Year 3 report: 2020

USDA APHIS VS National Animal Health Laboratory Network (NAHLN) Antimicrobial Resistance Pilot Project

Contents

Executive Summary

The National Animal Health Laboratory Network (NAHLN) antimicrobial resistance (AMR) pilot project aims to demonstrate the viability of implementing a sampling stream for monitoring AMR profiles in animal pathogens routinely isolated by U.S. veterinary clinics and diagnostic laboratories. The project provides AMR profiles for livestock/production and companion animals that are clinically ill, compared to other national surveillance programs that evaluate healthy animals.

This report describes AMR data collected and funded by the United States Department of Agriculture (USDA) during the third year of the pilot, January 1 – December 31, 2020. In 2020, 27 laboratories participated; 26 are NAHLN member laboratories and 1 is associated with a U.S. college of veterinary medicine. This is a 12.5% increase from the 24 laboratories enrolled during the second pilot year.

Bacterial isolates were selected to represent both pathogens of veterinary importance and zoonotic bacteria monitored through other national food-borne pathogen surveillance systems. Data from 5,396 isolates were submitted in 2020 despite the ongoing coronavirus pandemic. This represents a marginal increase of 0.43% in data collected over the previous pilot project year. Isolates surveyed in 2020 included:

- *Escherichia coli (E. coli)* – 2,749 isolates across all animal species
- *Salmonella enterica* spp. – 380 isolates from cattle
- *Mannheimia haemolytica (M. haemolytica)* – 566 isolates from cattle
- *Streptococcus suis (S. suis)* – 167 isolates from swine
- *Pasteurella multocida (P. multocida)* – 56 isolates from poultry (chicken and turkeys)
- *Streptococcus equi (S. equi)* – 75 isolates from horses
- *S. equi* ssp. *zooepidemicus (S. zooepidemicus)* – 369 isolates from horses
- *Staphylococcus intermedius* group – 1,034 isolates from dogs and cats

The project evaluated antimicrobial resistance for antibiotics with animal- and bacterial-specific breakpoints, as reported in *VET01S Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals, 5th edition* (The Clinical and Laboratory Standards Institute (CLSI), 2020).

Notable results from 2020 demonstrate that AMR in most production animals (swine, poultry, and horses) remains stable relative to submissions from previous years of the pilot project (2018 and 2019). In cattle, pan-susceptibility in *M. haemolytica* isolates was observed to be slightly lower than in previous years (63.3%, 358/566 isolates in 2020, compared to 69.4% in 2019 and 65.3% in 2018). We additionally observed a simultaneous increase in frequency of isolates (22.6%, 128/566) that were resistant to three or more classes of antimicrobials, thus meeting the multi-drug resistance (MDR) definition. This is an increase in MDR isolates recovered in 2018 (18.7%, 71/380) and 2019 (16.5%, 101/612),

Conversely, data from companion animal-sourced bacterial isolates shows a decreasing trend in AMR. More specifically, non-urinary tract (non-UTI)-associated canine *E. coli* resistance against gentamicin and fluoroquinolones showed the strongest overall decreasing trend (p = 0.077, pairwise t-tests for each individual drug across 2018 – 2020).

Introduction

Antimicrobial resistance remains one of the most serious global health threats to animals and humans. In 2015, the President of the United States released a National Action Plan for Combatting Antibiotic Resistant Bacteria (CARB), calling for collaborative action by the U.S. Government to strengthen our resources to address increasing AMR observed in both animals and humans.

The USDA continues to actively participate in CARB activities outlined in the National Action Plan for Combating Antibiotic-Resistant Bacteria (CARB), 2020-2025. This plan has four major goals: 1) prioritize infection prevention and control to slow the spread of resistant infections and reduce the need for antibiotic use; 2) support innovative approaches to developing and deploying diagnostic tests and treatment strategies; 3) expand efforts to understand antibiotic resistance in the environment; 4) focus on collecting and using data to better understand where resistance is occurring and support the development of new diagnostics and treatment options, and 5) to advance international coordination.

The NAHLN AMR pilot is aligned with CARB Goal 2 (Strengthen National One Health Surveillance Efforts to Combat Resistance), Objective 1.1 (Expand surveillance through existing systems to monitor antibiotic resistance from multiple sources across One Health) and provides information on AMR within sick animal populations. Third-year goals of the NAHLN AMR pilot project continue to be: monitor AMR profiles in animal pathogens routinely isolated by veterinary clinics and diagnostic laboratories across the U.S. for trends in antimicrobial resistance phenotypes and genotypes; identify new or emerging antimicrobial resistance profiles; help monitor the continued usefulness of antimicrobials over time; and provide information back to participating laboratories, other federal agencies, and industry stakeholders regarding these trends.

As in previous years, participating laboratories selected isolates obtained from routine clinical cases and performed antimicrobial susceptibility testing (AST) using a commercially available testing platform (Sensititre™). Laboratories then submitted the data to the NAHLN for monitoring. This report outlines the results of this monitoring.

In 2020, laboratories in the pilot were also invited for the first time to participate in whole genome sequencing and submit data for a subset of isolates they provided AST data on. This data will be reported separately.

Materials and Methods

Laboratory enrollment

For the third year of the NAHLN AMR pilot project, 27 laboratories were enrolled from the following states: Alabama, California, Colorado, Florida, Georgia, Indiana, Iowa, Kansas, Kentucky, Louisiana, Michigan, Minnesota, Missouri, Mississippi, Nebraska, New York, North Dakota, Ohio, Pennsylvania, South Dakota, Tennessee, Texas, Washington, and Wisconsin (Figure 1). Twenty-six of these laboratories are State or University-associated veterinary diagnostic laboratories with membership in the NAHLN; one laboratory was not a NAHLN member but is associated with a U.S. college of veterinary medicine.

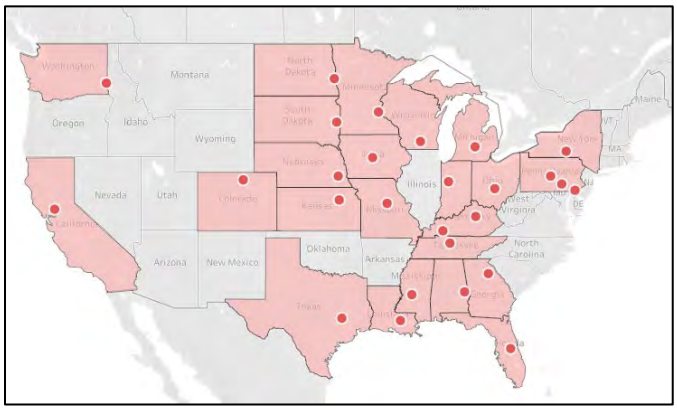


Figure 1. Geographic distribution of participating laboratories (points) in the contiguous U.S. (lower 48 states) for the 2020 NAHLN AMR pilot project. States of participating laboratories are shaded in red.

Pathogens monitored

Pathogens monitored for 2020 remained the same from 2019 (Table 1). As with prior years, participating laboratories were instructed to select isolates for inclusion in the AMR pilot project based on the following criteria: i) identification to the genus and species level (and serotype level for *S. enterica*) using commonly accepted veterinary microbiology laboratory techniques, ii) association with clinical disease or diagnostic findings, and iii) association with unique animal sources (no more than one isolate from the same herd/flock, farm/household, or owner). Additionally, to minimize local or regional bias in the aggregate dataset, laboratories were asked to submit data from no more than 40 isolates for each pathogen-host category, except for the *E. coli, M. haemolytica,* and *S. intermedius* group categories, which were capped at 65 isolates each per laboratory.

Table 1. Pathogen/animal species and number of categories for monitoring.

Bacterial pathogen	Host animal species
Escherichia coli	cattle, swine, poultry, horses, dogs, cats
Mannheimia haemolytica	cattle
Pasteurella multocida	poultry (chicken, ducks, turkeys)
Salmonella enterica	cattle
Staphylococcus intermedius group*	dogs, cats
Streptococcus equi	horses
Streptococcus equi ssp. *zooepidemicus***	horses, swine, dogs
Streptococcus suis	swine

* Includes *S. intermedius, S. pseudintermedius* and *S. delphini*.

** Isolates from companion animals (cats and dogs) were also collected as part of a larger surveillance project.

Antimicrobial Susceptibility Testing and Reporting

AST was conducted as in previous AMR pilot project years using the Sensititre™ broth microdilution platform (Thermo Fisher Scientific, Waltham, MA) and commercially available Sensititre™ microdilution plates according to manufacturer's instructions. Plate usage was based on bacterial pathogen and host animal species (Table 2).

Table 2. Sensititre™ plates used to identify antimicrobial susceptibility testing in each bacterial pathogen-host animal species for Year 3 of the AMR pilot project.

