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Antimicrobial Resistance in Bacteria Isolated from U.S. Goat, Sheep, and Lamb Cecal Content Samples: A FSIS NARMS Study

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Summary

The demand for goat, sheep, and lamb meat is increasing in the U.S. and globally. Unfortunately, in these animals, the prevalence of bacterial antimicrobial resistance (AMR) at slaughter is poorly understood. AMR is a global public health threat that may result in treatment failure and deaths in humans and animals. To address knowledge gaps, the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) conducted this first nationwide cecal (intestinal) sampling study under the National Antimicrobial Resistance Monitoring System (NARMS). From February 2020 to September 2022, FSIS collected a total of 1,025 cecal samples from goat, sheep and lamb from 449 FSIS-regulated slaughter establishments. The recovery of *Salmonella* of public health importance was low. Analysis showed that 91% of *Salmonella*, 23% of *Campylobacter*, 61% of *Enterococcus*, and 48% of generic *E. coli* found were not resistant (were pan-susceptible) to the antimicrobials tested. Resistance to 1-2 antimicrobial classes was highest in *Campylobacter* (74%), followed by *Enterococcus* (52%), generic *E. coli* (30%), and *Salmonella* (8%). Resistance to quinolones (ciprofloxacin and nalidixic acid) and/or tetracycline was exhibited in *Campylobacter*. Resistance to tetracycline was highest among *Salmonella*, generic *E. coli*, and *Enterococcus*. Multi-drug resistance (resistant to three or more classes of antimicrobial drugs) was highest in generic *E. coli* (9%), followed by *Campylobacter* (3%), *Salmonella* (≤ 1%), and *Enterococcus* (≤ 1%). A host-adapted *Salmonella* IIIb 61:k:1,5,(7) (enterica subspecies *diarizonae*) that can cause serious illnesses in sheep and lamb, was recovered in disproportion-

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ally higher numbers from cecal samples of sheep (63%) and lamb (52%) compared to goats (2%). More than 40% of cecal *Campylobacter* isolates from goat and sheep were resistant to quinolones (ciprofloxacin and nalidixic acid). This study provides a representative national snapshot of AMR occurrence in pathogens (*Salmonella*, *Campylobacter*) and indicator bacteria (generic *E. coli*, *Enterococcus*) from goat, sheep, and lamb collected from cecal content at the time of slaughter.

Key Words: Antimicrobial Resistance; National Antimicrobial Resistance Monitoring System; *Salmonella* IIIb 61: K: 1,5, (7); *Campylobacter*; generic *E. coli*; *Enterococcus*.

Introduction

Food-producing animals are a valuable source of macronutrients, including protein, micronutrients, and a variety of edible and inedible byproducts. In the U.S. alone, animal-derived foods currently provide energy (24% of total), protein (48%), essential fatty acids (23-100%), and essential amino acids (34-67%) in people's diet (White and Hall, 2017). Based on data from 2022, compared to the per capita U.S. consumption of major meat sources such as pork (56 lbs.), poultry (113 lbs.) or beef (59 lbs.), the per capita consumption of goat, sheep, and lamb meat is significantly lower at 0.25 lbs. for goat meat and 1.3 lbs. for lamb and mutton (Statista — beef, pork, poultry, lamb, and mutton). It is noteworthy that the popularity and demand of sheep and lamb is growing among U.S. ethnic populations in urban areas (Harvest Returns, 2023). The demand for and consumption of goat, sheep, and lamb meat are also increasing globally. By 2030, sheep meat as a source of dietary protein is expected to grow by 15.7% (OECD-FAO 2021). According to Mazinani, global sheep production is nearing 9 million tons, and ranks fourth after pork, poultry, and beef (Mazinani, 2020).

While food-producing animals are important sources of nutrients they can also be reservoirs for zoonotic pathogens. According to the Centers for Disease Control and Prevention (CDC), estimates are that animals spread more than 6 out of every 10 known human infectious diseases and 3 out of every 4 new or emerging infectious diseases in people come from animals (CDC, About Zoonotic Diseases). Some of these pathogens can cause foodborne infections and may be resistant to antimicrobials (*i.e.*, exhibit antimicrobial resistance, or AMR). Infections with AMR pathogens in humans are difficult to treat and can result in unexpected treatment failures and even death (CDC, 2019).

To protect the health of people and animals, zoonotic foodborne pathogens and AMR need to be managed effectively with the goal of reducing AMR to meet national and international AMR reduction targets (WHO, 2021). In food-producing animals, this requires a One Health type approach that encompasses 'farm to fork' components of farming, processing, distribution, and consumption to prevent, detect, and control hazards from pathogens of animal origin (Abebe et al., 2020) (WOAH, n.d.). This requires robust, well-designed, multifaceted national surveillance systems for detecting pathogens and AMR. Countries with well-designed national level AMR surveillance systems include the U.S., the European Union (European Commission, 2023), Canada (CARSS, 2023), Australia (AUS, 2019) and New Zealand (New Zealand Ministry of Health, 2017). While some of these surveillance systems use a unified farm to fork approach (such as the Canadian Integrated Program for Antimicrobial Resistance)(CIPARS), other countries (such as the U.S.) use separate surveillance systems designed to capture pathogen and AMR trends at different points from farm to fork.

In the U.S., the U.S. Department of Agriculture (USDA) monitors AMR and animal pathogens with the Animal and Plant Health Inspection Service (APHIS) responsible for studies of on-farm pathogens, and AMR through the National Animal Health Monitoring System (NAHMS) (APHIS, 2022, 2024). The Food Safety and Inspection Service (FSIS) studies pathogens and AMR from cecal and food samples collected from poultry, swine, and cattle at slaughter and processing (FSIS, 1996). FSIS analyzes pathogens and AMR from cecal and food samples in collaboration with the National Antimicrobial Resistance Monitoring System (FSIS NARMS, n.d.). Within the U.S. Department of Health and Human Services, the U.S. Food and Drug Administration NARMS (FDA NARMS, n.d.) program studies pathogens and AMR in retail samples of meat products from poultry, swine, and cattle, the final stage in "farm to fork." While these AMR studies are in different populations and at different stages of livestock and poultry production, together they provide a national snapshot of AMR in food-producing animals and animal-derived foods in the U.S. Studies at the regional or local level help to assess and address changes in pathogens and AMR (EFSA et al., 2021; Herawati et al., 2023).

