resistance genes (ARGs) between food animals, their meats, and humans. NARMS has gained much information on AMR bacteria in dairy cattle ceca and ground beef, among other animals and food animal products(47). We chose to expand the NARMS survey by adding veal meat, which is typically produced from young dairy breed calves. Standing at 0.3 lbs. per capita per year, veal meat consumption in the United States is lower than other cattle-derived meats. In 2020 alone, the U.S. produced 68.9 million pounds of veal, compared to over 27 billion pounds of beef (43). However, veal meat could pose a risk for AMR foodborne infection. Foodborne pathogens of great public health concern, such as *Salmonella*, have been found on veal hides, carcasses, and pre-chill samples (2, 44).

Additionally, studies have suggested that the prevalence of AMR bacteria in cattle is higher in younger animals (15), which may in part be related to the common practice of administering antimicrobials to calves to treat and prevent infections that result from their high susceptibility to disease and stress. The few surveys conducted in the United States suggest that veal calves are a potential source of AMR bacteria (2, 20, 33, 34). We conducted a NARMS pilot study to estimate the prevalence of resistant *Salmonella, E. coli, Enterococcus,* and *Campylobacter* isolated from fresh retail veal purchased in grocery outlets across the United States, and describe the genetic resistance, virulence, and plasmid profiles of select isolates. We also wanted to identify variables (e.g. veal cut, country of origin, and others) that might be associated with the occurrence of AMR bacteria. This information can be used to inform the potential development of a retail veal meat surveillance program in NARMS and to establish a baseline for the AMR status of retail veal meat sold in the U.S. We also intend to compare our results with other sources collected for the NARMS program.

MATERIALS AND METHODS

Veal sampling.

Veal samples were collected between April and December 2018 at retail supermarkets in the following states: Colorado, Georgia, Maryland, New York, Oregon, Pennsylvania, South Carolina, South Dakota, and Tennessee. Each site aimed to purchase 10 samples per month for an expected total of 480 samples by the end of the study. However, the actual number of veal samples purchased was based on product availability at the supermarkets sampled for the NARMS retail meat program and resulted in a total of 358 samples being collected. Only fresh, raw veal was collected and included the following products: ground, stew meat, cutlet, scallopini, and other or unidentified. Other demographic variables, including country of origin, meat color as surrogates for predominately grain-fed (red) vs. predominately formula/milk-fed (pink/white), and state of collection, were collected for all samples.

Bacterial isolation and identification.

Based on an expected low prevalence of bacteria shown in retail studies from Canada, every purchased sample was tested for *Salmonella* and *Campylobacter*, whereas approximately one-third were tested for commensal *E. coli* and *Enterococcus*. The NARMS retail meat methodology was used to isolate all four bacteria (46). Briefly, 25 g of veal were suspended in 250 mL buffered peptone water for 15 minutes. For *Salmonella*, buffered peptone water suspensions were enriched with Rappaport-Vassiliadis medium for 24 hours before streaking onto XLT-4 agar for isolation. For *Campylobacter*, buffered peptone water suspensions were enriched with Bolton broth for 24 hours in a microaerophilic atmosphere, before streaking onto Campy-Cefex Agar for isolation. For *E. coli*, buffered peptone water suspensions were enriched with MacConkey broth for 24 hours and streaked onto MacConkey agar for isolation. For *Enterococcus*, buffered peptone water suspensions were enriched with Enterococcosel broth for 24 hours and streaked onto Enterococcosel agar for isolation. All organisms were streaked to blood agar plates for purity. Typical colonies were selected and stored in Brucella broth with 15% glycerol for shipment to FDA where they were confirmed using the VITEK 2 Compact (bioMerieux, France) or WGS. *Salmonella* serovars were predicted *in silico* using the SeqSero tool (53).

Antimicrobial susceptibility testing.

At FDA, isolates were tested for susceptibility testing using broth microdilution (Sensititre System, TREK Diagnostic Systems Inc., Westlake, OH). *E. coli* and *Salmonella* isolates were tested against an antibiotic panel (Sensititre panel CMV4AGNF) using Clinical and Laboratory Standards Institute (CLSI) methods (7, 8). Antibiotic classes tested were: Aminoglycosides (gentamicin, streptomycin), β-Lactam/β-Lactamase Inhibitor Combinations (amoxicillin-clavulanic acid), Carbapenems (meropenem), Cephems (cefoxitin, ceftriaxone), Folate Pathway Inhibitors (sulfisoxazole and trimethoprim-sulfamethoxazole), Macrolides (azithromycin), Penicillins (ampicillin), Phenicols (chloramphenicol), Quinolones (ciprofloxacin, nalidixic acid), and Tetracyclines (tetracycline). *Enterococcus* isolates were tested against an antibiotic panel (Sensititre panel CMV4AGP) that included the following classes and drugs: Aminoglycosides (gentamicin, streptomycin), Glycopeptides (vancomycin), Glycylcyclines (tigecycline),

Lipopeptides (daptomycin), Macrolides (erythromycin), Nitrofurans (nitrofurantoin), Orthosomycins (avilamycin), Oxazolidones (linezolid), Penicillins (ampicillin), Phenicols (chloramphenicol), Streptogramins (quinupristin-dalfopristin), Quinolones (ciprofloxacin), and Tetracyclines (tetracycline). With the exception of ciprofloxacin, interpretation of MIC values was based on CLSI clinical breakpoints, when available (9); otherwise NARMS provisional cutoffs were used for streptomycin (*E. coli* and *Salmonella*, MIC   32 μg/mL), azithromycin (MIC   32 μg/mL), and tigecycline (MIC   0.25 μg/mL) (47). We included *E. coli* and *Salmonella* isolates with decreased susceptibility to ciprofloxacin (MIC   0.12 μg/mL) in our resistance calculations.

Identification of resistance and virulence genes.

WGS was conducted on *E. coli* and *Salmonella* isolates that were resistant to azithromycin or ceftriaxone or had decreased susceptibility to ciprofloxacin. These drugs are considered first line therapies for the treatment of severe salmonellosis (4, 35). *Enterococcus* isolates were not sequenced because none were resistant to first line therapies for enterococcal infections (vancomycin, tigecycline, daptomycin, and linezolid). All strains of *E. coli* (n=9) and *Salmonella* (n=2) were sequenced by Illumina MiSeq using v3 reagent kits (Illumina, San Diego, CA, USA) with 2x300 bp paired-end reads. The libraries were prepared with Nextera XT kit by Illumina. The raw sequences were assembled *de novo* using CLC Genomic Workbench (version 10.0). Seven of the *E. coli* and both *Salmonella* strains were also sequenced using Pacific Biosciences technology on the Sequel platform with sequencing kit 3.0, as described (39). DNA libraries were prepared using a 10 kb template preparation protocol with SMRTbell template prep kit v 1.0. The reads were assembled using PacBio Hierarchical Genome Assembly Process (HGAP4.0) or Microbial Assembly pipeline and contigs were circularized by Circlator (6, 19). BioSample accession numbers for all isolates are listed in Table S1.

The AMR, heavy metal resistance, biocide resistance and virulence genes were identified in the assembled genomes with AMRFinder Plus 3.8 (https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder/). Because AMRFinder Plus 3.8 does not include genes in the *spv*RABCD operon, the sequence of the *spv*RABCD operon was extracted from the pSDVr (pOU1115) plasmid (Accession DQ115388). A local blastn search was performed to determine the existence of *spv*RABCD operon. Plasmid typing was determined by PlasmidFinder 2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder/). Multi-Locus Sequence Types (MLST) were identified through MLST 2.0 (23) (https://cge.cbs.dtu.dk/services/MLST/).

Statistical analysis.