Pathogen \ Host	Cattle	Swine	Poultry	Horses	Cats	Dogs
E. coli	BoPo6F or BoPo7F	BoPo6F or BoPo7F	Avian1F	Equin1F or Equin2F	CompGN1F	CompGN1F
S. enterica	BoPo6F or BoPo7F	*N/A**	*N/A*	*N/A*	*N/A*	*N/A*
M. haemolytica	BoPo6F or BoPo7F	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
P. multocida	*N/A*	*N/A*	Avian1F	*N/A*	*N/A*	*N/A*
S. intermedius group	*N/A*	*N/A*	*N/A*	*N/A*	CompGP1F	CompGP1F
S. suis	*N/A*	BoPo6F or BoPo7F	*N/A*	*N/A*	*N/A*	*N/A*
S. equi	*N/A*	*N/A*	*N/A*	Equin1F or Equin2F	*N/A*	*N/A*
S. zooepidemicus	*N/A*	BoPo6F or BoPo7F	*N/A*	Equin1F or Equin2F	*N/A*	*N/A*

* *N/A* = not applicable

Minimum inhibitory concentration (MIC) data were compiled across all participating laboratories for each bacterial pathogen-host animal combination monitored. MIC data are provided for all antimicrobials present on the commercial plates used in this pilot, regardless of whether there is a label or indication for therapeutic, preventative, or control type of use. Susceptible, intermediate, and resistant interpretations are only provided for those antimicrobials with established pathogen-specific and host animal-specific clinical breakpoints according to *VET01S* standards (CLSI, 2020).

E. coli and *S. intermedius* group isolates from companion animals (dogs and cats) were differentiated into isolates cultured from UTIs and all other, non-UTI sources (e.g., skin, soft tissue, or other body sites) to more accurately apply MIC breakpoint interpretations to the standards set forth in the *VET01S* (CLSI, 2020; Figure 2). *S. intermedius* group isolates were further separated into oxacillin-sensitive (OX^S) and oxacillin-resistant (OX^R) groups based on human-derived breakpoints (Figure 2). This distinction was made to identify isolates potentially carrying methicillin-resistant genetic elements, thus rendering isolates resistant to additional β-lactam antimicrobials, including penicillins and extended spectrum β-lactam cephalosporins.

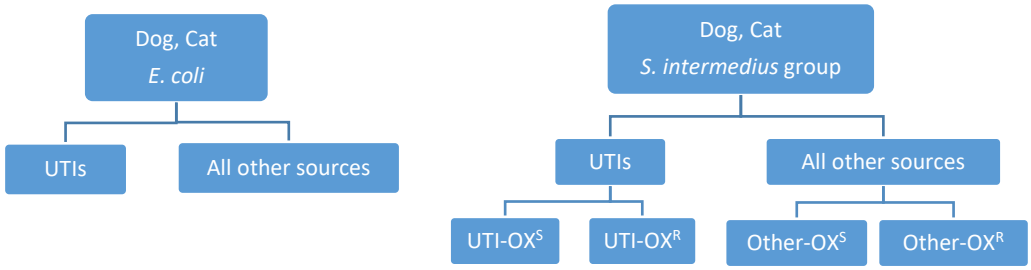


Figure 2. Breakdown of companion animal (dog and cat) bacterial isolates selected for antimicrobial susceptibility testing (AST). *S. intermedius* group isolates sourced from urinary tract infections were further separated into oxacillin-sensitive (OxS) and oxacillin-resistant (OxR) groups based on human-derived breakpoints.

Epidemiological data reported

Participating laboratories were required to assign a unique identifier to each isolate to eliminate all personally identifiable information associated with a sample submitted to NAHLN. Additional descriptive and epidemiological information reported for each isolate included the following:

- Purpose of submission (e.g., general diagnostic)
- Bacterial organism (genus, species, and serotype)
- Date of isolation
- Host animal (common and scientific genus-species names)
- Host animal state of origin
- Isolate sampling source (specimen/source tissue, e.g., oropharyngeal swab or feces)
- Final case diagnosis or results

Whole genome sequencing data

Participating laboratories were provided the opportunity to sequence isolates used for the pilot project in 2020 and submit sequencing data to National Veterinary Services Laboratories (NVSL) as part of the pilot. There were 16 laboratories enrolled in the sequencing portion of the pilot, but competing priorities resulting from the global SARS-CoV-2 (COVID-19) pandemic limited participation in 2020 to 13 laboratories, who sequenced a total of 192 isolates. An additional 956 isolates were sequenced at NVSL, resulting in approximately 21.3% of all isolates being sequenced and analyzed for the presence of antimicrobial resistance genes.

Results

The data provided in this report represent isolates recovered from routine diagnostic samples submitted to participating laboratories between January 1 and December 31, 2020. The NAHLN received data submissions from 5,396 isolates in 2020, a slight increase of 0.43% compared to the previous AMR pilot project year total of 5,373 submissions. Although each participating laboratory could submit up to 720 isolates across all 16 bacterial pathogen-animal host species categories, several factors could impact the number of data points submitted to the NAHLN. These include but are not limited to i) regional differences in animal populations, ii) availability of resources, and iii) variability in annual case load. Additionally, the ongoing COVID-19 pandemic presented a wide variety of challenges to participating laboratories, including disruption of laboratory supply chains, staffing, and other resource limitations.

The pilot project goal of 200 isolates per bacterial pathogen-host animal category was met or exceeded in 2020 for all bovine and canine isolates, poultry *E. coli*, equine *E. coli* and *S. zooepidemicus* isolates, and feline *E. coli* isolates (Figure 3). Of the 27 laboratories participating in the 2020 AMR pilot project, eight laboratories met or exceeded individual goals for isolate submissions; an additional six laboratories met 90% of their estimated annual submission goals. Participating laboratories averaged 208 isolate submissions in 2020 within a range of 47 to 384 submissions from any given group, representing a sustained increase over the first pilot project year in tandem with increased enrollment. As we had observed in previous AMR pilot project years, fewer than 100 submissions of the following isolates were received in 2020: poultry *P. multocida*, equine *S. equi*, and feline *S. intermedius* group (Figure 3).

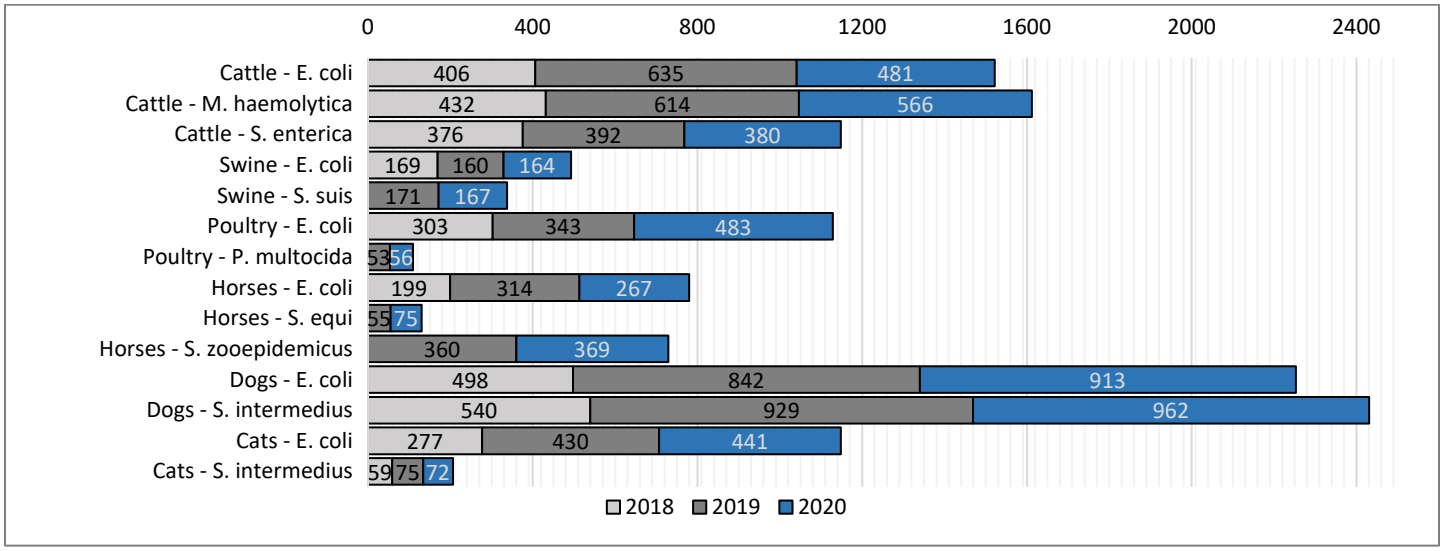


Figure 3. Overall numbers of isolates submitted each year of the pilot project by participating laboratories to the NAHLN AMR Pilot Project, by host animal and bacterial pathogen category.

Continual updates to the list of participating laboratories, MIC data, and antimicrobial sensitivity (sensitive-intermediate-resistant, or SIR tables) for the AMR pilot project can be found at the USDA APHIS | Antimicrobial Resistance (AMR) Dashboard.

Cattle

Information on production type (dairy, beef) and age was not collected from participating laboratories by the NAHLN. Aggregate data on MICs represent antimicrobial agents found on both the BoPo6F and BoPo7F plates and result in differing isolate numbers for specific antibiotics.