In the U.S., national level surveillance for zoonotic pathogens and AMR has historically focused on the major meat producing species of poultry, swine, and cattle. APHIS periodically studies zoonotic pathogens and AMR in fecal samples of goat, sheep, and lamb through NAHMS focusing on farm level production. Studies of the minor meat producing species of goat, sheep, and lamb at slaughter are limited, leaving a data gap in this area. Recognizing this data gap, in 2017, FDA's Science Board recommended that the microbial hazards of concern in these food-producing animals and their potential risk to human health and food safety be studied further (FDA, 2017). In February 2020, in collaboration with FDA, FSIS initiated the NARMS expansion surveillance projects. These included a study of AMR in *Salmonella*, *Campylobacter*, generic *Escherichia coli* (*E. coli*), and *Enterococcus* spp. isolated from cecal samples collected from goat, sheep, and lamb. This was the first nationwide AMR study in these minor species at slaughter.

Materials and Methods

Sampling Design

The FSIS Annual Sampling Plan (FSIS, 2024a) outlines the Agency's overall strategy for directing sampling resources in a given fiscal year. It identifies changes planned for various sampling programs and aligns goals and measures with sampling activities and results. The FSIS NARMS part of the sampling program is based on classes of animals slaughtered and annual slaughter volumes. For cecal sampling, FSIS

NARMS uses a statistical design based upon establishment slaughter volume and predicted positive rates to reach a target number of bacterial isolates. In this study, sampling task frequencies were assigned based upon 12 months of slaughter volume data for each class and included up to four samples per month for the establishments with the top 25% (≥ 75%) of slaughter volume, up to two per month for the next 25% (≥ 50% and < 75%), and up to one sample per month for the remaining 50% of eligible establishments (< 50%) (FSIS, 2024b). For this cross-sectional study, cecal samples were collected from goat, sheep, and lamb at FSIS-regulated establishments throughout the U.S. that slaughter at least 10 animals/year/slaughter class. Sampling occurred from February 2020 to September 2022. Due to COVID-19 pandemic disruptions to staffing availability, cecal samples were not collected in April, May, and part of June 2020. This study provided 1,025 goat, sheep, and lamb cecal samples collected from 449 FSIS-regulated establishments.

Bacterial Isolation and Confirmation

Samples were collected from the cecum (pl. ceca), a small blind pouch located at the intersection of the small and large intestine and sent to the FSIS Eastern Laboratory for microbiological analysis (FSIS, 2022b). The number of samples screened for each organism varied due to differences in expected recovery rates. Recovery and isolation of pathogens from cecal samples are described in the FSIS Microbiology Laboratory Guidebook Chapter 31 (FSIS, 2024c.) with a summary of methods used provided here. Cecal contents were enriched in Buffered Peptone Water (BPW) and incubated overnight. For *Salmonella*, enriched cecal samples were screened through a BAX® system real-time PCR Assay Kits (Dupont Nutrition and Health) and presumptive positives were carried forward to selective enrichment and plating media. For *Campylobacter*, enriched BPW was inoculated into double-strength Bolton enrichment broth, incubated, streaked to a Modified Charcoal-Cefoperazone-Deoxycholate Agar plate, and screened for typical colonies. For generic *E. coli*, an aliquot from BPW was streaked on Eosin Methylene Blue Agar media and screened for typical colonies. For *Enterococcus*, an aliquot of the enriched BPW was transferred into Enterococcusel™ broth, incubated, streaked to Enterococcosel™ agar, and screened for typical colonies. For each of the four enteric bacteria, a single presumptive positive isolate was streaked to Trypticase Soy Agar with 5% Sheep Blood plates and confirmed by Bruker® MALDI Biotyper. Bacterial isolates were further characterized for AMR.

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing was performed using the Clinical and Laboratory Standard Institute methods (CLSI, 2018 and 2020). Susceptibility testing was performed through broth microdilution (Sensititre System™, Thermo Fisher Scientific) using antibiotic panels CMV5AGNF for *Salmonella* and generic *E. coli*, CMVCAMPY for *Campylobacter* and CMV4AGP4 for *Enterococcus* that includes antimicrobial drugs selected based upon their importance in human and veterinary medicine. The interpretation of minimal inhibitory concentrations (MICs) was based upon the Clinical and Laboratory Standards Institute (CLSI) M100 (CLSI, 2020) clinical breakpoints. For ciprofloxacin, isolates with decreased susceptibility (MIC ≥ 0.12 μg/mL) were also included in total resistance calculations. For those without CLSI breakpoints, NARMS provisional cutoffs were used: streptomycin (generic *E. coli* and *Salmonella*, MIC ≥ 32 μg/mL), azithromycin (MIC ≥ 32 μg/mL), and tigecycline (MIC > 0.25 μg/mL). For *Campylobacter*, epidemiological cutoff values (ECOFFs) were based upon EUCAST recommendations (EUCAST, n.d.). The interpretive criteria used for susceptibility testing are in Appendix A, Tables A1-A3; susceptibility definitions are in Appendix B.

Statistical Analyses

Basic descriptive analyses, including contingency tables, simple proportions, pie charts and bar graphs, were used to portray the distribution of antimicrobial susceptibility detected for the four targeted bacteria (*Salmonella*, *Campylobacter*, generic *E. coli*, and *Enterococcus*) and their antimicrobial susceptibility patterns.

Results

Sample distribution based upon volume of slaughter facility

The distribution of samples collected by establishment slaughter volume is shown in Table 1. The distribution of collected samples based on the establishment's slaughter volume was 90% for the top 25%, 7% for the next 25%, and 3% for the bottom 50%. A total of 1,025 cecal samples were collected: 349 goat, 319 sheep, and 357 lamb samples.

Recovery of Bacteria

Cecal samples were screened for the microbes listed in Table 2 for goat, sheep and lamb. *Salmonella* was recovered at 12% (n=43) in goat, 34% (n=107) in sheep, and 21% (n=75) in lamb. A greater number of cecal samples were positive for *Campylobacter* than *Salmonella* with 26% (n=46) goat, 36% (n=58) sheep, and 38% (n=70) lamb samples being *Campylobacter* positive. Levels of generic *E. coli* and *Enterococcus* were high (≥ 78%).

Table 1. Distribution of number of samples and percent based on establishment slaughter volume, 2020-2022.

Slaughter volume	Number of establishments sampled[1]	Number of samples	Percentage of total
Top 25%	104	926	90%
Next 25%	56	73	7%
Bottom 50%	25	26	3%
Total	185	1,025	100%

[1] There are 27 establishments that are counted more than once because they were categorized differently based on commodity and year.

Table 2. Number of positive isolates per number of samples screened for each organism and slaughter class, 2020-2022.