Statistical analyses were performed using SAS version 9.4 (SAS, Cary, NC). For the source comparisons, statistical differences between proportions were determined using Fisher's exact 2-way test, and a p value   0.05 was considered statistically significant. Although, statistically speaking the sample sizes were big enough for comparison, we determined that the minimum power of each test was 80.6%. For analysis of risk factors for contamination and resistance, the data were first analyzed by running descriptive statistics to important categorical variables in the data. Regression analysis (PROC LOGISTIC) was conducted

to identify relationships between variables and: 1) presence of bacteria or, 2) resistance to at least one antimicrobial. In the first step, a bivariate screening was performed to identify possible factors associated with the two dependent variables. Variables with p < 0.2 were included in the multivariate regression model. For the multivariate model, odds ratios were reported only for variables with a significance level of p   0.05. Odds ratios with 95% CI were used to describe the magnitude of the correlation. Participating NARMS laboratories in Colorado and South Dakota partnered to conduct testing and were combined for the statistical analysis on state variables.

RESULTS

Prevalence of bacteria.

Salmonella was recovered from 2 (0.6%) of the 358 samples collected (Table 1). Both isolates were independently recovered from retail veal packages purchased from two South Carolina supermarkets at different time points. Both were serovar Dublin. *E. coli* and *Enterococcus* isolation was higher, with a recovery rate of 21.1% (49/232) and 53.5% (121/226), respectively (Table 1). The top two *Enterococcus* species were *E. faecalis* (76.9%), and *E. faecium* (13.2%). No *Campylobacter* were recovered from veal.

Antimicrobial resistance.

Both serovar Dublin isolates were resistant to multiple drug classes, including β-lactam/β-lactamase inhibitors, penicillins, cephems, phenicols, aminoglycosides, sulfonamides, and tetracyclines (Table S1). Both were susceptible to macrolides and fluoroquinolones.

Among *E. coli*, the prevalence of resistance to one or more antimicrobials was 65.3% and half (51%) of the *E. coli* isolates were MDR (resistant to at least three or more classes of antibiotics) (Figure 1). Resistance to individual drugs was mixed. Tetracycline resistance was the most abundant, and present in 63.2% (31/49) of isolates, followed by sulfisoxazole (49%, 24/49), ampicillin and streptomycin (45%, 22/49), trimethoprim-sulfamethoxazole (40.8%, 20/49) and chloramphenicol (30.6%, 15/49). Resistance was much lower among ciprofloxacin, azithromycin, and ceftriaxone. Six isolates (12.2%) had reduced susceptibility to ciprofloxacin, six isolates (12.2%) were resistant to ceftriaxone, and two isolates (4.1%) were resistant to azithromycin. One isolate (2%) was resistant to all three antibiotics. No meropenem-resistant isolates were found.

Approximately 74% of all *Enterococcus* spp. were resistant to at least one antimicrobial, while 14.1% were MDR (Figure 2). Tetracycline resistance was the most abundant in *Enterococcus* (60.3% of all enterococcal species combined), followed by streptomycin (19%, 23/121), erythromycin (14.9%, 18/121), and chloramphenicol (8.3%, 10/121). Approximately 3% (4/121) of *Enterococcus* were resistant to ciprofloxacin and less than 1% were resistant to gentamicin (1/121) and ampicillin (1/121). Resistance to quinupristin-dalfopristin, which was only interpreted for non- E. *faecalis* species, was 78.6%. All *Enterococcus* spp. isolates were susceptible to vancomycin, tigecycline, daptomycin, and linezolid, drugs commonly used to treat penicillin resistant enterocccal infections (22).

Individual AMR prevalence levels were compared with retail beef data and dairy cecal data from samples collected by NARMS in 2018 (Figures 1 and 2) (49). In general, *E. coli* and *Enterococcus* isolates from veal were more likely to be resistant to at least one antimicrobial class and at least three antimicrobial classes (p <0.05) than isolates from dairy cattle ceca or retail ground beef. Additionally, *E. coli* from veal had a higher prevalence of resistance (p < 0.05) than the other sources to twelve of the fourteen individual drugs tested. There were not enough data to compare sources for *E. coli* resistance to azithromycin and meropenem. *Enterococcus* isolates from veal were significantly more resistant (p < 0.05) than isolates from both retail ground beef and dairy cattle ceca to only four of the fourteen drugs tested. Veal isolates were less likely to be resistant to quinupristin-dalfopristin than isolates from dairy cattle ceca, but there was no difference compared to retail ground beef isolates. There were not enough data to compare sources for *Enterococcus* resistance to linezolid, nitrofurantoin, vancomycin, avilamycin, and tigecycline.

Resistance genes and virulence genes.

E. coli are known reservoirs of resistance genes (40) that could potentially be transferred to other pathogenic Gram-negative bacteria, particularly *Salmonella* (3, 31). Because ciprofloxacin, ceftriaxone, and azithromycin are considered first line therapies for the treatment of severe *Salmonella* infections (4, 35), we selected nine *E. coli* with decreased susceptibility to ciprofloxacin, or resistance to ceftriaxone or azithromycin for sequencing, in order to identify potentially transmissible ARGs. Both *Salmonella* were also sequenced. Among the eleven isolates, 41 unique ARGs were identified (Table 2). ARGs were correlated with resistance phenotypes for drugs tested, with the exception of streptomycin resistance genes which were present in two isolates (VPS18EC0802 and VPS18EC1101) with no corresponding phenotype (MICs 8-16 µg/ml), an already well-described phenomenon (38), and one isolate (VPS18EC1077) with decreased susceptibility to ciprofloxacin but no identified quinolone resistance genes. Of the five *E. coli* isolates resistant to ceftriaxone, one (VPS18EC0801) carried an extended-spectrum β-lactamase (ESBL) gene ($bla_{CTX-M-55}$), three isolates (VSP18EC0467, VPS18EC0927, VPS18EC1077) had a bla_{CMY} gene, and one (VPS18EC0505) carried mutations in the promoter region of the chromosomal *ampC* gene. Azithromycin resistance was conferred by *mph*(A) (VPS18EC0801, VPS18EC0927); *mph* genes were also found in isolates (VSP18EC0467, VPS18EC1077) with reduced susceptibility to azithromycin (MIC= 16µg/mL; Table S1). Interestingly, the gene *mph*(B) in VPS18EC1077 has not previously been reported in retail meat *E. coli* collected for NARMS. Three of the six isolates with decreased susceptibility to ciprofloxacin had mutations in the *gyr*A gene, while two isolates had *qnr* genes (*qnr*B19 in VPS18EC0676, *qnr*S1 in VPS18EC0801).

Tetracycline resistance genes were found in all eleven sequenced isolates, with *tet*(A) being the most prevalent in seven isolates, followed by *tet*(A)/*tet*(M) in two isolates, and one isolate each with *tet*(B) and *tet*(A)/*tet*(B). Eight of the nine *E. coli* isolates carried dihydrofolate reductase (*dfrA*) genes in addition to *sul1* to confer trimethoprim-sulfamethoxazole resistance. Many *E. coli* isolates carried more than one (up to nine) aminoglycoside resistance genes; VPS18EC0505 had multiple copies of the same aminoglycoside genes (*aph(6)-Id, aph(3'')-Ib, aph(3')-Ia*) spread across the chromosome

and a plasmid. The *aadA5* and *aadA2* genes were present in two isolates that were susceptible to streptomycin with MICs of 8 μg/mL (VPS18EC0802) and 16 μg/mL (VPS18EC1101) (Table S1). We also identified genes and mutations that confer resistance to drugs absent from the susceptibility testing panel, including streptothricin (*sat2*), lincomycin (*lnu(F)*), rifampicin (*arr-2*) and fosfomycin (*uhpT_E350Q*) (Table 2). One isolate (VPS18EC0466) carried all ARGs on the chromosome. All other isolates harbored ARGs on plasmids or both plasmids and the chromosome.