Cattle – *Escherichia coli*

The AMR pilot project year 3 dataset represents 481 *E. coli* isolates, a 24.3% decrease from the previous year but an 18.3% increase from the first year (Figure 3). Ampicillin and ceftiofur are the only two antimicrobial agents with clinical breakpoints for *E. coli* in cattle, both for treating specific clinical indications (ampicillin for metritis; ceftiofur for mastitis). In 2020, there were five isolates associated with mastitis and no isolates associated with cases of metritis. None of the five mastitis isolates were resistant to ceftiofur, and comparisons to previous years of AMR pilot data were not conducted as a direct result. All MIC data for bovine *E. coli* isolates are in Table 3, Appendix A.

Trends for clinical signs or indications associated with bovine *E. coli* infections are shown in Table 4, Appendix A. Diarrhea/gastroenteric infections were again the majority (46.6%), followed by sepsis/septicemia (16.2%), and colibacillosis (10.0%) as well as pneumonia/respiratory infections (10.0%).

Cattle – *Salmonella enterica*

The AMR pilot project year 3 dataset represent 380 *Salmonella enterica* isolates, for which the MIC data is reported in Table 5, Appendix A. As in previous years, the most frequently identified *S. enterica* serotypes were Dublin, Cerro, Montevideo, and Typhimurium. These serotypes represented 64.5% of the dataset. Because many of the serotypes are infrequently reported, a closer look at the 15 most frequently isolated serotypes from 2018 – 2020 are shown in Figure 4, Appendix A. A full list of all serotypes recovered for 2020 is provided in Table 6, Appendix A.

Cattle – *Mannheimia haemolytica*

Data for 566 isolates were submitted in 2020, representing a 49.3% increase over the first pilot project year and a marginal decrease of 7.5% in submissions from 2019. As expected, all isolates were associated with pneumonia or respiratory disease. Of these, 63.3% (358/566) were pan-susceptible, slightly lower than both previous pilot project years in which the pan-susceptible percentages were 69.4% and 65.3% for 2019 and 2018, respectively. MIC values for all antimicrobials and antibiotic classes are shown in Table 7, Appendix A.

Resistance to individual antimicrobial agents remained stable relative to submissions from 2018 and 2019 (Figure 5, Appendix A), with resistance against chlortetracycline, oxytetracycline, and tetracycline trending towards an increase year over year.

In 2020, 8.3% (47/566) of the isolates were resistant to only one antimicrobial, and 2.6% (15/566) were resistant to two antimicrobials. Of the remaining isolates, 22.6% (128/566) were resistant to three or more classes of antimicrobials, thus meeting the MDR definition. This is an increase in MDR isolates compared to both 2018 (18.7%, 71/380) and 2019 (16.5%, 101/612). It should be noted that changes in number of isolates recovered each year and changes in participating laboratories using the BoPo6 and BoPo7 plates for phenotypic AST did occur, and thus the increase in the % MDR across 2018-2020 is most likely due to changes in data reporting versus a true increase in drug resistance. Fourteen of the 128 *M. haemolytica* isolates (10.9%) classified as MDR from 2020 were resistant to 11 of the 12 antimicrobials with bovine breakpoints, compared to 2019, where isolates resistant to 11 antimicrobials comprised 16.4% of the total. Table 8, Appendix A provides the complete analysis of antimicrobial resistance for the bovine *M. haemolytica* isolates.

Swine

As with the cattle isolates, swine MIC tables represent antimicrobial test results aggregated from both the BoPo6F and BoPo7F plates. Thus, total isolate numbers may differ between antimicrobials.

Swine – *Escherichia coli*

In 2020, AST profiles for 164 *E. coli* isolates recovered from swine samples were submitted, representing an increase of 5.1% and 14.7% when compared to submissions from 2019 and 2018, respectively. The MIC data for these isolates is shown in Table 9, Appendix B. As in previous pilot project years, most isolates were associated with diarrhea/gastroenteric infection (59.8%). Mixed or secondary infections were also closely associated with porcine *E. coli* infections in 2020 at a case prevalence of 10.4%, followed by pneumonia/respiratory infections at 7.9%. A complete list of clinical signs and diagnoses associated with porcine *E. coli* infections can be found in Table 10, Appendix B.

Swine – *Streptococcus suis*

Antimicrobial sensitivity test profiles for a total of 167 *S. suis* isolates were submitted in 2020. Corresponding MIC values for all antimicrobials are in Table 11, Appendix B. Pneumonia and other respiratory diseases accounted for 49.1% of all diagnoses reported for *S. suis* isolate submissions. Final diagnoses of central nervous system infection (e.g., meningitis and encephalitis) and sepsis/septicemia were the second and third most common at 15.6% and 14.4%, respectively. These findings are similar to the diagnostic prevalence rates among *S. suis* isolates recovered from swine in 2019. Additional diagnoses are presented in Table 12, Appendix B.

A retrospective AMR analysis of *S. suis* isolates submitted to the NAHLN for this pilot project (Figure 6, Appendix B) demonstrates that there are no significant changes in phenotypic resistance observed in 2020 isolates against 2019. Among all *S. suis* isolates recovered from swine in 2020, 6.6% (11/167) of the isolates were susceptible to all antimicrobials tested with SIR breakpoints established by the *Vet01S* (Table 13, Appendix B; CLSI, 2020). Resistance to at least one antimicrobial was observed in 51.5% (86/167) of the isolates at nearly the same rate as samples recovered in 2019. Another 33.5% (56/167) of isolates were resistant to the two tetracycline derivates with established SIR breakpoints, namely chlortetracycline and oxytetracycline. Nine isolates (5.4%, 9/167) recovered in 2020 were classified as phenotypically MDR, representing a non-significant increase over four *S. suis* isolates similarly characterized as MDR in 2019 ($p = 0.26$, OR = 2.32, Fisher's exact test). All nine MDR *S. suis* isolates were found to carry phenotypic resistance against tetracyclines, 3rd generation cephalosporins, and penicillins. Four of the nine MDR *S. suis* isolates additionally carried resistance against fluoroquinolones (enrofloxacin), and one isolate was found to be resistant against phenicols (florfenicol).

Poultry

The NAHLN AMR pilot project monitored *E. coli* and *P. multocida* isolates recovered from domestic chickens and turkeys in 2020 for AMR phenotypes against antibiotics on the commercially available Avian1F broth microdilution plate. Only SIR breakpoints for enrofloxacin have been established in *E. coli* isolates from poultry, but approval for use of enrofloxacin in all poultry was withdrawn by the FDA in 2005. As in previous years, MIC data are provided for all antimicrobials on these plates regardless of therapeutic use.

Poultry – *Escherichia coli*

Data from 483 poultry *E. coli* isolates (326 from chickens, 157 from turkeys) was submitted in 2020, representing an increase of 29.1% from laboratory submissions in 2019 and 77.6% in 2018. MIC data is presented as aggregate data for all *E. coli* isolates recovered from chickens and turkeys (Table 14, Appendix C), from chickens alone (Table 15, Appendix C), and from turkeys alone (Table 16, Appendix C). Antimicrobial resistance to enrofloxacin remained steady at 1.7% (8/483), compared to relative rates of resistance in 2019 (1.9%, 7/374) and 2018 (1.1%, 3/272).

All diagnoses associated with poultry *E. coli* infections are provided in Table 17, Appendix C. For chickens, the top three clinical signs/indications were sepsis/septicemia (19.9%, 65/326), reproductive tract infections (18.0%,

59/326) and equal numbers of peritonitis and liver/kidney/spleen infections (9.8%, 32/326 each respectively). For turkeys, the top three clinical signs/indications were pneumonia/respiratory infections (30.6%%, 48/157), general health concerns (21.7%, 34/157), and sepsis/septicemia (11.5%, 18/157).

Poultry – *Pasteurella multocida*

A total of 56 isolates (41 from chickens, 15 from turkeys) were submitted by participating laboratories in 2020. MIC data is presented as aggregate data for all *P. multocida* isolates recovered from chickens and turkeys in Table 18, Appendix C, from chickens alone (Table 19, Appendix C), and from turkeys alone (Table 20, Appendix C).

The most common clinical signs/indications associated with *P. multocida* isolates recovered from poultry in 2020 remained consistent with results from previous years of the pilot (Table 21, Appendix C). The most common diagnoses in chickens were fowl cholera (36.6%, 15/41) and sepsis/septicemia (26.8%, 11/41). The most common diagnoses in turkeys were fowl cholera (40%, 6/15) and reproductive tract infections (26.7%, 4/15).

Horses

Doxycycline and enrofloxacin breakpoint interpretive values in the *Vet01S* (CLSI, 2020) for *E. coli*, *S. equi, and S. zooepidemicus* isolates recovered from horses are: susceptible (S) ≤0.12 μg/ml; intermediate (I) =0.25 μg/ml; and resistant (R) ≥0.5 μg/ml (CLSI, 2020). However, the doxycycline dilutions on the Equin1F plate range from 2 to 16 μg/ml whereas dilutions on the Equin2F plate range from 0.12 to 8 μg/ml. Therefore, isolates reported with a MIC readout of ≤2 μg/ml on the Equin1F plate are unable to be interpreted as either sensitive or intermediate against doxycycline. Similarly, enrofloxacin dilutions on the Equin1F plate range from 0.25 to 2 μg/ml and only those isolates with a MIC value at or above 0.5 μg/ml can be interpreted as resistant. Isolates reported with a MIC readout of ≤0.25 μg/ml are unable to be interpreted as either sensitive or intermediate as a direct consequence.