Organism	Goat			Sheep			Lamb		
	No. of samples screened[1]	No. of positives	% positive	No. of samples screened[1]	No. of positives	% positive	No. of samples screened[1]	No. of positives	% positive
Salmonella	349	43	12%	319	107	34%	357	75	21%
Campylobacter	175	46	26%	159	58	36%	186	70	38%
Generic *E. coli*	103	84	82%	87	72	83%	105	90	86%
Enterococcus	98	79	81%	84	69	82%	100	78	78%

[1] Not all samples collected were screened for all organisms; hence, the number of samples screened vary. For generic *E. coli* and *Enterococcus*, lower number of samples were screened due to their high rate of recovery (percent positive) while recovery of *Salmonella* and *Campylobacter* was relatively lower.

Distribution of *Salmonella* Serotypes

The distribution and diversity of *Salmonella* serotypes by slaughter class is shown in Table 3 and Figure 1. Nine predominate serotypes (each comprising ≥2% and ≥3% of the total serotypes isolated in sheep and lamb, respectively) were recovered from sheep and lamb. *Salmonella* enterica subsp. diarizonae serotype IIIb 61:k:1,5,(7) (herein referred to as *Salmonella* serotype IIIb 61:k:1,5,(7)) was the most frequent serotype isolated (63% of *Salmonella* isolates, n=67) in sheep and (52% of *Salmonella* isolates, n=39) in lamb. Other serotypes were observed at lower levels: for sheep, Muenster (6%, n=6) and Typhimurium (4%, n=4), for lamb, Typhimurium (7%, n=5) and I 4,[5],12:i:- (5%, n=4). In total, the top three serotypes accounted for over half of the total number of *Salmonella* isolates, 73% in sheep and 64% in lamb.

Twelve predominant *Salmonella* serotypes (each comprising ≥5% of the total serotypes isolated) were recovered in goat cecal samples with the top three serotypes recovered being: Muenster (16%, n=7), Montevideo (9%, n=4), and Anatum (7%, n=3) that accounting for 32% of the total number of *Salmonella* isolates. The diversity and the distribution of *Salmonella* serotypes are shown in Figure 1.

Distribution of *Campylobacter* Species

The distribution of *Campylobacter* species by slaughter class is shown in Table 4. *C. coli* was the predominant species accounting for 65% (n=30) of *Campylobacter* isolates in goat, 69% (n=40) in sheep, and 53% (n=37) in lamb. *C. jejuni* was present in goat (35%, n=16) and sheep (31%, n=18) with a higher proportion of lamb samples 47% (n=33) tested having *C. jejuni* (Table 4).

Distribution of *Enterococcus* species

The distribution of *Enterococcus* species by slaughter class is shown in Table 5. The most frequent species observed among all slaughter classes was *Enterococcus* hirae with similar percentages: 47% (n=37) in goat, 42% (n=29) in sheep, and 49% (n=38) in lamb. *Enterococcus faecalis* ranked second in goat

Table 3. *Salmonella* serotype distribution for goat, sheep, and lamb, 2020-2022.

Goat (N=349)			Sheep (N=319)			Lamb (N=357)		
Serotype	**n**	**%**	**Serotype**	**n**	**%**	**Serotype**	**n**	**%**
Muenster	7	16%	IIIb 61:k:1,5,(7)	67	63%	IIIb 61:k:1,5,(7)	39	52%
Montevideo	4	9%	Muenster	6	6%	Typhimurium	5	7%
Anatum	3	7%	Typhimurium	4	4%	I 4,[5],12:i:-	4	5%
Infantis	3	7%	I 4,[5],12:i:-	4	4%	Anatum	2	3%
Typhimurium	2	5%	Anatum	3	3%	Altona	2	3%
Altona	2	5%	Montevideo	2	2%	Reading	2	3%
Bredeney	2	5%	Altona	2	2%	Muenchen	2	3%
Agona	2	5%	Muenchen	2	2%	Derby	2	3%
Panama	2	5%	Newport	2	2%	Chester	2	3%
Kiambu	2	5%	-	-	-	-	-	-
Kentucky	2	5%	-	-	-	-	-	-
Adelaide	2	5%	-	-	-	-	-	-
Others	10	23%	Others	15	14%	Others	15	20%
Total	43	100%	Total	107	100%	Total	75	100%

N = total number of samples screened, n= number of isolates, Others = include serotypes with a single occurrence

Figure 1. *Salmonella* serotype diversity for goat, sheep, and lamb, 2020-2022.

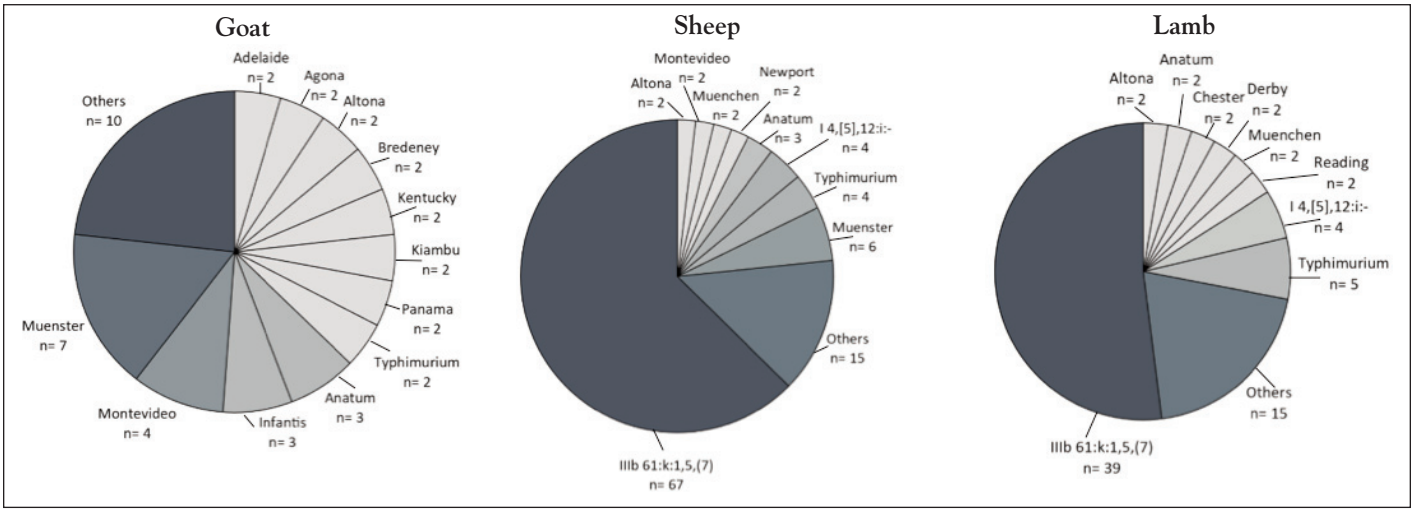

Table 4. *Campylobacter* species distribution for goat, sheep, and lamb, 2020-2022.