WGS data were also characterized for the presence of disinfectant resistance genes (DRGs), heavy metal resistance genes (HMRGs), and virulence genes. Four unique DRGs and 17 unique HMRGs were identified. DRGs (*qac* and *emrE*) were found in seven of the nine *E. coli* isolates but were not found in either *Salmonella* isolate (Table 2). The *emrE* gene was chromosomally located but the *qac* genes were housed on plasmids that also contained ARGs. HMRGs *ter, ars, and mer,* conferring resistance to tellurium, arsenic and mercury, respectively, were found in five *E. coli* and were distributed between chromosomes and plasmids. In *Salmonella*, the commonly identified gold resistance genes, *golS* and *golT*, were found on the chromosome and *mer* genes were found on an IncA/C2 plasmid in one isolate. The *fieF* gene which encodes the iron efflux transporter was found in all 9 *E. coli* and both *Salmonella*. Thirty-three different virulence genes were identified among the nine *E. coli* isolates. We found one STEC isolate (VPS18EC0467), carrying the stx_{1a} gene ($stxA_{1a}$ and $stxB_{1a}$) (Table 2). Additional genes commonly associated with the Enterohemorrhagic *E. coli* (EHEC) pathotype (*eaeA, stx2, nleB, nleF,* and *espK*) and other intestinal pathogenic subtypes were not found. The *spv*RABCD virulence operon was detected in both *Salmonella* serovar Dublin isolates (Table 2).

Plasmid types and sequence types.

Sequence types (ST) were diverse, with eight unique STs identified among the nine *E. coli* tested. However, none of the isolates were the highly resistant type ST131 or other pandemic lineages (ST95, ST393, ST69, ST95 and ST73) (Table 2) (32). There was also great diversity in plasmid number and type. All nine *E. coli* carried at least one plasmid; one isolate (VPS18EC0801) carried seven plasmids. Several plasmids were hybrids, containing sequences with homology to two to three replicons of different plasmid types. IncF replicons were the most commonly detected replicons in *E. coli* (7/9). Col plasmids were also identified in six of the nine *E. coli*. The *qnr*B19 gene was found in a Col pHAD28 plasmid. Consistent with previous reports, both *Salmonella* serovar Dublin isolates contained IncA/C2, IncFII(S), and IncX1 replicons (Table 2). VPS18S1796 had one hybrid plasmid, while VPS18S0911 appeared to have a separate IncA/C2 plasmid and a hybrid IncFII(S)-IncX1 plasmid.

Demographic analysis.

Demographic variables including country of origin, meat color (as an indicator of grain vs. formula-fed), state of collection, and veal cut were collected for all samples. Over 96% of samples collected were produced in the United States (Table 3). Approximately 85% of the retail veal samples with color information collected were pink/white in color (Table 3), indicating a formula/milk fed-diet (29). Collection was unevenly distributed among the

participating states, owing to variability in months of participation as well as availability of veal in supermarkets. Ground veal comprised the single largest proportion (30.2%) of veal cuts collected. Since the pilot study would be used to assess the feasibility and appropriate parameters for routine surveillance, all variables were tested for association with bacterial isolation and AMR. Associations were initially measured through a bivariate analysis to determine need for inclusion in a multivariate regression model. Only meat cut and state were significantly associated with the presence of *E. coli* and *Enterococcus* in veal samples. When controlling for state, ground veal was 5-16 times more likely to harbor *E. coli* than all other meat cuts (Table 4). *Enterococcus* were more likely to be recovered from ground veal than stew meat (OR 2.6, 95% CI 1.2-6.0) and veal scallopini (OR 3.0, 95% CI 1.4-6.6). There was no effect of state or meat cut on resistance to one or more drugs in either bacteria.

DISCUSSION

In this study, we assessed the prevalence of AMR *Salmonella, E. coli, Enterococcus,* and *Campylobacter* isolated from fresh retail veal purchased in grocery outlets across the United States. We report no contamination with *Campylobacter* and a low prevalence of *Salmonella* contamination among retail veal samples. Comparable to other studies (10, 12), retail veal samples were more likely to be contaminated with *Enterococcus* (particularly *E. faecalis* which constituted 77% of enterococcal species) and *E. coli* In our study, we found that there was a high proportion of AMR (63-79%) among both *E. coli* and *Enterococcus* and a moderate occurrence of MDR *E. coli* (51%). This is consistent with other studies showing high resistance among *E. coli* and *Enterococcus* isolated from veal feces and hide swabs (12, 20, 33). Tetracycline resistance was observed most frequently among *E. coli* and *Enterococcus* spp. isolates, similar to its occurrence in other retail meats collected for NARMS. All *Enterococcus* isolates were susceptible to antimicrobials used as a first-choice to treat human infections. This is a positive finding as resistance to these drugs limits treatment options.

We found that both *Salmonella* isolates were resistant to at least one first-choice therapy for salmonellosis. Both isolates harbored MDR bla_{CMY} plasmids, conferring resistance to cephalosporins, but were susceptible to both azithromycin and fluoroquinolones. Both bla_{CMY} plasmids contained IncA/C signatures, which are generally associated with cattle sources (14). The *spv*RABCD operon was also found on these MDR plasmids, consistent with previous reports showing linkages in *Salmonella* serovar Dublin isolates between the operon and hybrid plasmids containing IncF, IncA/C, and IncX types (17).

Eighteen percent (9/49) of *E. coli* isolates were resistant to ceftriaxone, azithromycin, or ciprofloxacin. Genes conferring resistance to those drugs were found on plasmids in six of the nine isolates. This finding is concerning as in vitro studies have shown that conjugative transfer of antibiotic resistant plasmids between *E. coli* and *Salmonella* is possible (3, 31). The most predominant of the cephalosporinase-encoding genes was bla_{CMY}, which is characteristic for food isolates in the United States. However, we also identified an ESBL-encoding gene, $bla_{CTX-M-55}$. While $bla_{CTX-M-55}$ has been identified in U.S. isolates, its finding is relatively rare compared to other ESBL genes (28), whereas in Asian countries, $bla_{CTX-M-55}$ is the second most common ESBL-encoding gene (25). The plasmid containing

$bla_{CTXM-55}$ (VPS18EC0801) also housed $qnrS1$ and $mph(A)$, making this plasmid a major risk to human health. Only one other isolate (VPS18EC0676) had a PMQR gene, $qnrB19$, and it was the sole resistance determinant on a small Col plasmid. The widespread distribution of PMQR through Col plasmids is well characterized in *Enterobacteriacea* (13, 16, 26, 30, 41). One of the *E. coli* isolates (VPS18EC0467) carried stx_{1a}, and also carried an IncA/C plasmid with bla_{CMY-2} and $mph(A)$. The finding of STEC in veal is not surprising. Surveillance of slaughtered veal carcasses conducted by the U.S. Department of Agriculture Food Safety Inspection Service showed that 9.9% of pre-chill veal carcasses tested were positive for non-O157 STEC (44). However, in our case, with only an stx_{1a} gene and no attachment genes (*eae*, or *saa* genes), this particular STEC is of low or unknown risk for causing infection. It is worth noting that STEC infections are not treated with antimicrobials, due to the potential risk of antibiotic treatment exacerbating illness (35). However, resistance genes can aid in outbreak tracking and identification of STEC strains (1).