Additionally, separate breakpoints have been established for adult animals and foals for amikacin; all information provided in Appendix D is based on adult breakpoints. Participating laboratories also incorporated the antimicrobial sensitivity test against minocycline, which is exclusively found on the Equin2F plate, in 2020. As with the other animal species, summary MIC data is given for all antimicrobials found on the equine (Equin1F and Equin2F) AST plates, regardless of therapeutic use.

Horses – *Escherichia coli*

AST results and MIC data from 267 equine isolates were submitted in 2020, representing an 11.6% decrease in submissions from 2019 and an increase of 41.3% over the first pilot project year (Table 22, Appendix D). Seven antimicrobials have breakpoints established for *E. coli* from horses: amikacin, gentamicin, cefazolin, enrofloxacin, ampicillin, doxycycline, and minocycline. The updated *Vet01S* (CLSI, 2020) reference included newly released breakpoints for ampicillin (S ≤0.25 μg/ml, I =0.5 μg/ml, and R ≥1 μg/ml) and cefazolin (S ≤2 μg/ml, I =4 μg/ml, R ≥8 μg/ml). The addition of ampicillin breakpoints resulted in the inclusion of several counts of MDR isolates in 2020, thus accounting for the large increase observed from 2019 to this year and rendering comparisons over time non-equivalent.

Resistance to individual antimicrobial agents remained stable relative to submissions from 2018 and 2019, with resistance against doxycycline trending towards an increase year over year (Figure 7, Appendix D). While doxycycline is present on the Equin1F and 2F plates, the lack of lower MIC dilutions present on the Equin1F plate leads to more samples being categorized as NI (no interpretation) rather than S, I, or R. In contrast, the Equin2F plate does have low-MIC dilutions that facilitate precise SIR interpretations. The increase in doxycycline observed is therefore likely due to the increase in utilization of the Equin2F plate among participating laboratories, rather than a true increase in doxycycline resistance among equine *E. coli* isolates.

As in previous years, reproductive tract infections accounted for the vast majority (51.3%, 137/267) of all *E. coli* infections identified in 2020, followed by abscess/skin/wound infections (15.0%, 40/267). Table 23, Appendix D contains more information on types of infections associated with *E. coli* isolates recovered from horses. In 2020, 56% (150/267) of the isolates were resistant to only one antimicrobial, and 22% (60/267) were resistant to two

antimicrobials (Table 24, Appendix D). Of the remaining isolates, 21% (55/267) were resistant to three or more classes of antimicrobials, thus meeting the MDR definition.

Horses – *Streptococcus equi* subsp. *equi* and *Streptococcus equi* subsp. *zooepidemicus*

In 2020, participating laboratories submitted AST data from 75 *S. equi* isolates recovered from horses (Table 25, Appendix D). For *S. zooepidemicus* and *S. equi*, there are seven antimicrobials with breakpoints established in horses. These antimicrobials are amikacin, cefazolin, enrofloxacin, ampicillin, penicillin, doxycycline, and minocycline; the *Vet01S* (CLSI, 2020) additionally establishes interpretive breakpoints for ceftiofur in *S. zooepidemicus* isolates from horses Ampicillin breakpoints for I and R interpretations have not been established in horses. As a direct result, only MIC values obtained from five antimicrobials—amikacin, enrofloxacin, penicillin, doxycycline, and minocycline—could potentially be interpreted as resistant. All SIR interpretations and MDR tallies of *S. equi* isolates recovered from horses reported in Appendix D are based on MIC breakpoints in the *Vet01S* (CLSI, 2020).

Levels of AMR in *S. equi* isolates recovered from horses remained high against amikacin (78.7%, 59/75 isolates) and enrofloxacin (92.0%, 69/75 isolates; Figure 8, Appendix D). Resistance to doxycycline was very low in comparison to the previous year of the pilot project at 2.6% (2/75 isolates), and resistance to penicillin was observed to rise (from 1.8% in 2019 to 5.3% in 2020, representing 4/75 isolates). As we previously noted for equine *E. coli* isolates, the lack of lower MIC dilutions on the Equin1F plate for cefazolin, enrofloxacin, and doxycycline led to a significant proportion of samples being categorized as NI rather than S, I, or R. In addition, only MIC readouts from the Equin2F plate would lead to any SIR interpretations. As a result, interpretations of the same data reported in 2019 (where MIC values $\leq$2 μg/mL for doxycycline were incorrectly interpreted as resistant) are markedly different for cefazolin, enrofloxacin, and doxycycline in this report.

Clinical signs and diagnoses associated with *S. equi* infections in horses are provided in Table 26, Appendix D. Pneumonia/respiratory infections represented the majority (65.3%) of all cases, and together with abscess/skin/wound infections accounted for 89.3% (67/75) of all cases.

Participating laboratories submitted AST data from 369 *S. zooepidemicus* isolates; corresponding MIC data can be found in Table 27, Appendix D. *Streptococcus zooepidemicus* isolates showed high levels of resistance against amikacin (84.3%, 311/369 isolates) and enrofloxacin (96.5%, 356/369 isolates), and no significant change in resistance against doxycycline (18.4%, 68/369 isolates). Minocycline resistance was observed in isolates tested on the Equin2F platform for the first time in this pilot project at 25.0% (9/36 isolates). Additionally, cefazolin and penicillin resistance continued to remain present in low frequencies at 1.9% (7/369 isolates) and 2.4% (9/369 isolates), respectively (Figure 9, Appendix D).

Clinical signs and diagnoses associated with *S. zooepidemicus* isolates recovered from horses are provided in Table 28, Appendix D. Reproductive tract, pneumonia/respiratory, and abscess/skin/wound infections continued to account for almost 75% of all cases at 31.2%, 29.0%, and 14.6%, respectively.

In 2020, 11.9% (44/369) of the isolates were resistant to only one antimicrobial, namely enrofloxacin, and 70.5% (260/369) were resistant to two antimicrobials (Table 29, Appendix D). Of the remaining isolates, 16.5% (61/369) were resistant to three or more classes of antimicrobials, thus meeting the MDR definition. Prevalence of MDR isolates was not reported in previous pilot project years.

Dogs

Escherichia coli and *Staphylococcus intermedius* group isolates continued to be monitored in dogs for 2020. The data reported here are split into two categories per bacterial pathogen by the source of infection, namely UTIs and non-UTIs.

Dogs – *Escherichia coli*

For 2020, AST data from 913 *E. coli* isolates recovered from dogs were submitted, representing a sustained increase of 8.4% over submissions from 2019 (*n* = 842) and 98.9% over submissions from 2018 (n = 459; Figure 3).

Isolates were split into those recovered from UTIs (*n* = 590; Table 30, Appendix E), an increase of 8.3% over 2019 and 89.1% over 2018, and those associated with all other (non-UTI) infections (*n* = 323; Table 32, Appendix E), an increase of 9.5% over 2019 and 73.7% over 2018.

As in previous years, extended spectrum β-lactamase (ESBL) production for *E. coli* isolates with MIC values ≥8 μg/mL for cefpodoxime or ≥2 μg/mL for ceftazidime are identified in Table 30, Appendix E.

Dogs – *E. coli* – urinary tract infections

The number of isolates that met the criteria for ESBL screening based on cefpodoxime and ceftazidime MIC values was similar to 2019, with 84 isolates meeting the criteria of > 8 ug/ml for cefpodoxime and 60 isolates with >2 ug/ml for ceftazidime in 2020. These represent moderate increases of 6.3% (77) ESBL screens for cefpodoxime and 1.7% (61) for ceftazidime, respectively, from 2019.

While there appears to be a bimodal distribution of MIC values for enrofloxacin and marbofloxacin suggesting the presence of antimicrobial resistance genetic determinants (Table 30, Appendix E), overall prevalence of resistant isolates in the pilot project over the past three years (Figure 10, Appendix E) show a decreasing trend. Evaluation of whole genome sequencing data for these bacterial populations may help resolve this discrepancy. For other antibiotics, resistance to all antimicrobial agents except for gentamicin also show a decreasing trend from 2018 to 2020 ($p = 0.077$, pairwise *t*-tests for each individual drug across 2018 – 2020). Resistance to amikacin remained the lowest out of all drugs with SIR interpretable data, at 0.8% (5/590 isolates) in 2020. Conversely, resistance to cephalexin remained the highest out of all drugs with SIR interpretable data, at 15.9% (94/590 isolates).

Most *E. coli* isolates recovered from dogs with UTIs were pan-susceptible to all antimicrobial agents, at a frequency of 79.0% (466/590) in 2020 (Table 31, Appendix E). Among the remaining isolates, 12.7% (75/590) were resistant to only one antibiotic class; 6.3% (37/590) were resistant to two antibiotic classes, namely cephalosporins and fluoroquinolones; and only 2.0% (12/590) of *E. coli* isolates recovered from dogs with UTIs met the requirements for MDR classification. .

Dogs – *E. coli* – non-urinary tract infections

MIC data for *E. coli* isolates recovered in 2020 from canine non-UTI infections show persistently high resistance to amoxicillin/clavulanic acid (99.1%, 320/323 isolates) and ampicillin (99.4%, 321/323 isolates) (Table 32, Appendix E; Figure 11, Appendix E).