	Goat (N=175)		Sheep (N=159)		Lamb (N=186)	
***Campylobacter* Species**	**n**	**%**	**n**	**%**	**n**	**%**
coli	30	65%	40	69%	37	53%
jejuni	16	35%	18	31%	33	47%
Total	46	100%	58	100%	70	100%

N = total number of samples screened, n= number of isolates

(19%, n=15) and lamb (15%, n=12) and third in sheep (19%, n=13). *Enterococcus gallinarum* ranked second in sheep (23%, n=16) and third in goat (16%, n=13) and lamb (12%, n=9).

AMR in Microbes Recovered from Cecal Samples

The distribution of bacterial isolates and antimicrobial resistance for goat, sheep, and lamb are shown in Figure 2 and Table 6. Most *Salmonella* isolates from the three slaughter classes combined (91%, n=204) were pan-susceptible, with 8% (n=18) resistant to 1-2 classes of antimicrobials and 1% (n=3) showing multi-drug resistance (MDR). A similar trend was observed for pan-susceptible *Salmonella* isolates in individual slaughter classes: 88% (n=38) in goat, 93% (n=99) in sheep, and 89% (n=67) in lamb (Table 6). One MDR *Salmonella* isolate was found in sheep, two in lamb, and none in goat (Table 6).

In contrast to *Salmonella*, most (74%, n=128) *Campylobacter* isolates from goat, sheep, and lamb tested were resistant to 1-2 classes of antimicrobials while 23% (n=40) were pan-susceptible, and only 3% (n=6) were MDR (Figure 2). Resistance to 1-2 classes of antimicrobials among the individual slaughter classes was similar: 76% (n=35) in goat, 72% (n=42) in sheep, and 73% (n=51) in lamb (Table 6).

A majority (61%, n=150) of generic *E. coli* isolates were pan-susceptible for goat, sheep, and lamb combined, followed by 30% (n=73) of the isolates being resistant to 1-2 classes and 9% (n=23) being MDR (Figure 2). When generic *E. coli* was examined individually in goat, sheep, and lamb, pan-susceptibility was 62% (n=52) in goat, 63%

Table 5. *Enterococcus* species distribution for goat, sheep, and lamb, 2020-2022.

Goat (N=98)			Sheep (N=84)			Lamb (N=100)		
Species	**n**	**%**	**Species**	**n**	**%**	**Species**	**n**	**%**
faecalis	15	19%	gallinarum	16	23%	faecalis	12	15%
gallinarum	13	16%	faecalis	13	19%	gallinarum	9	12%
durans	7	9%	faecium	5	7%	faecium	7	9%
casseliflavus	3	4%	casseliflavus	3	4%	casseliflavus	6	8%
faecium	3	4%	mundtii	2	3%	durans	3	4%
mundtii	1	1%	durans	1	1%	mundtii	3	4%
Total	79	100%	Total	69	100%	Total	78	100%

N = total number of samples screened, n = number of isolates

Figure 2. Distribution of aggregated bacterial AMR categories for goat, sheep, and lamb combined, 2020-2022.

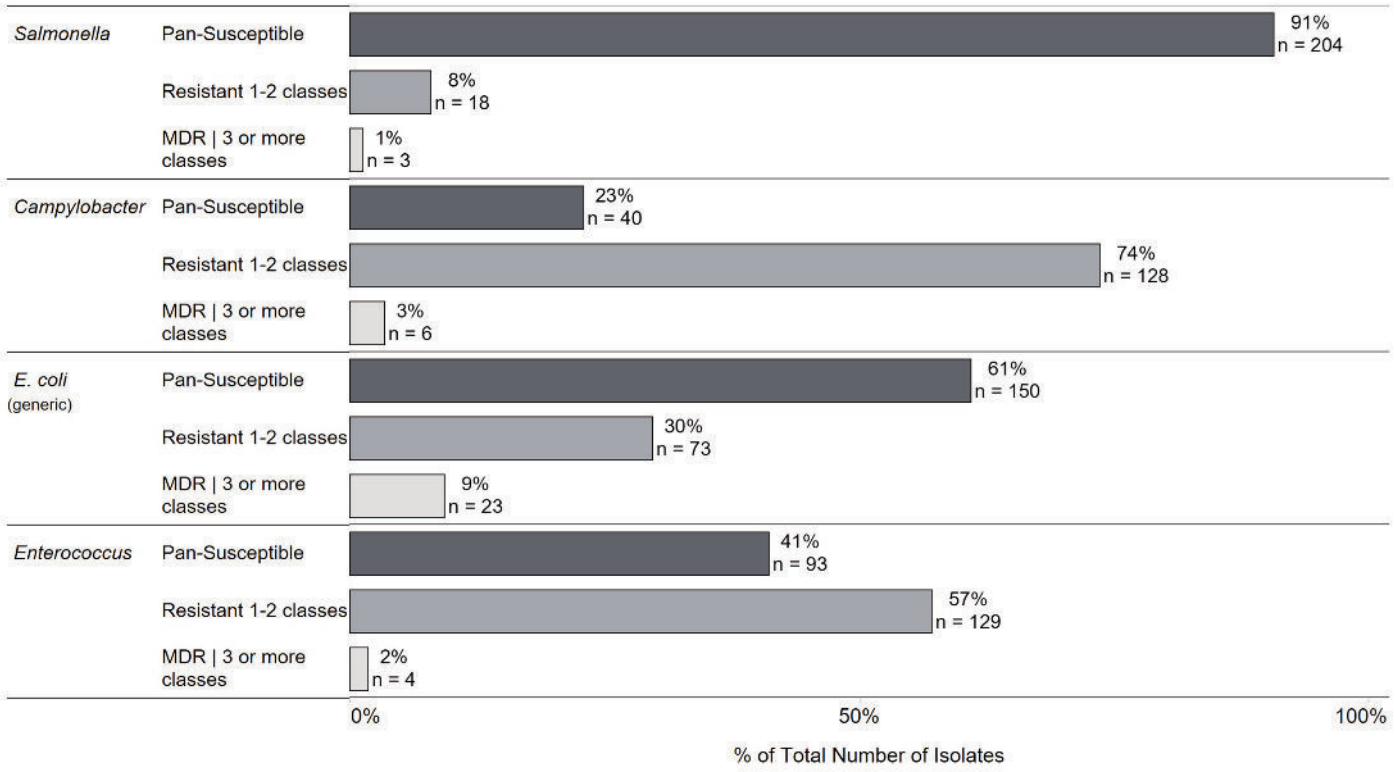

Table 6. Distribution of bacterial isolates by slaughter class and AMR category, 2020-2022.