The retail veal products demonstrated much higher resistance to most drugs compared to retail ground beef products or dairy cattle ceca collected for NARMS in the same year (Figures 1 & 2). Retail ground beef may not be an equivalent comparator as a portion of ground beef sold at retail come from cull bulls and beef cattle, however much of U.S. ground beef is supplied by cull dairy cows. We also found that veal meat had elevated resistance compared to dairy cattle ceca. Comparison of ceca and retail meat are understandably limited because they are two different sample types. However, it should be noted that this dichotomy has played out in other studies as well. Other studies have shown an age-related decline in resistant *E. coli* in calves. This finding is thought to be related to a number of factors, including selective advantage of resistant strains in calves (21), exposure to residues in waste milk from adult dairy cattle, and use of medicated milk replacers in calves (36). While there currently is no systematic collection of the amounts of antimicrobials used in veal in the United States, other countries collecting these data have shown that veal calves consume more antimicrobials than adult dairy cattle and beef cows (11, 51). This could be ascribed to the calves' higher susceptibility to infection (5). Other factors could play a role in age-related resistance as well, including differences in gastrointestinal physiology and diet transition (24). Veal calves lack functional rumens, and their esophageal groove shunts milk/replacer directly to the abomasum, bypassing the rumen, whereas solid feed given to older cattle enter the rumen (50). The fecal microbiome and resistome would reflect those differences (24, 34). Production type specific practices, including variation in antimicrobial usage, are also likely to play a role in the differences we observed between veal calves and ground beef. Fluoroquinolones are not FDA-approved for use in veal calves (45), and are prohibited from extralabel use in food producing animals (48). We observed isolates resistant to this antibiotic. This could be the result of meat contact with contaminated surfaces in the slaughter or packaging plant or co-selection of resistant strains by the more commonly used tetracycline drugs that are administered to veal calves in medicated milk replacers (27).

Isolates harbored a multitude of genes conferring resistance to heavy metals (gold, mercury, arsenic, tellurium). Also present was the *fieF* gene, encoding cation efflux pumps for excretion of zinc and iron. These metals would commonly be found as nutritional supplements in milk replacers (iron) or fattening agents in animal feed (zinc). A surprising

omission was resistance genes to copper which is also used as an additive in animal feed. Disinfectant resistance genes were also widespread in our study and are common in other slaughterhouse-derived products collected for NARMS.

E. coli sequence types were wide-ranging. None were the highly drug resistant Extraintestinal Pathogenic *E. coli* (ExPEC) type, ST131, however two of the sequence types (ST101 and ST10) have also been detected in patients suspected to have uropathogenic *E. coli* infections (52). Sequence types ST101, ST345, ST10, ST349, and ST23 are not uncommon in dairy cattle or retail foods and have been associated with other retail meats collected for NARMS, including chicken breast, ground turkey, and pork chop (37). Since only nine *E. coli* were sequenced, the pathogenicity and sequence types of the other 40 *E. coli* isolates is unknown, however it is clear from this study that non-pathogenic *E. coli* can act as reservoirs for resistance genes of human importance. We did not sequence enough isolates to detect associations between resistance types and plasmid replicon types or ST of the host bacteria.

Milk-fed (white/pink) veal represent the majority of the veal industry in the United States (42); and this was also evident in our study. Milk-fed veal represented 85% of veal collected in supermarkets, whereas predominately grain-fed (red) veal (calves fed formula for the first 2 months and then switched to grain until their finishing weight has been reached) represented only 15.4% of veal collected in supermarkets. We hypothesized that differences in raising practices of the two types of veal may have some effect on AMR levels, but we could not assess that relationship due to the insufficient diversity of the samples. We did not find a relationship between resistance to at least 1 drug and any of the variables we measured, however we did find a relationship between the prevalence of *E. coli* and *Enterococcus* and cut of veal meat. Ground veal were significantly more likely to be contaminated with these bacteria than most other cuts sampled, signifying a potential target for future surveillance. It should be noted that ground veal may incorporate some meat from bob veal calves, which are the youngest (up to 1 month old) to be harvested and are typically used for hot dogs and other fabricated meats (18). Bosilevac et al. found that bob veal hides and carcasses tested at slaughter had increased prevalence of *Salmonella* compared to formula-fed veal, which was attributed to the challenges of removing bob veal hides (2). Those same challenges may result in bob veal, and subsequently ground veal product, having higher levels of contamination with *E. coli* and *Enterococcus*.

In conclusion, we assessed the prevalence of AMR *Salmonella, E. coli, Enterococcus,* and *Campylobacter* isolated from fresh retail veal purchased in grocery outlets across the United States. *Campylobacter* were absent from the veal meats we sampled, and *Salmonella* contamination was rare, but *Enterococcus* and *E. coli* were prevalent and were more likely to be isolated from ground veal product. Veal meats showed significantly higher levels of AMR compared to retail ground beef and dairy cattle ceca. *Salmonella* and *E. coli* were found to harbor genes that confer resistance to medically important antimicrobials. These genes included AmpC (5 of the 9 *E. coli* tested and both *Salmonella*), ESBL (1/9 *E. coli*), PMQR (2/9 *E. coli*), and *mph* (4/9 *E. coli*). These findings suggest that veal meats are reservoirs for resistance determinants. The results of this study can be used to develop a retail veal sampling program in the United States.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

REFERENCES

1. Bielaszewska M, Mellmann A, Zhang W, Köck R, Fruth A, Bauwens A, Peters G, and Karch H. 2011. Characterisation of the Escherichia coli strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. Lancet Infect Dis. 11:671–6. [PubMed: 21703928]

2. Bosilevac JM, Zhilyaev S, Wang R, Luedtke BE, Wheeler TL, and Koohmaraie M. 2019. Prevalence and Characterization of Salmonella Present during Veal Harvest. J Food Prot. 82:775–784. [PubMed: 30986365]

3. Card RM, Cawthraw SA, Nunez-Garcia J, Ellis RJ, Kay G, Pallen MJ, Woodward MJ, and Anjum MF. 2017. An in vitro chicken gut model demonstrates transfer of a multidrug resistance plasmid from Salmonella to commensal Escherichia coli. MBio. 8:e00777–17. [PubMed: 28720731]

4. Centers for Disease Control and Prevention. 2019. Salmonella: Information for Healthcare Professional and Laboratories. Available at: https://www.cdc.gov/salmonella/general/technical.html. Accessed April 29, 2021.

5. Chase CCL, Hurley DJ, and Reber AJ. 2008. Neonatal Immune Development in the Calf and Its Impact on Vaccine Response. Veterinary Clinics of North America: Food Animal Practice. 24:87–104. [PubMed: 18299033]

6. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, and Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods. 10:563–9. [PubMed: 23644548]

7. Clinical and Laboratory Standards Institute. 2016. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria: Third edition. CLSI Document M45-Ed3. Wayne, PA.

8. Clinical and Laboratory Standards Institute. 2018. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Eleventh Edition. CLSI document M07-Ed11. Wayne, PA.

9. Clinical and Laboratory Standards Institute. 2019. Performance Standards for Antimicrobial Susceptibility Testing: Thirtieth Edition. CLSI document M100-Ed30. Wayne, PA.

10. Cook A, Reid-Smith RJ, Irwin RJ, McEwen SA, Young V, and Ribble C. 2011. Antimicrobial resistance in Campylobacter, Salmonella, and Escherichia coli isolated from retail grain-fed veal meat from Southern Ontario, Canada. J Food Prot. 74:1245–51. [PubMed: 21819650]

11. Danish Programme for Surveillance of Antimicrobial Consumption and Resistance in Bacteria from Food Animals Food and Humans (DANMAP). 2019. DANMAP 2018-Use of antimicrobial agents and occurence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark.