Among the 1st generation cephalosporins, cephalexin resistance was significantly higher than cefazolin (75.5%, 244/323 and 26.6%, 86/323, respectively). Resistance to 3rd generation cephalosporins remained stable at 22.3% (72/323) for cefpodoxime and 13.9% (45/323) for ceftazidime. Bimodal distributions of the MIC data for many cephalosporins, including cefazolin, cefovecin, cefpodoxime, and cephalexin suggest that the presence of genetic factors conferring AMR is contributing to this observation. However, resistance to gentamicin and the fluoroquinolones showed an overall decreasing trend ($p = 0.077$, pairwise *t*-tests for each individual drug across 2018 – 2020) in Figure 11, Appendix E.

In 2020, only one non-UTI *E. coli* isolate (0.3%) was pan-susceptible to all antimicrobials tested in the CompGN1F panel. The number of MDR isolates observed in the non-UTI dog *E. coli* category increased slightly from 66.9%, (214/320 isolates) in 2019 to 76.8% (248/323 isolates) in 2020. Among the MDR isolates, one was resistant to 12 of the 13 antimicrobials with canine breakpoints (apart from amikacin); 4 isolates were resistant to 11 antimicrobials; and 14 isolates were resistant to 10 antimicrobials.

Abscess/skin/wound infections remained the most common clinical signs and indications associated with non-UTI *E. coli* infections in dogs (29.1%), followed by reproductive tract infections (18.0%) and otitis/ear infections (13.0%). Additionally, pneumonia/respiratory infections emerged as a predominant source of non-UTI *E. coli* isolates (12.7%). Table 33, Appendix E provides information on additional clinical diagnoses associated with non-UTI *E. coli* isolates recovered from dogs.

Dogs – *Staphylococcus intermedius* group

The *Staphylococcus intermedius* group category, which includes *S. intermedius, S. pseudintermedius,* and *S. delphini*, consistently received the most submissions across all bacterial isolate-host animal categories again in 2020, with 962 isolates (Figure 3). As with *E. coli*, isolates were broadly separated by clinical signs/indications into those associated with UTIs (*n* = 206), and non-UTIs (*n* = 756). Each isolate category was further subdivided into oxacillin resistant (OxR) or oxacillin sensitive (OxS) based on human breakpoint values (S ≤0.25 μg/mL, R ≥0.5 μg/mL), as no oxacillin breakpoints has been established for dogs.

Oxacillin resistance, which is associated with the presence of the *mecA* gene (known to confer methicillin resistance), was considered here to be indicative of an isolate's resistance to methicillin. If resistant to methicillin, the isolate was also considered resistant to all beta-lactam, third generation cephalosporin, and penicillin-class antimicrobials, regardless of actual MIC values.

Dogs – *Staphylococcus intermedius* group – urinary tract infections

Participating laboratories submitted 206 isolates from canine clinical cases associated with UTIs, of which 58 isolates were OxR and 148 were OxS. Six antimicrobials with MIC breakpoints have been established for canine Staphylococcal infections (amikacin, amoxicillin/clavulanic acid, cefazolin, enrofloxacin, marbofloxacin, and pradofloxacin), although the *Vet01S* (CLSI, 2020) does not specify I and R interpretations for amoxicillin/clavulanic acid in dog UTIs and resistance was therefore not calculated for this antimicrobial agent. Additionally, amikacin may be under-reported due to the range of dilutions on the Sensititre™ CompGP1F sensitivity plate, which does not include dilutions needed for MIC breakpoints and subsequent SIR interpretations ≤16 μg/mL.

Dogs – *Staphylococcus intermedius* group – urinary tract infections – Oxacillin sensitive

Of the *S. intermedius* group isolates associated with dog UTIs, 70.8% (148/209) were OxS using the human breakpoint value of S <0.25 μg/mL (Table 35, Appendix E). This is a reduction in sensitivity compared to 2019, where 76.8% (133/173) of isolates were identified as oxacillin sensitive.

Canine UTI *S. intermedius* isolates from 2020 demonstrated a range of resistance profiles (Figure 12, Appendix E), from a low of 0.0% for amikacin to a high of 10.1% (15/148) for enrofloxacin. Resistance to fluoroquinolones did increase from 2019 to 2020 (4.5% for enrofloxacin; 4.7% for marbofloxacin; and 3.3% for pradofloxacin), although it should be noted that this increase is not consistent from 2018 to 2019 and requires further study. In addition, no MDR isolates were identified among the OxS *S. intermedius* group isolates associated with dog UTIs, consistent with data from previous years.

Dogs – *Staphylococcus intermedius* group – urinary tract infections – Oxacillin resistant

Using the human oxacillin breakpoint value of R ≥0.5 μg/mL, 58 of the 206 *S. intermedius* group isolates associated with dog UTIs were OxR in 2020 (28.2%) (Table 36, Appendix E). This represents a notable increase in OxR *S. intermedius* group isolates from dog UTIs reported in 2019 (23.1%, 40/173 isolates).

Only two classes of antimicrobials, aminoglycosides and fluoroquinolones, were evaluated for resistance trends since all other antimicrobial classes are automatically classified as resistant due to the oxacillin resistance phenotype. Notably, resistance to individual fluoroquinolone drugs increased from 2019 to 2020: enrofloxacin resistance increased by 15.9% (44/58 isolates in 2020; 24/40 isolates in 2019), marbofloxacin resistance increased by 14.1% (43/58 isolates in 2020; 24/40 isolates in 2019), and pradofloxacin resistance increased by 12.4% (42/58 isolates in 2020; 24/40 isolates in 2019; Figure 13, Appendix E). While it appears that amikacin resistance has decreased over the past three years, this may be attributable to the increase in total number of OxR *S. intermedius* group isolates reported from dog UTIs in 2018 (n = 10) compared to 2019 and 2020 (n = 40 and n = 58, respectively). (Figure 13, Appendix E).

Dogs – *Staphylococcus intermedius group* – non-urinary tract infections

In 2020, 756 *S. intermedius* group isolates associated with non-UTI infections were recovered from dogs. This is a slight decrease of 2.7% from isolates in this category submitted in 2019 (*n* = 777), and a sustained increase of 82.6% over submissions from 2018 (*n* = 414).

Dogs – *Staphylococcus intermedius group* – non-urinary tract infections – Oxacillin sensitive

Of the *S. intermedius* group isolates associated with canine non-UTI infections, 61.8% (467/756) were OxS. This is a slight decrease in non-UTI *S. intermedius* group isolates recovered from dogs in 2019 (62.4%, 485/777). Minimum inhibitory concentration values for all antimicrobials tested are given in Table 37, Appendix E. Resistance patterns from 2018 to 2020 remained relatively stable (Figure 14, Appendix E). As in previous years, ampicillin was the most frequently observed resistant phenotype in 2020 among all antimicrobials (31.3%, 146/467 isolates). Amoxicillin/clavulanic acid and amikacin resistance remained low in 2020 relative to all other antimicrobials, at 0.2% and 1.3% respectively.

Only 27/467 (5.8%) of OxS *S. intermedius* group isolates from dog non-UTIs were classified as MDR, a decrease of 4.3% from the 2019 pilot project year (10.1%, 49/485). Again, we did not identify any samples with resistance phenotypes to more than four antimicrobial classes; resistance to penicillins (ampicillin), tetracyclines (doxycycline and tetracycline), and lincosamides (clindamycin) were the most frequently observed phenotypes (Table 38, Appendix E). The presence of one particular resistance phenotype (against tetracyclines, lincosamides, and penicillins) among MDR isolates suggest that genetic element(s) such as the *erm* gene family, which confer resistance to multiple antimicrobial agents that share similar mechanisms of action, may interact with genes that confer resistance to penicillins.

Over half of all OxS *S. intermedius group* isolates were associated with abscess/skin/wound infections (typically pyoderma, 54.4%, 254/467), followed by otitis/ear infections (24.2%, 113/467). All clinical signs and diagnostic indications associated with these samples are presented in Table 39, Appendix E.

Dog – *Staphylococcus intermedius* group – non-urinary tract infections – Oxacillin resistant

38.2% (289/756) of *S. intermedius* group isolates associated with dog non-UTIs were OxR, a slight increase from 2019 (37.6%, 292/777). Data from MIC testing against antimicrobial agents is shown in Table 40, Appendix E. This group of isolates exhibited clear and consistent levels of AMR against all antimicrobials tested (except amikacin), with rates of resistance averaging 80.5% across fluoroquinolones, linosamides, and tetracyclines as shown in Figure 15, Appendix E.

The prevalence of MDR in the OxR *S. intermedius* group of isolates recovered from dog non-UTIs remained the highest observed across all animal/pathogen species (69.9%, 202/289). These data represent a 7.6% increase in MDR observed compared to last year (62.3% in 2019), although the resistance phenotypes observed among MDR isolates remained consistent. Pan-resistance to all fluoroquinolones, lincosamides, and tetracyclines tested was found in 92.6% (187/202) of MDR isolates; two additional isolates exhibited resistance to amikacin (Table 41, Appendix E).