	Goat (n=43)		Sheep (n=107)		Lamb (n=75)	
***Salmonella* (N=225)**	**n**	**%**	**n**	**%**	**n**	**%**
Pan-Susceptible	38	88%	99	93%	67	89%
Resistant 1-2 classes	5	12%	7	6%	6	8%
MDR (3 or more classes)	0	0%	1	1%	2	3%
	Goat (n=46)		**Sheep (n=58)**		**Lamb (n=70)**	
***Campylobacter* (N=174)**	**n**	**%**	**n**	**%**	**n**	**%**
Pan-Susceptible	8	17%	13	22%	19	27%
Resistant 1-2 classes	35	76%	42	72%	51	73%
MDR (3 or more classes)	3	7%	3	5%	0	0%
	Goat (n=84)		**Sheep (n=72)**		**Lamb (n=90)**	
Generic *E. coli* (N=246)	**n**	**%**	**n**	**%**	**n**	**%**
Pan-Susceptible	52	62%	45	63%	53	59%
Resistant 1-2 classes	22	26%	22	31%	29	32%
MDR (3 or more classes)	10	12%	5	7%	8	9%
	Goat (n=79)		**Sheep (n=69)**		**Lamb (n=78)**	
***Enterococcus* (N=226)**	**n**	**%**	**n**	**%**	**n**	**%**
Pan-Susceptible	36	46%	29	42%	28	36%
Resistant 1-2 classes	40	51%	39	57%	50	64%
MDR (3 or more classes)	3	4%	1	1%	0	0%

N= total number of isolates for slaughter classes combined; n = number of isolates in each slaughter class

(n=45) in sheep, and 59% (n=53) in lamb. For generic *E. coli*, resistance to 1 -2 antimicrobial drug classes was 26% (n=22) in goat, 31% (n=22) in sheep, and 32% (n=29) in lamb (Table 6). In goat, sheep, and lamb, MDR in generic *E. coli* was 12% (n=10), 7% (n=5) and 9% (n=8), respectively (Table 6).

Overall, 41% (n=93) of the *Enterococcus* isolates were pan-susceptible, 57% (n=129) were resistant to 1-2 classes, and 2% (n=4) of the *Enterococcus* isolates were MDR (Figure 2). The distribution of pan-susceptible *Enterococcus* isolates in goat, sheep, and lamb was 46% (n=36), 42% (n=29), and 36% (n=28), respectively. *Enterococcus* isolates resistant to 1-2 antibiotics were highest in lamb (64%, n=50), followed by sheep (57%, n=39) and goat (51%, n=40) (Table 6). Goat samples contained three *Enterococcus* isolates (4%) with MDR, while sheep had one MDR isolate (1%), and lamb had 0 MDR isolates (Table 6).

Antimicrobial susceptibility for bacterial isolates is shown in Table 7. Tetracycline resistance was found in 12% (n=5) of goat, 8% (n=8) of sheep, and 11% (n=8) of lamb *Salmonella* isolates.

Table 7. The number of bacterial isolates from goat, sheep, and lamb, and antimicrobial susceptibility testing results, 2020-2022.

Antimicrobial Class	Antimicrobial	*Salmonella*			*Campylobacter*			Generic *E. coli*			*Enterococcus*		
		Goat n=43	Sheep n=107	Lamb n=75	Goat n=46	Sheep n=58	Lamb n=70	Goat n=84	Sheep n=72	Lamb n=90	Goat n=79	Sheep n=69	Lamb n=78
Aminoglycosides	Gentamicin (C)	-	-	-	0	1	0	0	1	1	0	0	0
	Streptomycin (C)	-	-	-	-	-	-	-	-	-	2	1	0
β-Lactam/ β-Lactamase Inhibitor Combinations	Amoxicillin/ Clavulanic Acid (C)	0	0	0	-	-	-	0	0	0	-	-	-
Carbapenems	Meropenem (C)	0	0	0	2	1	0	0	0	0	-	-	-
Cephems	Cefoxitin (H)	0	0	0	-	-	-	0	0	0	-	-	-
	Ceftriaxone (C)	0	0	0	-	-	-	1	0	1	-	-	-
Folate Pathway Inhibitors	Sulfisoxazole (I)	3	3	3	-	-	-	19	7	14	-	-	-
	Trimethoprim/ Sulfamethoxazole (C)	0	1	0	-	-	-	4	1	0	-	-	-
Glycopeptides	Vancomycin (C)	-	-	-	-	-	-	-	-	-	0	0	0
Glycylcycline	Tigecycline (C)	-	-	-	-	-	-	-	-	-	0	0	0
Lincosamides	Clindamycin (H)	-	-	-	2	2	4	-	-	-	-	-	-
	Lincomycin (NC)	-	-	-				-	-	-	0	0	0
Lipopeptides	Daptomycin (C)	-	-	-				-	-	-	2	0	1
Macrolides	Azithromycin (C)	0	1	0	0	2	1	1	0	0	-	-	-
	Erythromycin (C)	-	-	-	0	1	0	-	-	-	4	0	1
Nitrofurans	Nitrofurantoin (H)	-	-	-	-	-	-	-	-	-	0	2	2
Orthosomycin	Avilamycin (NC)	-	-	-	-	-	-	-	-	-	0	0	0
Oxazolidinones	Linezolid (C)	-	-	-	-	-	-	-	-	-	0	0	0
Penicillins	Ampicillin (H)	0	1	2	-	-	-	7	3	6	0	0	0
Phenicols	Chloramphenicol (H)	0	1	0	-	-	-	9	4	8	1	0	0
	Florfenicol (H)	-	-	-	0	0	0	-	-	-	-	-	-
Polymyxin	Colistin (C)	0	0	0	-	-	-	0	0	0	-	-	-
Quinolones	Ciprofloxacin (C)	0	1	1	21	27	17	3	1	1	1	0	01
	Nalidixic Acid (C)	0	0	1	21	26	17	1	1	0	-	-	-
Streptogramins	Quinupristin/ Dalfoprisitn (H)	-	-	-	-	-	-	-	-	-	25	24	30
Tetracyclines	Tetracycline (H)	5	8	8	33	38	47	32	27	36	30	24	31

Note: Blank fields (no values) represent/denote antibiotics not tested for specific bacteria. FDA classifies antimicrobials into critically important (C), highly important (H), important (I) and not classified (NC) based on their human medical importance. See FDA's Guidance For Industry #152 for additional information. For ciprofloxacin resistance, isolates with decreased susceptibility (MIC > 0.12 μg/mL) were also included in total resistance calculations.