12. Di Labio E, Regula G, Steiner A, Miserez R, Thomann A, and Ledergerber U. 2007. Antimicrobial resistance in bacteria from Swiss veal calves at slaughter. Zoonoses Public Health. 54:344–52. [PubMed: 18035972]

13. Fiegen U, Klein G, de Jong A, and Kehrenberg C. 2017. Detection of a Novel qnrB19-Carrying Plasmid Variant Mediating Decreased Fluoroquinolone Susceptibility in Salmonella enterica Serovar Hadar. Microb Drug Resist. 23:280–284. [PubMed: 27404660]

14. Folster JP, Tolar B, Pecic G, Sheehan D, Rickert R, Hise K, Zhao S, Fedorka-Cray PJ, McDermott P, and Whichard JM. 2014. Characterization of blaCMY plasmids and their possible role in source attribution of Salmonella enterica serotype Typhimurium infections. Foodborne Pathog Dis. 11:301–6. [PubMed: 24484290]

15. Gaire TN, Scott HM, Sellers L, and Volkova V. 2017. Age-dependency of antimicrobial resistance in fecal bacteria of animals – A scoping review. The 98th Conference of Research Workers in Animal Diseases (CRWAD), Chicago, IL, US.

16. Hammerl JA, Beutlich J, Hertwig S, Mevius D, Threlfall EJ, Helmuth R, and Guerra B. 2010. pSGI15, a small ColE-like qnrB19 plasmid of a Salmonella enterica serovar Typhimurium strain carrying Salmonella genomic island 1 (SGI1). J Antimicrob Chemother. 65:173–5. [PubMed: 19861336]

17. Hsu CH, Li C, Hoffmann M, McDermott P, Abbott J, Ayers S, Tyson GH, Tate H, Yao K, Allard M, and Zhao S. 2019. Comparative Genomic Analysis of Virulence, Antimicrobial Resistance, and Plasmid Profiles of Salmonella Dublin Isolated from Sick Cattle, Retail Beef, and Humans in the United States. Microb Drug Resist. 25:1238–1249. [PubMed: 31149890]

18. Humane Society Veterinary Medical Association. 2018. Facts on Veal Calves. Available at: https://www.hsvma.org/facts_veal_calves#.X2y5G4t7mUl. Accessed August 18, 2020.

19. Hunt M, Silva ND, Otto TD, Parkhill J, Keane JA, and Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. Genome Biol. 16:294. [PubMed: 26714481]

20. Hutchinson H, Finney S, Muñoz-Vargas L, Feicht S, Masterson M, and Habing G. 2017. Prevalence and Transmission of Antimicrobial Resistance in a Vertically Integrated Veal Calf Production System. Foodborne Pathog Dis. 14:711–718. [PubMed: 28915068]

21. Khachatryan AR, Hancock DD, Besser TE, and Call DR. 2004. Role of calf-adapted Escherichia coli in maintenance of antimicrobial drug resistance in dairy calves. Appl Environ Microbiol. 70:752–7. [PubMed: 14766551]

22. Kristich CJ, Rice LB, and Arias CA. 2014. Enterococcal Infection-Treatment and Antibiotic Resistance. In Gilmore MS, et al. (ed.), Enterococci: From Commensals to Leading Causes of Drug Resistant Infection, Boston.

23. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, and Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol. 50:1355–61. [PubMed: 22238442]

24. Liu J, Taft DH, Maldonado-Gomez MX, Johnson D, Treiber ML, Lemay DG, DePeters EJ, and Mills DA. 2019. The fecal resistome of dairy cattle is associated with diet during nursing. Nature Communications. 10:4406.

25. Lupo A, Saras E, Madec JY, and Haenni M. 2018. Emergence of blaCTX-M-55 associated with fosA, rmtB and mcr gene variants in Escherichia coli from various animal species in France. J Antimicrob Chemother. 73:867–872. [PubMed: 29340602]

26. Moreno-Switt AI, Pezoa D, Sepúlveda V, González I, Rivera D, Retamal P, Navarrete P, Reyes-Jara A, and Toro M. 2020. Corrigendum: Transduction as a Potential Dissemination Mechanism of a Clonal qnrB19-Carrying Plasmid Isolated From Salmonella of Multiple Serotypes and Isolation Sources. Front Microbiol. 11:547. [PubMed: 32318037]

27. Mzyk DA, Gehring R, Tell LA, Vickroy TW, Riviere JE, Ragan G, Baynes RE, and Smith GW. 2017. Considerations for extralabel drug use in calves. J Am Vet Med Assoc. 250:1275–1282. [PubMed: 28509644]

28. National Center for Biotechnology Information. 2016. The NCBI Pathogen Detection Project [internet]. National Library of Medicine (US), National Center for Biotechnology Information, Bethesda (MD).

29. Ngapo TM, and Gariépy C. 2006. Factors affecting the meat quality of veal. Journal of the Science of Food and Agriculture. 86:1412–1431.

30. Pallecchi L, Riccobono E, Sennati S, Mantella A, Bartalesi F, Trigoso C, Gotuzzo E, Bartoloni A, and Rossolini GM. 2010. Characterization of Small ColE-Like Plasmids Mediating Widespread Dissemination of the qnrB19 Gene in Commensal Enterobacteria. Antimicrobial Agents and Chemotherapy. 54:678–682. [PubMed: 20008783]

31. Poole T, Callaway T, Norman K, Scott H, Loneragan G, Ison S, Beier R, Harhay D, Norby B, and Nisbet D. 2017. Transferability of antimicrobial resistance from multidrug-resistant Escherichia coli isolated from cattle in the USA to E. coli and Salmonella Newport recipients. Journal of global antimicrobial resistance. 11:123–132. [PubMed: 28801276]

32. Riley LW 2014. Pandemic lineages of extraintestinal pathogenic Escherichia coli. Clin Microbiol Infect. 20:380–90. [PubMed: 24766445]

33. Salaheen S, Kim SW, Cao H, Wolfgang DR, Hovingh E, Karns JS, Haley BJ, and Van Kessel JAS. 2019. Antimicrobial Resistance Among Escherichia coli Isolated from Veal Calf Operations in Pennsylvania. Foodborne PathogDis. 16:74–80.

34. Salaheen S, Kim SW, Hovingh E, Van Kessel JAS, and Haley BJ. 2021. Metagenomic Analysis of the Microbial Communities and Resistomes of Veal Calf Feces. Frontiers in Microbiology. 11:609950. [PubMed: 33633694]

35. Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, Langley JM, Wanke C, Warren CA, Cheng AC, Cantey J, and Pickering LK. 2017. 2017 Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea. Clinical Infectious Diseases. 65:e45–e80. [PubMed: 29053792]

36. Springer HR, Denagamage TN, Fenton GD, Haley BJ, Van Kessel JAS, and Hovingh EP. 2019. Antimicrobial Resistance in Fecal Escherichia coli and Salmonella enterica from Dairy Calves: A Systematic Review. Foodborne Pathog Dis. 16:23–34. [PubMed: 30481058]

37. Tadesse DA, Li C, Mukherjee S, Hsu CH, Bodeis Jones S, Gaines SA, Kabera C, Loneragan GH, Torrence M, Harhay DM, McDermott PF, and Zhao S. 2018. Whole-Genome Sequence Analysis of CTX-M Containing Escherichia coli Isolates from Retail Meats and Cattle in the United States. Microb Drug Resist. 24:939–948. [PubMed: 30148698]

38. Tyson GH, Li C, Ayers S, McDermott PF, and Zhao S. 2016. Using whole-genome sequencing to determine appropriate streptomycin epidemiological cutoffs for Salmonella and Escherichia coli. FEMS Microbiology Letters. 363:fnw009. [PubMed: 26781915]

39. Tyson GH, Li C, Ceric O, Reimschuessel R, Cole S, Peak L, and Rankin SC. 2019. Complete Genome Sequence of a Carbapenem-Resistant Escherichia coli Isolate with blaNDM-5 from a Dog in the United States. Microbiology Resource Announcements. 8:e00872–19. [PubMed: 31439705]

40. Tyson GH, Li C, Hsu C-H, Bodeis-Jones S, and McDermott PF. 2019. Diverse fluoroquinolone resistance plasmids from retail meat E. coli in the United States. Frontiers in microbiology. 10:2826. [PubMed: 31866986]

41. Tyson GH, Tate HP, Zhao S, Li C, Dessai U, Simmons M, and McDermott PF. 2017. Identification of Plasmid-Mediated Quinolone Resistance in Salmonella Isolated from Swine Ceca and Retail Pork Chops in the United States. Antimicrob Agents Chemother. 61:e01318–17. [PubMed: 28784677]

42. U.S. Department of Agriculture. 2013. Veal from Farm to Table. Available at: https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/meat/veal-farm-table. Accessed August 10, 2020.