Most OxR *S. intermedius* group isolates from non-UTI dog sources were associated with abscess/skin/wound/abscess infections (66.1%), followed by otitis/ear infections (17.3%) as in previous years. The remaining clinical signs and diagnoses associated with these samples are shown in Table 42, Appendix E.

Cats

As with dogs, the data reported here are split into two categories per bacterial pathogen (*E. coli* or *S. intermedius* group) by the source of infection, namely UTIs and non-UTIs. Data is provided for all antimicrobials found on the CompGN1F and CompGP1F plates, regardless of therapeutic use for the pathogens surveyed.

Cats – *Escherichia coli*

Data from 441 *E. coli* isolates were submitted in 2020. Most (322/441, 73.0%) were associated with UTIs, with the remainder (119/441, 27.0%) from infection sites other than the urinary tract. However, non-UTI isolates increased by 5.2% this year (27.0%, 119/441 isolates in 2020, up from 21.8%, 95/435 isolates in 2019).

Candidates identified for ESBL screening in 2020 included 9.1% (40/441) of isolates with MIC values confirmed >8 μg/mL for cefpodoxime, and 5.2% (23/441) of isolates with MIC values confirmed >2 μg/mL for ceftazidime (Table 43, Appendix F).

Cats – *E. coli* – urinary tract infections

Only two antimicrobials have MIC breakpoints established for urinary tract infections in cats: amoxicillin/clavulanic acid and cefovecin. Established MIC breakpoints for ampicillin were removed in the *Vet01S* (CLSI, 2020); additionally, I and R breakpoints for amoxicillin/clavulanic acid have also been removed. As a result, antimicrobial resistance cannot be evaluated except for cefovecin, which remained stable in 2020 (10.2%, 33/322 isolates; Table 43, Appendix F). Several isolates met criteria for ESBL screening: 9.9% (32/322) based on cefpodoxime and 7.1% (23/322) for ceftazidime.

Cats – *E. coli* – non-urinary tract infections

Minimum inhibitory concentration values for all 119 *E. coli* isolates recovered from feline non-UTIs can be found in Table 44, Appendix F. Resistance to fluoroquinolones continued to climb slightly by 0.3% in 2020 (Figure 16, Appendix F), again likely due to changes in sampling numbers rather than a true increase in the detection of isolates resistant to these drugs. Ampicillin and amoxicillin/clavulanic acid resistance remained relatively stable at 99.2% for each drug, although it should be noted that this may represent a slight increase in susceptibility against beta-lactams in 2020.

Common clinical sign and diagnostic indications for *E. coli* isolates from cat non-UTIs were diarrhea/gastroenteric infections (22.7%, 27/119) and abscess/skin/wound infections (21.8%, 26/119). All clinical signs and diagnoses associated with *E. coli* isolates from cat non-UTI sources are in Table 45, Appendix F.

Seven *E. coli* isolates from cat non-UTI sources (5.9%, 7/119) were classified as MDR based on their resistance to all three antimicrobial classes (B-lactam combination agents, fluoroquinolones, and penicillins) represented in Table 46, Appendix F.

Cats – *Staphylococcus intermedius* group

As in previous years, *S. intermedius* group isolates were not recovered from cats in large numbers in 2020. Only 72 isolates were submitted for this category, a slight decrease from submissions in 2019 (*n* = 75) and an increase from submissions in 2018 (*n* = 59). As with *S. intermedius* group isolates recovered from dogs, isolates were separated by clinical signs/indications into those associated with UTIs (*n* = 21), and non-UTIs (*n* = 51). Each isolate category was further subdivided into OxR or OxS based on human breakpoint values for oxacillin (S ≤0.25 μg/mL, R ≥0.5 μg/mL), as no oxacillin breakpoints has been established for cats.

Cats – *S. intermedius* group – urinary tract infections

29.2% of *S. intermedius* group isolates (21/72) were associated with cat UTIs in 2020, of which one-third (7/21 isolates) were OxR and two-thirds (14/21 isolates) were OxS.

Cats – *S. intermedius* group – urinary tract infections – Oxacillin sensitive

Only two antimicrobials have breakpoints established for *Staphylococcus* spp. Isolates recovered from UTIs in cats; these are amoxicillin/clavulanic acid and ampicillin. Three of 14 isolates (21.4%) were resistant to ampicillin and none were resistant to amoxicillin/clavulanic acid, largely consistent with previous years of the pilot project (Table 47, Appendix F).

Cats – *S. intermedius* group – urinary tract infections – Oxacillin resistant

Minimum inhibitory concentration values against all antimicrobial agents tested are shown in Table 48, Appendix F.

Cats – *S. intermedius* group – non-urinary tract infections

70.8% of *S. intermedius* group isolates (51/72) were associated with cat non-UTIs in 2020, of which the majority (58.8%, 30/51) were OxS and the remainder (41.2%, 21/51) were OxR. This represents a slightly biased distribution in comparison to the even split in OxS and OxR *S. intermedius* group isolates recovered from cat non-UTIs in 2019 (50.0%, 22/44 in each category).

Cats – *S. intermedius* group – non-urinary tract infections – Oxacillin sensitive

Interpretive MIC breakpoint values are shown in Table 49, Appendix F for six antimicrobials including oxacillin. It is important to note that due to the very low sample numbers of OxS *S. intermedius* isolates in the cat non-UTI category, fluctuations in rates of resistance against beta-lactams, fluoroquinolones, and penicillins shown in Figure 17, Appendix F represent changes on the order of two isolates at the most. Therefore, changes in resistance rates largely depended on the denominator (isolates tested in this category) and do not reflect the true population.

Clinical signs and diagnostic indications for OxS isolates are consistent with previous years, with most isolates associated with abscess/skin/wound infections (56.7%, 17/30; Table 50, Appendix F), followed by otitis/ear infections (26.7%, 8/30) and pneumonia/respiratory infections (13.3%, 4/30).

Cats – *S. intermedius* group – non-urinary tract infections – Oxacillin resistant

Like dogs, the observed resistance to other antimicrobials in this group of OxR *S. intermedius* group isolates was considerably higher against all fluoroquinolones tested (Table 51, Appendix F). We observed resistance of 81.0% (17/21 Isolates) against enrofloxacin, marbofloxacin, and pradofloxacin, an increase of two to four isolates from the previous pilot project year (Figure 18, Appendix F).

Summary

This report provides an initial look at AMR trends over the last three years for six animal species, covering both livestock and companion animals. While resistance appears to be stable or even potentially decreasing in livestock, interpretation of the MIC data is limited due to the lack of interpretive breakpoints for many important antimicrobials used in animals.

One challenge that we continued to face was the ability to collect sufficient isolates for certain bacterial pathogens in order to accurately predict AMR trends at a national level. Because many variables may affect submission numbers, including the number and type of diagnostic cases submitted to a laboratory in a given year, it will be important to consistently monitor these pathogens over the next two years to determine if this trend continues and is statistically significant.

Goals for the upcoming year are to incorporate genetic monitoring of antimicrobial resistance genes (genotype), and to compare bacterial genotypes to antimicrobial susceptibility MIC profiles (phenotypes) for antibiotics tested in this pilot.

References:

1. CLSI, 2018. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals* (4th ed, Supplement VET08). Clinical and Laboratory Standards Institute (CLSI); Wayne, PA.
2. CLSI, 2020. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals* (5th ed, Supplement VET01S). Clinical and Laboratory Standards Institute (CLSI); Wayne, PA.
3. USDA, 2019. USDA APHIS AMR Pilot Project Report, Year 1. https://www.aphis.usda.gov/animal_health/nahln/downloads/2018%20APHIS%20AMR%20Pilot%20Project%20EOY%20Report-05.01.2019.pdf
4. USDA, 2020. USDA APHIS AMR Pilot Project Report, Year 2. https://www.aphis.usda.gov/animal_health/nahln/downloads/2019_APHIS_AMR_PilotProject_EOY_Report.pdf

APPENDIX A: MIC Distributions and Clinical Signs for *E. coli, S. enterica,* and *M. haemolytica* in Cattle

Table 3. Minimum inhibitory concentration (MIC) distribution (μg/ml) for *Escherichia coli* isolates recovered from cattle in 2020.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	>1	≤2	2	>2	≤4	4	>4	≤8	8	>8	16	>16	32	>32	64	>64	≤256	>256
Aminocyclitol	Spectinomycin	481															16			258		46		23	138		
Aminoglycoside	Gentamicin	481						403				13			1			8		10	46						
	Neomycin	481												312				11		12		20	126				
Cephalosporin	Ceftiofur	481		73			218		22			7			6			46	109								
Fluoroquinolone	Danofloxacin	481	355		4		16		8	98																	
	Enrofloxacin	481	352		10		13		8			3	95														
Folate pathway	Sulfadimethoxine	481																								192	289
antagonist	Trimethoprim/Sulfa	481									319		162														
Lincosamide	Clindamycin	481		1																1	479						
Macrolide	Gamithromycin	199						2				2			35			121	39								
	Tidipirosin	199						3				6			100			79		6	5						
	Tilmicosin	481												1				2		2	197	16		142	121		
	Tulathromycin	481						1				3			32		154	139		113		17		6	16		
	Tylosin	481				1										2							478				
Penicillin	Ampicillin	481		2			1		10			103			96			5		4	260						
	Penicillin	481	1		1				1									5	473								
Phenicol	Florfenicol	481					1		1			42			194			56	187								
Pleuromutilin	Tiamulin	481							1									2		2		31	445				
Tetracycline	Chlortetracycline	282				3			21			46			13			11	188								
	Oxytetracycline	282				1			43			33			1				204								
	Tetracycline	199				2			33			34			2			5	123								

1: Bovine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Total number of isolates for each antibiotic reflect a combination of the BoPo6F and BoPo7F plates. Not all antibiotics in the table are present on both plates, leading to differences in total numbers of isolates. 3: Ceftiofur breakpoints have been established for mastitis cases only for *E. coli* infections in cattle. 4: Ampicillin breakpoints have been established for metritis cases only for *E. coli* infections in cattle. 5: Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentration on BoPo6F and BoPo7F plates = 2/38 μg/mL.