Tetracycline resistance was found in 72% (n=33) of goat, 66% (n=38) of sheep, and 67% (n=47) of lamb *Campylobacter* isolates; 38% (n=32) of goat, 38% (n=27) of sheep, and 40% (n=36) of lamb *E. coli* isolates; and 38% (n=30) of goat, 35% (n=24) of sheep, and 40% (n=31) of lamb *Enterococcus* isolates.

Campylobacter had the highest percent of isolates resistant to the quinolones with 46% (n=21) of goat, 47% (n=27) of sheep, and 24% (n=17) of lamb isolates resistant to ciprofloxacin and 46% (n=21) of goat, 45% (n=26) of sheep, and 24% (n=17) of lamb isolates resistant to nalidixic acid.

For generic *E. coli*, sulfisoxazole resistance was 23% (n=19) of goat, 10% (n=7) of sheep, and 16% (n=14) of lamb isolates. *Enterococcus* resistance to quinupristin/dalfopristin was observed at 32% (n=25) of goat, 35% (n=24) of sheep , and 38% (n=30) of lamb isolates.

Discussion

In the U.S., goat, sheep, and lamb are considered to be minor species of food-producing animals (New Animal Drugs for Minor Use and Minor Species, 2022). Globally, these animals are important sources of meat as well as milk and fiber. Minor species meat consumption varies greatly and is influenced by cultural, dietary, economic, social, and geographic factors (Mazinani, 2020). Given the importance of minor species to U.S. agriculture, APHIS periodically conducts voluntary on-farm national studies under the NAHMS program. These studies gather health and management related information including antimicrobial use (AMU). A proportion of studies include AMR testing from fecal samples collected from animals on the operations participating in the study (APHIS, 2023, 2024; Dargatz et al., 2015; Gensler et al., 2024). In addition, several regional or convenience AMU/AMR studies have been conducted with goat, sheep, and lamb (Atlaw et al., 2022; CDFA, 2019; Cheney et al., 2015; Roug et al., 2013; Xia et al., 2019). While the APHIS on-farm studies provide a national snapshot of AMR and the convenience studies do the same at a state or regional level, a representative national snapshot of AMR in goat, sheep, and lamb at slaughter or in retail meats in the U.S. was lacking. This FSIS NARMS study is the first of its kind to provide national AMR information from FSIS-regulated slaughter establishments for goat, sheep, and lamb. This paper examined cecal samples from federally regulated goat, sheep, and lamb slaughter establishments for *Salmonella*, *Campylobacter*, generic *E. coli*, and *Enterococcus* and associated AMR.

The FSIS cecal sampling program, administered under FSIS NARMS, provides a nationally representative means to monitor trends in AMR in pathogens (*Salmonella*, *Campylobacter*) and indicator organisms (generic *E. coli* and *Enterococcus* spp.). FSIS NARMS routinely includes major meat-producing animals (poultry, swine, and cattle). This study expanded the ability of FSIS to monitor minor meat-producing animals such as goat, sheep, and lamb for trends in AMR or pathogens at the point of slaughter.

Both NAHMS and NARMS studies gather AMR information from sheep and goats; however, each has its own method of collecting data, selecting animals, and testing samples that fits their respective purpose. NAHMS conducts nationally representative and voluntary on-farm studies examining animal health and management practices. Operations that complete two questionnaires and meet the size requirements are eligible to participate in the animal testing phase, during which up to 25 sheep or goats meeting specific age and breeding class requirements are sampled. The Goat 2019 Study was conducted in 24 states that represented 76% of U.S. goat operations with >5 adult goats and 80% of the adult goats in the U.S. (APHIS, 2019). The 2011 Sheep Study was conducted in 22 states that represented 86% of the U.S. ewe inventory and 70% of U.S. farms with ewes (APHIS, 2013). Only operations with 20 or more ewes on January 1, 2010 and that completed two questionnaires were eligible to participate in biologic collection. FSIS NARMS studies collect cecal samples at slaughter from establishments that slaughter at least 10 animals per slaughter class of goat, sheep, or lamb per year nationwide. The NAHMS and FSIS NARMS studies are representative and provide a means to monitor AMR trends in goat, sheep, and lamb on-farm and at slaughter.

CDC estimates that *Salmonella* causes about 1.35 million infections, 26,000 hospitalizations, and 420 deaths in the U.S. every year, with food identified as the source of most of these illnesses (CDC *Salmonella*). Although the number of reported *Salmonella* outbreaks and illnesses related to consumption of goat, mutton, and lamb meat is very low, additional work is needed to assess public health risks (CDC NORS). The FSIS NARMS expansion study found that *Salmonella* serotype IIIb 61:k:1,5,(7) accounted for over half of the isolates in sheep (63%) and lamb (52%). A 2011 NAHMS on farm sheep study collected 1,133 composite fecal samples (up to six animals per sample, five samples per farm), of which 370 (32.7%) were positive for *Salmonella*. The *Salmonella* serotype IIIb 61:-:1,5, [7] accounted for 94.6% of the isolates (APHIS, 2013). This serotype is thought to be host adapted to sheep. In other sheep studies, *Salmonella* serotype IIIb 61:k:1,5,(7) was found to cause chronic proliferative rhinitis (Lacasta et al., 2012; Meehan, 1992), orchitis and epididymitis (Ferreras et al., 2007), and stillbirths in sheep (Davies et al., 2001). *Salmonella* serotype IIIb 61:k:1,5,(7) has been found in higher numbers in sheep than goats (Alvseike and Skjerve, 2002; Bonke et al., 2012) and has differences in regional and seasonal prevalence (Davies et al., 2001). Although *Salmonella* IIIb 61:k:1,5,(7) infections in humans are not common, reports show that this serotype is capable of causing humans illness. *Salmonella* IIIb 61:k:1,5,(7) infections have been reported in individuals who traveled internationally (Hall, 1992), immunocompromised individuals (Hoag and Sessler, 2005), and individuals who have handled reptiles (CDC, 2003; Parihar, 2020). In 2009, there were 86 laboratory-confirmed *Salmonella* IIIb 61:k:1,5,(7) isolates from human sources reported to the CDC compared with over 30,000 subspecies enterica isolates (CDC, 2011).