43. U.S. Department of Agriculture. 2021. All Meat Statistics. Available at: https://www.ers.usda.gov/data-products/livestock-meat-domestic-data/. Accessed March 15, 2021.

44. U.S. Department of Agriculture. 2016. The Nationwide Microbiological Baseline Data Collection Program: Beef-Veal Carcass Survey August 2014-December 2015. U.S. Department of Agriculture, Washington, D.C.

45. U.S. Food and Drug Administration. 2020. Animal Drugs @FDA Available at: https://animaldrugsatfda.fda.gov/adafda/views/#/search. Accessed August 18 2020.

46. U.S. Food and Drug Administration. 2019. NARMS Retail Meat Surveillance Laboratory Protocol. Available at: https://www.fda.gov/media/93332/download. Accessed August 22, 2020.

47. U.S. Food and Drug Administration. 2019. The National Antimicrobial Reistance Monitoring System: NARMS Integrated Report, 2016-2017. U.S. Department of Health and Human Services, Food and Drug Administration, Rockville, MD.

48. U.S. Food and Drug Administration. 2020. Drugs prohibited for extralabel use in animals. 21 Code of Federal Regulations § 530.41. Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=530.41. Accessed April 28, 2021.

49. U.S. Food and Drug Administration. 2020. The National Antimicrobial Reistance Monitoring System: NARMS Integrated Report, 2018. U.S. Department of Health and Human Services, Food and Drug Administration, Rockville, MD.

50. University of Minnesota Extension. Date, 2021, The ruminant digestive system. Available at: https://extension.umn.edu/dairy-nutrition/ruminant-digestive-system. Accessed April 28, 2021.

51. Veldman K, Wit B, Pelt W, Heederik D, and Dj M. 2017. MARAN 2017: Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2016. Combined with NETHMAP-2017: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands.

52. Yamaji R, Friedman CR, Rubin J, Suh J, Thys E, McDermott P, Hung-Fan M, and Riley LW. 2018. A Population-Based Surveillance Study of Shared Genotypes of Escherichia coli Isolates from Retail Meat and Suspected Cases of Urinary Tract Infections. mSphere. 3:e00179–18. [PubMed: 30111626]

53. Zhang S, Yin Y, Jones MB, Zhang Z, Kaiser BLD, Dinsmore BA, Fitzgerald C, Fields PI, and Deng X. 2015. Salmonella serotype determination utilizing high-throughput genome sequencing data. Journal of clinical microbiology. 53:1685–1692. [PubMed: 25762776]

HIGHLIGHTS

- *Salmonella*, *E. coli* and *Enterococcus* were found in veal meats collected for NARMS.

- AMR levels were higher in retail veal than in ground beef and dairy cattle ceca.

- bla^{CMY}, bla_{CTX-M}, *mph*, and *qnr* genes and *gyrA* mutations were found.

- An stx_{1a}-containing *E. coli* was also found.

- The majority of *Salmonella* and *E. coli* contained the IncF plasmid type.