Clinical signs/Indications	Counts in 2020	% of Counts	Clinical signs/Indications	Counts in 2020	% of Counts
Diarrhea/Gastroenteric infection	224	46.6	Neonatal infection	5	1.0
Sepsis/Septicemia	78	16.2	Reproductive tract infection	5	1.0
Colibacillosis	49	10.2	Peritonitis/Polyserositis	4	0.8
Pneumonia/Respiratory infection	48	10.0	Liver/Kidney/Spleen infection	4	0.8
Undetermined	30	6.2	Endocarditis/Epicarditis/Pericarditis	3	0.6
Abscess/Skin/Wound infection	9	1.9	Urinary tract infection	2	0.4
Abortion/Placental infection	7	1.5	Otitis/Ear infection	1	0.2
Other*	6	1.2	Arthritis/Joint/Bone infection	1	0.2
Mastitis	5	1.0	Total	481	

*Other diagnoses included hemorrhagic diathesis (1), hemosiderosis (1), polyserositis (1), and salmonellosis (3).

Table 5. Minimum inhibitory concentration (MIC) distribution (μg/ml) for *Salmonella enterica* isolates recovered from cattle in 2020.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	>1	≤2	2	>2	≤4	4	≤8	8	>8	16	>16	32	>32	64	>64	≤256	>256
Aminocyclitol	Spectinomycin	380																	89		220		57	14		
Aminoglycoside	Gentamicin	380						368				6			2				1	3						
	Neomycin	380												294			4		1		1	80				
Cephalosporin	Ceftiofur	380		1			57		167			2			6		23	124								
Fluoroquinolone	Danofloxacin	380	316		17		33		12	2																
	Enrofloxacin	380	316		9		42		9			2	2													
Folate pathway antagonist	Sulfadimethoxine	380																							167	213
	Trimethoprim/Sulfa	380									357		23													
Lincosamide	Clindamycin	380																		380						
Macrolide	Gamithromycin	212										2			49		149	12								
	Tidipirosin	212													7		127		54	24						
	Tilmicosin	380																	1	211			19	149		
	Tulathromycin	380													6	121	64		149		38		2			
	Tylosin	380																				380				
Penicillin	Ampicillin	380					14		179			22					1			164						
	Penicillin	380										1			16		162	201								
Phenicol	Florfenicol	380		1					7			110			104		2	156								
Pleuromutilin	Tiamulin	380																				380				
Tetracycline	Chlortetracycline	168				1			47			28			4		2	86								
	Oxytetracycline	168				14			54			11			2			87								
	Tetracycline	212				21			66			30			3		1	91								

1: No antimicrobial breakpoints have been established. 2: Total number of isolates for each antibiotic reflect a combination of the BoPo6F and BoPo7F plates. Not all antibiotics in the table are present on both plates, leading to differences in total numbers of isolates. 3: Trimethroprim/sulfamethoxazole (abbrev. Sulfa) concentration on BoPo6F and BoPo7F plates = 2/38 ug/mL.

Figure 4. The 15 most prevalent serotypes observed in *Salmonella enterica* isolates recovered from cattle in 2018 – 2020.

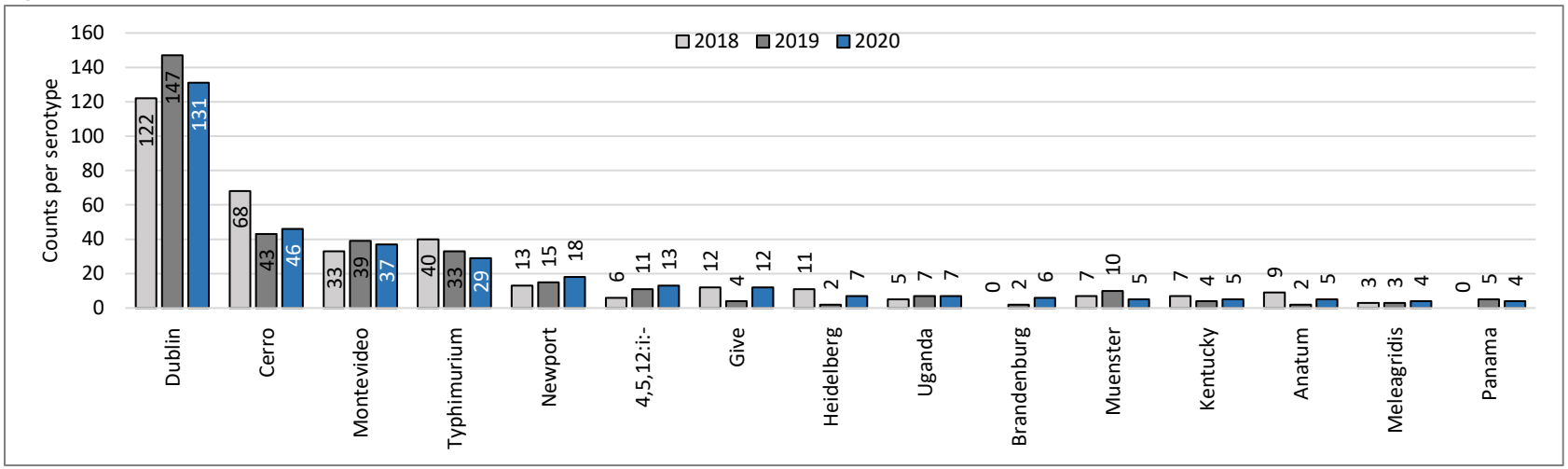


Table 6. Serotype counts and prevalence of all *Salmonella enterica* isolates recovered from cattle in 2020, listed from left to right in decreasing order.

Serotype	Counts	% of Counts	Serotype	Counts	% of Counts	Serotype	Counts	% of Counts	Serotype	Counts	% of Counts
Dublin	131	17.3	Kentucky	5	0.7	Bovismorbificans	2	0.3	Derby	1	0.1
Cerro	46	6.1	Anatum	5	0.7	Infantis	2	0.3	Idikan	1	0.1
Montevideo	37	4.9	Meleagridis	4	0.5	Liverpool	2	0.3	Bareilly	1	0.1
Typhimurium	29	3.8	Panama	4	0.5	Kiambu	2	0.3	Hartford	1	0.1
Newport	18	2.4	Thompson	4	0.5	Othmarschen	2	0.3	Havana	1	0.1
4,5,12:i:-	13	1.7	Muenchen	3	0.4	Bredeney	2	0.3	Litchfield	1	0.1
Give	12	1.6	Mbandaka	3	0.4	Agona	1	0.1	Braenderup	1	0.1
Heidelberg	7	0.9	Saintpaul	3	0.4	Oranienburg	1	0.1	Cubana	1	0.1
Uganda	7	0.9	Non-motile	3	0.4	Senftenberg	1	0.1	4,12:i:-	1	0.1
Brandenburg	6	0.8	Worthington	3	0.4	London	1	0.1	Poona	1	0.1
Muenster	5	0.7	Schwarzengrund	2	0.3	Orion	1	0.1	Total*	377	

*Total does not include 3 NT (non-typable) isolates.

Table 7. Minimum inhibitory concentration (MIC) distribution (µg/ml) for *Mannheimia haemolytica* isolates recovered from cattle in 2020.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	>1	≤2	2	>2	≤4	4	≤8	8	>8	16	>16	32	>32	64	>64	≤256	>256
Aminocyclitol	Spectinomycin	566														14			100		346		4	102		
Aminoglycoside	Gentamicin	566						78				339			54		8		14	73						
	Neomycin	566												210			211		29		12	104				
Cephalosporin	Ceftiofur	566		546			13		3						1			3								
Fluoroquinolone	Danofloxacin	566	432		10		12		16	96																
	Enrofloxacin	566	437		6		19		10			2	92													
Folate pathway	Sulfadimethoxine	566																							377	189
antagonist	Trimethoprim/Sulfa	566									559		7													
Lincosamide	Clindamycin	566		1			1		1			6			25		283		133	116						
Macrolide	Gamithromycin	453						310				48			7		8	80								
	Tidipirosin	453						293				57			28		13		3	59						
	Tilmicosin	566									43			38	197		124		41	88	2		3	30		
	Tulathromycin	566						2				2			29	348	31		28		21		14	91		
	Tylosin	566				1			1			1					5		11		67	480				
Penicillin	Ampicillin	566		471			36		4			6			1		6		8	34						
	Penicillin	566	243		146		91		27			4			3		6	46								
Phenicol	Florfenicol	566		14			270		171			36			15		8	52								
Pleuromutilin	Tiamulin	566				2			1			6			18		144		313		67	15				
Tetracycline	Chlortetracycline	113				27			29			14			9		19	15								
	Oxytetracycline	113				44			15			1					5	48								
	Tetracycline	453				210			108			2			8		13	112								

1: Bovine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Total number of isolates for each antibiotic reflect a combination of the BoPo6F and BoPo7F plates. Not all antibiotics in the table are present on both plates, leading to differences in total numbers of isolates. 3: Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentration of BoPo6F and BoPo7F plates = 2/38 µg/mL.