Salmonella recovered from goats in the FSIS NARMS expansion study were more diverse than in lamb and sheep, with Muenster (16%), Montevideo (9%), Anatum (7%), and Infantis (7%) making up the top four serotypes. Similarly, the NAHMS goat 2019 study of 4,918 fecal samples from 332 farm operations in the U.S. showed a low prevalence of *Salmonella* (0.7%) and a broad range of serotypes in goat species. The

top five *Salmonella* serotypes in the NAHMS study included Bareilly, Uganda, Newport, Poona, and Manhattan (Hempstead, et. al., 2022). These findings differ from those of the FSIS NARMS expansion study. These differences may be due to multiple factors, including (but not limited to) sample selection, sample sources (fecal vs. cecal samples), and laboratory methodology.

The CDC estimates that 1.5 million people in the U.S. become ill from *Campylobacter* infection every year. (CDC, 2024). *Campylobacter jejuni* is one of the most common bacterial causes of human foodborne illness (UW Madison, 2015) and, according to the CDC, *C. jejuni* causes 90% of human cases of *Campylobacter* (CDC, 2024). Less common species, such as *C. coli*, *C. upsaliensis*, *C. fetus*, and *C. lari* also infect people. In this FSIS NARMS study, *C. coli* was found in the majority of goat, sheep, and lamb samples (>50% of the *Campylobacter* organisms isolated). In the 2011 APHIS NAHMS on-farm study, among the *Campylobacter* species isolated from sheep and lamb fecal samples, the predominant species was *C. jejuni* (APHIS, 2014). In one study of retail meat, *C. coli* was found to be the most prevalent *Campylobacter* isolated from goat meat (Rahimi et al., 2010), while other studies found *C. jejuni* to be more prevalent in sheep and goat meat (Gensler et al., 2024; Lazou, 2014; Mpalang et al., 2014; Scates et al., 2003). The differences in *Campylobacter* species (*C. coli*, *C. jejuni*) recovered in different studies are influenced by multiple factors as seen in *Salmonella*.

Generic *E. coli* and *Enterococcus* species are normal bacteria in the gastrointestinal tract. The NARMS program has historically used these bacteria as indicators to monitor for emerging trends in antimicrobial resistance in enteric bacteria. These bacteria have been found to play a role in the horizontal transfer and spread of antibiotic-resistant genes (ARG) and mobile elements, in both internal (intestinal) and shared external environments. The internal and external environments provide an opportunity for horizontal gene transfer where genetic determinants of AMR may be exchanged between commensals and opportunistic pathogens (Lerner et al., 2017). A cause-and-effect relationship between antimicrobial usage and AMR should not be automatically assumed since the transfer and spread of AMR may be mediated through mobile genetic elements; these may spread among microbial populations through triggers not directly related to antimicrobial use.

The inclusion of *Enterococcus* and generic *E. coli* testing in this study provides insight into the presence of AMR in goat, sheep, and lamb. These bacteria were isolated from >80% of cecal samples in all three classes of animals compared to 12% positive for *Salmonella* and 38% positive for *Campylobacter* and may provide insight into the presence of AMR.

Antimicrobial Resistance

Despite some gains in combating AMR, the CDC 2019 Antibiotic Resistance Threats Report (CDC, 2019) shows additional actions are needed to protect against AMR. There are over 2.8 million antibiotic-resistant human infections and 35,000 deaths attributed to antibiotic resistance each year. AMR is recognized as an increasing global public health threat. In this study, we found that there was a high proportion of pan-susceptibility among *Salmonella* (88% - 93%) and generic *E. coli* (60% - 63%) isolates from all three minor species. This proportion was lower among *Campylobacter* isolates (8% - 27%) and varied between goat (8%), sheep (22%), and lamb (27%), with a greater proportion of isolates (72%-76%) showing resistance to 1-2 antimicrobial drug classes. The proportion of AMR in *Enterococcus* isolates was somewhat evenly distributed across all three species between pan-susceptible (44%-51%) and resistant to 1-2 antimicrobial drug classes (48%-56%). The highest proportion of MDR was observed in *Campylobacter* (7%) and generic *E. coli* (8%) in goat cecal samples.

Resistance to tetracycline was the most common finding among the three minor species and among the four bacteria. Tetracycline resistance was higher in *Campylobacter*, generic *E. coli*, and *Enterococcus* isolates and lower in *Salmonella* isolates. Chopra and Roberts reported that increasing resistance to tetracycline was seen in a number of pathogenic, opportunistic, and commensal bacteria (Chopra and Roberts, 2001). The authors opined that this was mostly mediated by the genetic acquisition of tet genes and that this phenomenon followed the introduction of tetracyclines in the mid-20th century for clinical, veterinary, and agricultural use.

Resistance to the critically important quinolone antibiotics, ciprofloxacin and nalidixic acid, was observed in approximately half of the *Campylobacter* isolates from goat and sheep. In addition to tetracycline, resistance to other important antimicrobial drugs were seen, including chloramphenicol in *Salmonella* (sheep), generic *E. coli* (goat, sheep, and lamb), and *Enterococcus* (goat) and quinupristin/dalfopristin in *Enterococcus* (goat, sheep, and lamb). Whereas *Salmonella* showed resistance in 8% and MDR in 1% of isolates, generic *E. coli* showed resistance in 30% and MDR in 9% of isolates. *Campylobacter* and *Enterococcus* were similar to each other with 74% and 52% resistant and 3% and 0% MDR, respectively. Tetracycline resistance was high in all slaughter classes and *Campylobacter*, *Enterococcus* and generic *E. coli* showed higher levels of resistance to sulfisoxisole, penicillins and phenicols.

In the NAHMS goat 2019 study conducted by APHIS, 4,917 fecal samples were collected from 332 operations tested for *Salmonella* and AMR (Hempstead, et al., 2022). In this on farm study, fecal *Salmonella* prevalence was low (0.7%), and all the *Salmonella* tested were pan-susceptible. While *Campylobacter* and generic *E. coli* isolates showed varied degrees of pan-susceptibility (42.3% and 84.7%, respectively), the most frequent resistance seen in these organisms was to tetracycline (APHIS, 2023; Gensler et al., 2024).