Figure 1:
Comparison of antimicrobial resistance in *E.coli* isolates from retail veal, retail ground beef, and dairy cattle ceca *For these antimicrobials, there are significant differences between percentage of isolates resistant in retail veal and retail ground beef as well as between retail veal and dairy cattle ceca. ‡First line therapies for the treatment of complicated salmonellosis include ciprofloxacin, azithromycin, and ceftriaxone. Note: percent resistance to ciprofloxacin includes isolates with decreased susceptibility (MIC >= 0.12).
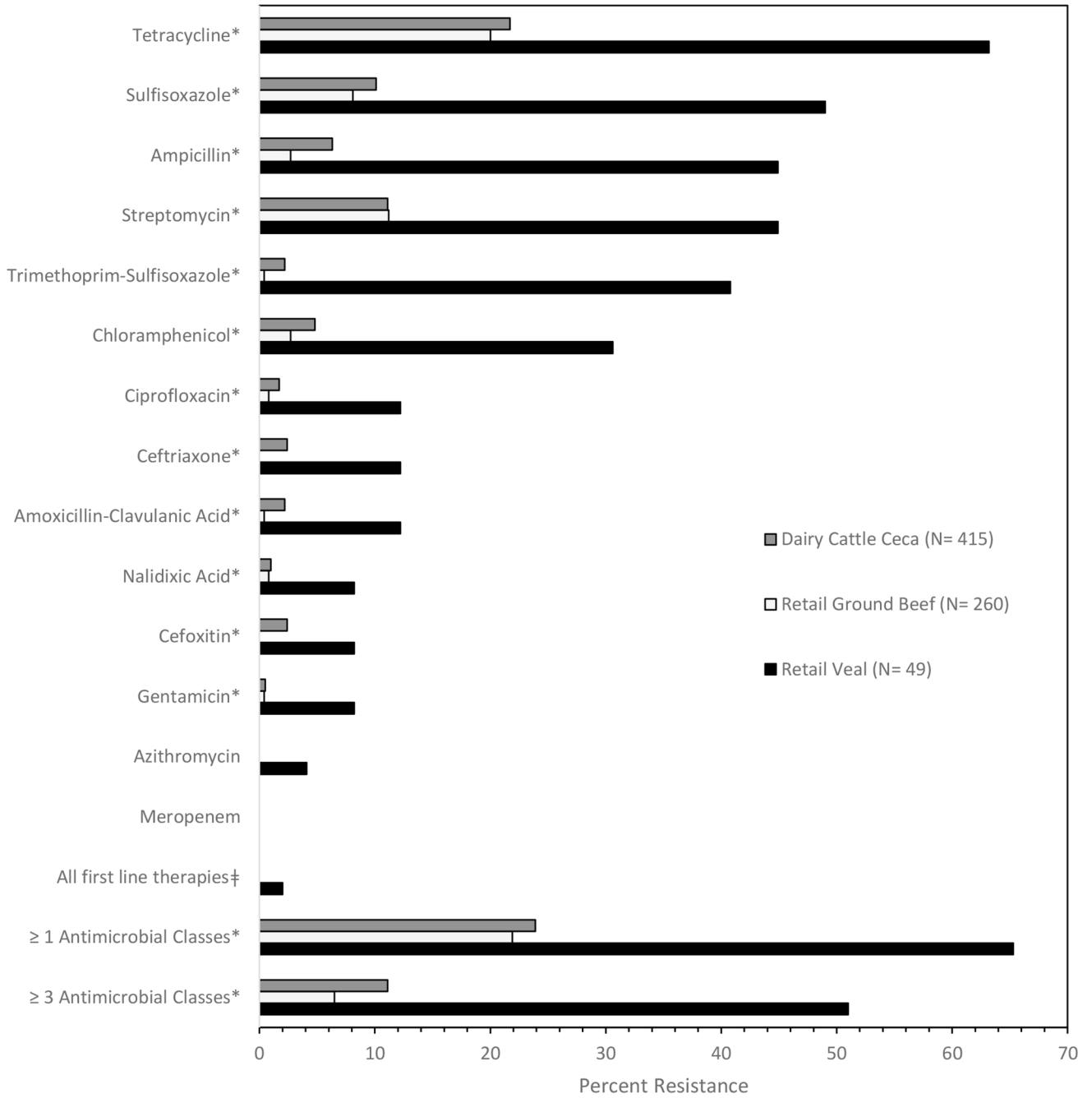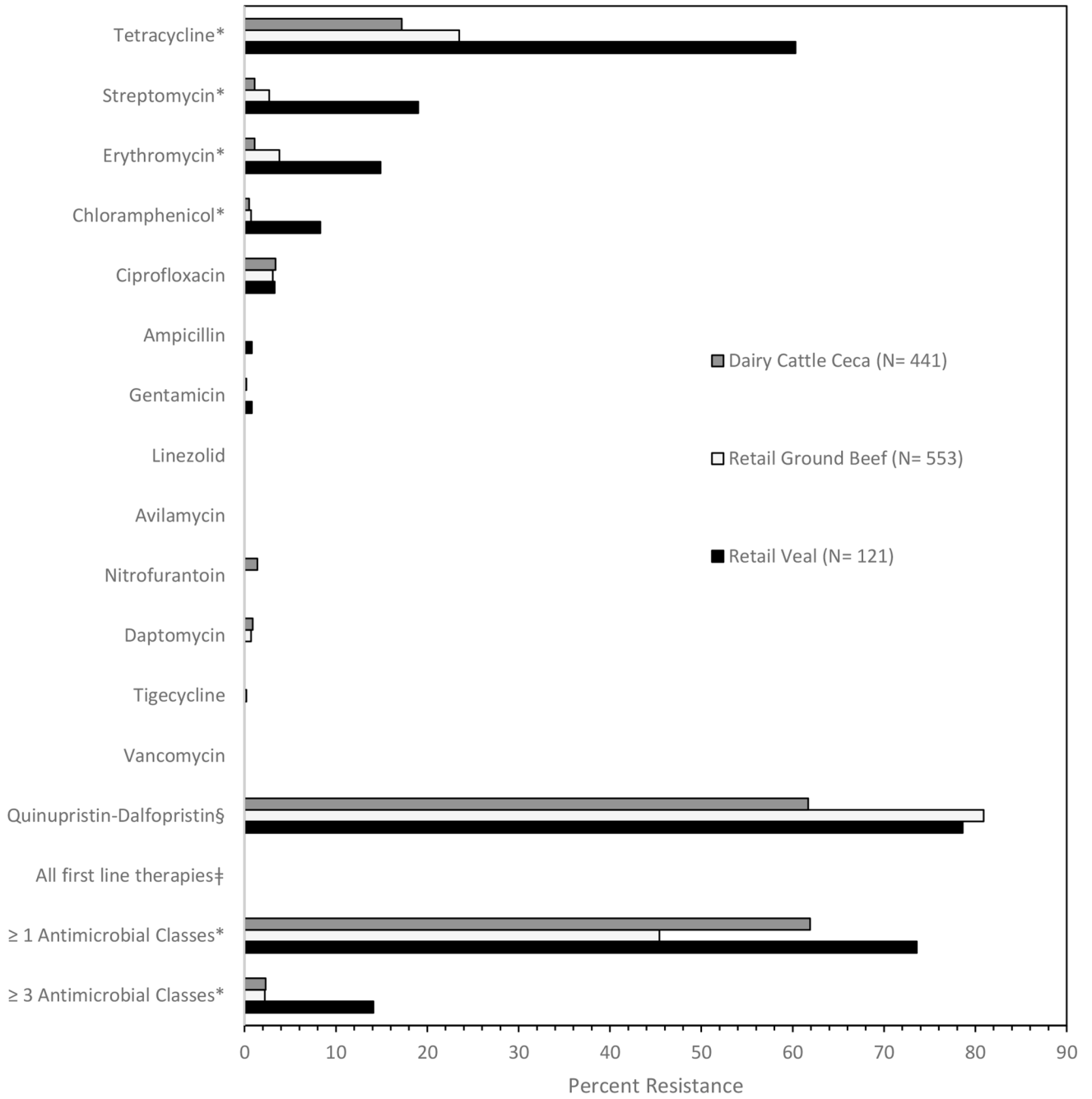
Figure 2:
Comparison of antimicrobial resistance in *Enterococcus* spp. isolates from retail veal, retail ground beef, and dairy cattle ceca *For these antimicrobials, there were significant differences between percentage of isolates resistant in retail veal and retail ground beef as well as between retail veal and dairy cattle ceca. § CLSI Quinupristin-Dalfopristin breakpoints are only available for non-*E. faecalis* species. For this drug the following denominators were used: Dairy Cattle Ceca (N= 363), Retail Ground Beef (N= 178), Retail Veal (N= 28). ‡First line therapies for the treatment of *Enterococcus* include

vancomycin and tigecycline (vancomycin-susceptible infections) and daptomycin and linezolid (vancomycin-resistant infections).

Table 1.

Prevalence of enteric bacteria in retail veal

Organism (No. of samples tested)	Total No. of Isolates (%)	Species/Serovar	No. of Isolates (%)
Enterococcus (N= 226)	121 (53.5)	---	--
		durans	7 (5.8)
		faecalis	93 (76.9)
		faecium	16 (13.2)
		gallinarum	3 (2.5)
		malodoratus	1 (0.8)
		unidentified	1 (0.8)
E. coli (N= 232)	49 (21.1)	---	--
Salmonella (N= 358)	2 (0.6)	Dublin	2 (100)
Campylobacter (N=358)	0	--	--

Table 2.

Antimicrobial Resistance Genes Found in Isolates Resistant to Clinically Important Drugs

Sample ID	Organism	MLST	Resistance Phenotype	Plasmid or Chromosome	Resistance Genes	Virulence Genes
VPS18EC0466	*E. coli*	278	CHL CIP NAL TET	Chromosome	*gyrA_S83L, floR, tet(A), emrE, arsC arsR, fieF*	*lpfA, f17a, lpfA, fdeC, espX1*
				Col156	--	--
				Col44011	--	--
VPS18EC0467	*E. coli*	101	AMC AMP FOX AXO CHL GEN STR FIS TET COT	Chromosome	*arsC arsR, fieF*	*lpfA, stxA1a, stxB1a, bmaE, lpfA, iss, espX1, fdeC, iutA, iucA, iha, espP, cdtB*
				IncA/C2	*mph(A), sul1, aac(3)-VIa, aadA1, floR, tet(A), aph(6)-Id, aph(3'')-Ib, sul2, blaCMY-2, dfrA12, qacEdelta1*	--
				IncFII-IncFIB	--	
VPS18EC0505	*E. coli*	23	AMC AMP AXO CHL CIP NAL STR FIS TET COT	Chromosome	*gyrA_S83L, ampC C42T, aph(3'')-Ib, aph(6)-Id, aph(3')-Ia, tet(B), sul2, adA1, sat2, dfrA1, terD, terZ, terW, arsC, arsR, fieF*	*ybtP, ybtQ, iutA, iha, iss, papG-II, papE, papC, ireA, lpfA, bmaE, astA, iucA, fdeC, espX1, papF*
				IncY	--	--
				IncA/C2	*floR, tet(A), aph(6)-Id, aph(3'')-Ib, sul2, aph(3')-Ia, sul1, aadA5, aadA6, qacEdelta1*	--
				IncB/O/K/Z	--	*epeA*
				Col RNAI	--	--
				Col RNAI	--	--
				Col (MG828)	--	--
VPS18EC0676	*E. coli*	349	AMP CIP STR FIS TET COT	Chromosome	*uhpT_E350Q, emrE, arsC, arsD, fieF*	*ybtP, ybtQ, iss, eilA, fdeC, espX1*
				IncQ1-IncFII-IncFIB	*tet(A), dfrA5, blaTEM-1, sul2, aph(3'')-Ib, aph(6)-Id, aph(3')-Ia, merC, merP, merT, merR*	*iroN, iutA, iss, mchF, cvaC, iss, iucA, iroE, epeA*
				IncB/O/K/Z	*aph(3'')-Ib, aph(6)-Id*	*epeA*
				ColRNAI	--	--
				Col8282	--	--
				Col(pHAD28)	*qnrB19*	--
				ColpVC	--	--
				Col(MG828)	--	--
VPS18EC0801	*E. coli*	345	AZI AMP AXO CHL CIP GEN	Chromosome	*arsC, arsR, fieF, terW, terZ, terD*	*iss, lpfA, fdeC, espX1*
				IncY	--	--
			STR FIS TET COT	IncFII-IncFIB	*dfrA12, aadA2, cmlA1, aadA1, tet(M), qacL*	--