In collaboration with the California Department of Agriculture goat operations in California were oversampled and thus represent a state-level subset of the NAHMS goat 2019 study operations (CDFA, 2019). Nearly 50 goat operations in California voluntarily submitted fecal samples for AST using a panel of drugs important to human health. In the study, fecal recoveries of *Salmonella* (2%) and *Campylobacter* (10%) were relatively low. Almost all the *Salmonella* isolates in these studies were pan-susceptible and only a few *Campylobacter* isolates exhibited resistance to ciprofloxacin and nalidixic acid, although these drugs are not used in goats. Compared to this study, we found 88% of *Salmonella* iso-

lates to be pan-susceptible and 17% of *Campylobacter* to be pan-susceptible. The difference in levels of pan-susceptible *Campylobacter* reflected an increase of isolates resistant to one or more classes of antibiotics (78%) and MDR (8%). Compared to the above study, 46% of *Campylobacter* showed resistance to the WHO highest priority critically important antimicrobials ciprofloxacin and nalidixic acid.

The differences in recovery of *Salmonella*, *Campylobacter*, *E. coli*, or *Enterococcus* among the different studies of minor species could be due to multiple factors. These include, but are not limited to, the points of sampling along the farm to fork continuum, type of sample (fecal vs. cecal samples), and the differences in testing methodologies.

Conclusion

The NARMS minor species cecal AMR study is the first study of its kind to address the AMR data gap at slaughter for goat, sheep, and lamb. Unlike the regional or convenience studies, this study provides a representative national snapshot of cecal AMR and enables comparisons between goat, sheep, and lamb at a national level. Although *Salmonella* and *Campylobacter* and their AMR can be major concerns in foods and food-producing animals, findings from this study indicate that in minor species, a vast majority of *Salmonella* and roughly half of the *Campylobacter* isolates were pan-susceptible. In addition, MDR in both *Salmonella* and *Campylobacter* was minimal to low. A notable resistance to quinolones (ciprofloxacin/nalidixic acid) in *Campylobacter* will need further study. Periodic monitoring of *Salmonella* serotypes in minor species is important to maintain awareness of AMR trends. From the AMR surveillance perspective, inclusion of generic *E. coli* and *Campylobacter* provide a good assessment of the potential for MDR and quinolone resistance. While this national cecal sample study of minor species helps to address the AMR data gap at the initial point of slaughter, a similar nationally representative study with minor species derived food products (meat) a similar nationally representative study with minor species derived food products (meat) would shed light on AMR in finished products.

Limitations of this cross-sectional study include the relatively small numbers of bacterial isolates recovered and AMR findings. Also, bacterial isolates and AMR from the final products or retail meats were not evaluated. A follow-up study of cecal and retail meats conducted in conjunction with an on-farm study may provide the opportunity to determine if findings from this study persist over time and if there is any association with the on farm and at retail findings. However, we acknowledge that differences between on farm, at slaughter, and at retail samples; the types and ages/stages of animals sampled; and the interim influences between animals on farm and animals at the end of the slaughter process may make these correlations challenging.

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Appendix A

Table A1. Interpretive Criteria Used for Susceptibility Testing of *Salmonella* and Generic *E. coli*.

			Breakpoints (µg/ml)		
Antimicrobial Class	**Antimicrobial Agent**	**Ranking[1]**	**Susceptible**	**Intermediate**	**Resistant**
Aminoglycosides	Gentamicin	C	≤ 4	8	≥ 16
Aminoglycosides	Streptomycin	C	≤ 16	8	≥ 16
β-Lactam/ β-Lactamase Inhibitor Combinations	Amoxicillin-Clavulanic Acid	C	≤ 8 / 4	N/A	≥ 32
Carbapenems	Meropenem	C	≤ 1	16 / 8	≥ 32 / 16
Cephems	Cefoxitin	H	≤ 8	2	≥ 4
Cephems	Ceftriaxone	C	≤ 1	16	≥ 32
Folate Pathway Inhibitors	Sulfisoxazole	I	≤ 256	2	≥ 4
Folate Pathway Inhibitors	Trimethoprim-Sulfamethoxazole	C	≤ 2 / 38	N/A	≥ 512
Macrolides	Azithromycin	C	≤ 16	N/A	≥ 4 / 76
Penicillins	Ampicillin	H	≤ 8	N/A	≥ 32
Phenicols	Chloramphenicol	H	≤ 8	16	≥ 32
Polymyxin	Colistin	C	N/A	16	≥ 32
Quinolones	Ciprofloxacin	C	≤ 0.06	≤ 2	≥ 4
Quinolones	Nalidixic acid	C	≤ 16	0.12-0.5	≥1
Tetracyclines	Tetracycline	H	≤ 4	N/A	≥ 32

[1] Ranking according to FDA's Guidance for Industry #152 (FDA, 2023): C - Critically important, H - Highly important, I - Important, NC - Not classified

Table A2. Interpretive Criteria Used for Susceptibility Testing of *Enterococcus*.

			Breakpoints (µg/ml)		
Antimicrobial Class	**Antimicrobial Agent**	**Ranking[1]**	**Susceptible**	**Intermediate**	**Resistant**
Aminoglycosides	Gentamicin	C	≤ 500	N/A	>500
	Streptomycin	C	≤ 512	N/A	≥ 1000
Glycopeptides	Vancomycin	C	≤ 4	8 -16	≥ 32
Glycylcycline	Tigecycline	C	≤ 0.25	N/A	≥ 0.5
Lipopeptides	Daptomycin (*E. faecium* only)	C	≤ 4	N/A	≥ 8
	Daptomycin (*Enterococcus* species other than *E. faecium*)	C	≤ 2	4	≥ 8
Macrolides	Erythromycin	C	≤ 0.5	1 - 4	≥ 8
Nitrofurans	Nitrofurantoin	H	≤ 32	64	≥ 128
Oxazolidinones	Linezolid	C	≤ 2	4	≥ 8
Orthosomycin	Avilamycin	NC	≤ 2	4	16
Penicillins	Ampicillin	H	≤ 8	N/A	≥ 16
Phenicols	Chloramphenicol	H	≤ 8	16	≥ 32
Quinolone	Ciprofloxacin	C	≤ 1	2	≥ 4
Streptogramins	Quinupristin/Dalfopristin	H	≤ 1	2	≥ 4
Tetracyclines	Tetracycline	H	≤ 4	8	≥ 16

[1] Ranking according to FDA's Guidance for Industry #152 (FDA, 2023). C - Critically important, H - Highly important, I - Important, NC - Not classified

Appendix B

The following categories were used to describe the susceptibility or resistance of enteric bacterial isolates to antimicrobial drug classes tested.

- *Pan-susceptible:* bacterial isolates that are susceptible to all antimicrobial drugs included in the NARMS testing panels.
- *Resistant 1-2 classes:* bacterial isolates resistant to antimicrobials in one or two drug classes.
- *Multi-Drug Resistant (MDR):* bacterial isolates resistant to antimicrobials in three or more drug classes.