Sample ID	Organism	MLST	Resistance Phenotype	Plasmid or Chromosome	Resistance Genes	Virulence Genes
				IncN-IncHI2A-IncHI2	*blaCTX-M-55, qnrS1, mph(A), arr-2, dfrA14, blaTEM-1, blaLAP-2, aac(3)-IId, aadA22, lnu(F), sul3, tet(A), floR, aph(3')-Ia, terD, terZ, terW*	--
				IncX4	--	--
VPS18EC0802	*E. coli*	109	AMP CIP NAL FIS TET COT	Chromosome	*gyrA _S83L, uhpT E350Q, sul2, sul1, aadA5, dfrA17, blaTEM-1, qacEdelta1, fieF, arsR, arsC*	*lpfA, iroN, iss, lpfA, astA, fdeC,espX1*
				IncFII	*tet(A), tet(M), qacG2*	--
				Col156	--	--
VPS18EC0927[a]	*E. coli*	10	AZI AMC AMP FOX AXO STR FIS TET COT	IncFII(29),IncFII, IncFIB(AP001918), ColRNAI,Col(MG828)	*tet(B), sul1, aadA5, dfrA17, mph(A), blaCMY, arsC, arsR, fieF*	*f17g, iss, cdtB, iutA, ybtP, ybtQ, capU*
VPS18EC1077[a]	*E. coli*	57	AMC AMP FOX AXO CHL CIP GEN NAL STR FIS TET COT	IncFIB(AP001918), IncFIA, Col(pHAD28)	*dfrA1, aph(3')-Ia, blaTEM-1, aph(6)-Id, aph(3'')-Ib, dfrA1, catA1, aac(3)-IIa, blaCMY, , dfrA1, dfrA36, aadA1, sat2, mph(A), sul2, mph(B), sul1, tet(M), tet(A), floR, arsC, arsR, fieF, merP, merC, merR, merT,*	*astA, papC, papH, ireA, iss, iroN, iroE, sfaS, sfaF, fdeC, espX1*
VPS18EC1101	*E. coli*	10	AMC AMP FOX AXO FIS TET COT	Chromosome	*emrE, arsC, arsR, fieF*	*fdeC, espX1*
				IncFIA-IncFIIB	*dfrA12, aadA2, sul1, blaCMY-2, aph(3')-Ia, tet(A),qacEdelta1*	--
VPS18S0911	*Salmonella.* Dublin		AMC AMP FOX AXO CHL STR FIS TET	Chromosome	*uhpT_E350Q, golS, golT, fieF*	--
				IncA/C2	*sul2, aph(3'')-Ib, aph(6)-Id, tet(A), floR, blaTEM, blaCMY-2, merR, merT, merP, merA, merB, merD, merE*	
				IncFII(S)-IncX1	--	*spvR, spvA, spvB, spcC, spvD*
VPS18S1796			AMC AMP AXO CHL	Chromosome	*uhpT_E350Q, golS, golT, fieF*	--
	Salmonella. Dublin		STR FIS TET	IncFII(S)-IncX1-IncA/C2	*blaCMY-2, sul2, aph(3'')-Ib, aph(6)-Id, tet(A), floR*	*spvR, spvA, spvB, spcC, spvD*

[a]Isolates were sequenced on the Illumina MiSeq only, therefore locations of genes are unknown. AMC= Amoxicillin-Clavulanic Acid; AMP= Ampicillin; AZI= Azithromycin; AXO= Ceftriaxone; CHL= Chloramphenicol; CIP= Decreased susceptibility to Ciprofloxacin; COT= Trimethoprim/Sulfamethoxazole; FIS= Sulfisoxazole; FOX= Cefoxitin; GEN= Gentamicin; NAL= Nalidixic Acid; STR= Streptomycin; TET= Tetracycline;

Table 3.

Bacterial prevalence and antimicrobial resistance by sample demographic

Demographic	Total No. of Samples Collected	No. of Samples Tested for *E. coli*	No. of Samples Positive for *E. coli* (%)	No. of *E. coli* Resistant to At Least 1 Drug (%)	No. of Samples Tested for *Enterococcus*	No. of Samples Positive for *Enterococcus* (%)	No. of *Enterococcus* Resistant to At Least 1 Drug
Country of Origin							
Netherlands	5	2	0	-	2	1 (50.0)	1 (100)
USA	347	226	49 (21.7)	32 (65.3)	220	118 (53.6)	112 (94.9)
USA and Canada	6	4	0	-	4	2 (50.0)	2 (100)
Meat Color							
Red	54	33	6 (18.2)	5 (83.3)	34	19 (55.8)	19 (100)
Pink/White	295	191	42 (22.0)	27 (64.3)	184	100 (54.3)	94 (94)
missing	*9*	*7*			*8*		
State of Collection							
CO & SD	16	8	1 (12.5)	0	8	6 (75.0)	6 (100)
GA	24	24	9 (37.5)	7 (77.8)	24	14 (58.3)	14 (100)
MD	54	31	7 (22.6)	5 (71.4)	32	17 (53.1)	16 (94.1)
NY	61	29	2 (6.9)	2 (100)	29	23 (79.3)	22 (95.7)
OR	21	11	2 (18.2)	0	12	8 (66.7)	8 (100)
PA	61	61	7 (11.5)	2 (28.6)	51	19 (37.3)	17 (89.5)
SC	64	35	9 (25.7)	5 (55.6)	35	13 (37.1)	12 (92.3)
TN	57	33	12 (36.4)	11 (91.7)	35	21 (60.0)	20 (95.2)
Veal cut							
Ground Veal	108	70	32 (45.7)	19 (59.4)	69	49 (71.0)	46 (93.9)
Other	84	53	3 (5.7)	2 (66.7)	48	26 (54.2)	25 (96.2)
Stew Meat	47	27	2 (7.4)	1 (50)	26	13 (50.0)	12 (92.3)
Veal Cutlet	66	42	7 (16.7)	6 (85.7)	42	21 (50.0)	20 (95.2)
Veal Scallopini	53	40	5 (12.5)	4 (80.0)	41	12 (29.3)	12 (100)

Table 4.

Comparative effect of veal cut on isolation of *E. coli* and *Enterococcus*

		Comparator (OR, 95% CI)			
		Other	Stew Meat	Veal Cutlet	Veal Scaloppini
E. coli	Ground Veal	**16.4 (4.2 – 63.7)**	**11.7 (2.4 – 56.1)**	**4.9 (1.8 – 13.3)**	**7.7 (2.4 - 24.1)**
Enterococcus	Ground Veal	2.0 (1.0 – 3.9)	**2.6 (1.2 – 6.0)**	1.8 (0.9 -3.5)	**3.0 (1.4 – 6.6)**

Bolded numbers are statistically significant Odds Ratios (OR)