Figure 5. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in *Mannheimia haemolytica* isolates recovered from cattle in 2018 – 2020.

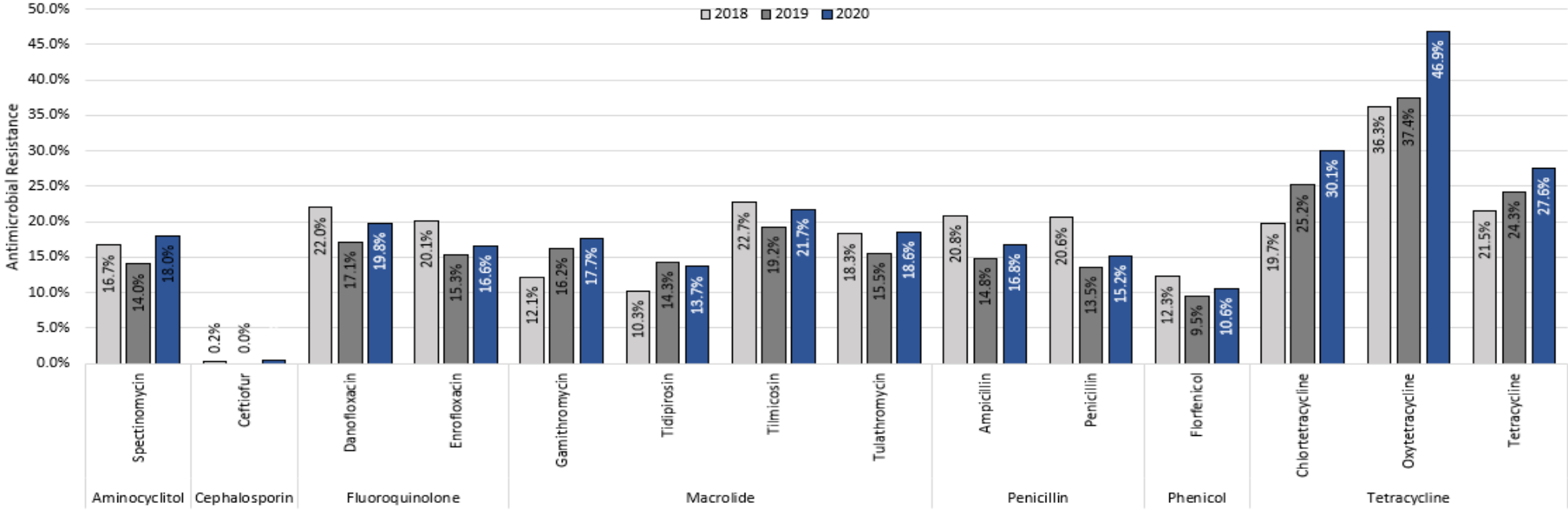


Table 8. Per-isolate and per-antibiotic resistance phenotypes for *Mannheimia haemolytica* isolates recovered from cattle in 2020.

Resistance phenotype (# antibiotics)		11	10	9	8	7	6	5	4	3	2	1	0
# Isolates with resistance phenotype		14	22	19	18	16	15	13	14	15	15	47	358
Aminocyclitol	Spectinomycin	14	21	19	16	8 (1)	9	4	8	2	1 (1)	0 (1)	0 (1)
Cephalosporin	Ceftiofur	0	0	0	0	3	0	0	0	0	0 (1)	0	0
Fluoroquinolone	Danofloxacin	14	22	19	18	13	11 (1)	4 (3)	5	2	2 (1)	2 (2)	0 (5)
	Enrofloxacin	14	22	19	17 (1)	9 (4)	6 (6)	2 (3)	3 (2)	2 (1)	0 (3)	0 (4)	0 (5)
Macrolide	Gamithromycin	14	17	11 (1)	13	9 (3)	6	4	2 (2)	4 (2)	0	0	0
	Tildipirosin	14	17	9 (1)	8	5 (2)	4	4 (1)	0 (2)	0 (1)	0	1 (2)	0 (4)
	Tilmicosin	14	22	19	18	16	12 (3)	10 (2)	3 (8)	7 (1)	2 (2)	0 (10)	0 (15)
	Tulathromycin	14	21 (1)	15 (2)	17 (1)	12 (3)	13 (2)	6 (4)	0 (3)	4 (1)	2	1 (2)	0 (2)
Penicillin	Ampicillin	14	22	14	6	5	2	6	9	7	5	5	0
	Penicillin	14	19 (3)	12 (6)	3 (10)	5 (5)	2 (5)	7 (2)	8 (2)	7 (1)	6 (1)	3 (5)	0 (51)
Phenicol	Florfenicol	14	10 (2)	10 (4)	6 (3)	8	5	1 (1)	2 (1)	0 (2)	2 (1)	2 (1)	0
Tetracycline	Chlortetracycline	0	5	5 (1)	5	3	5 (2)	4 (1)	2 (1)	2 (2)	3	0 (2)	0
	Oxytetracycline	0	5	6	5	4	8	6	6	5	3	5	0
	Tetracycline	14	17	13	12 (1)	12	7	7	8	3 (1)	4	28	0 (6)

Values for each antimicrobial agent listed in rows 3 - 14 represent # isolates resistant to each antimicrobial (column 2) with an overall phenotype of resistance against X antimicrobials (row 1). Parenthetical values represent # isolates with intermediate susceptibility against each antimicrobial (column 2), where applicable. Interpretive values are based on the *Vet01S* (CLSI, 2020).

APPENDIX B: MIC Distributions and Clinical Signs for *E. coli* and *S. suis* isolates in Swine

Table 9. Minimum inhibitory concentration (MIC) distribution (µg/ml) for *Escherichia coli* isolates recovered from swine in 2020.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	>1	≤2	2	>2	≤4	4	≤8	8	>8	16	>16	32	>32	64	>64	≤256	>256
Aminocyclitol	Spectinomycin	164														3			63		9		7	82		
Aminoglycoside	Gentamicin	164						89				3			1		10		6	55						
	Neomycin	164												98			2		3		5	56				
Cephalosporin	Ceftiofur	164		20			70		11			5			3		13	42								
Fluoroquinolone	Danofloxacin	164	83		6		12		9	54																
	Enrofloxacin	164	84		6		12		9			12	41													
Folate pathway	Sulfadimethoxine	164																							57	107
antagonist	Trimethoprim/Sulfa	164									118		46													
Lincosamide	Clindamycin	164																		164						
Macrolide	Gamithromycin	109										1			12		56	40								
	Tidipirosin	109										6			55		36		4	8						
	Tilmicosin	164																		109	2		27	26		
	Tulathromycin	164													4	80	24		32		6		6	12		
	Tylosin	164																				164				
Penicillin	Ampicillin	164		1					6			21			20		1			115						
	Penicillin	164	1														1	162								
Phenicol	Florfenicol	164										19			77		24	44								
Pleuromutilin	Tiamulin	164																	1		11	152				
Tetracycline	Chlortetracycline	55				2			5			4			2		5	37								
	Oxytetracycline	55				1			5			6						43								
	Tetracycline	109							9			7			1			92								

1: No antimicrobial breakpoints have been established. 2: Total number of isolates for each antibiotic reflect a combination of the BoPo6F and BoPo7F plates. Not all antibiotics in the table are present on both plates, leading to differences in total numbers of isolates. 3: Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentration on BoPo6F and BoPo7F plates = 2/38 µg/mL.

Table 10. Clinical signs/diagnostic indications associated with *Escherichia coli* isolates recovered from swine in 2020.

Clinical signs/Indications	Counts in 2020	% of Counts	Clinical signs/Indications	Counts in 2020	% of Counts
Diarrhea/Gastroenteric infection	98	59.8	Sepsis/Septicemia	3	1.8
Mixed/Secondary infection	17	10.4	Urinary tract infection	3	1.8
Pneumonia/Respiratory infection	13	7.9	Arthritis/Joint/Bone infection	2	1.2
Colibacillosis	9	5.5	Liver/Kidney/Spleen infection	2	1.2
Peritonitis/Polyserositis	6	3.7	Eye infection	1	0.6
Abscess/Skin/Wound infection	4	2.4	Abortion/Placental infection	1	0.6
Undetermined	4	2.4	Other*	1	0.6
*Other diagnoses included vasculitis (1).			Total	164	

Table 11. Minimum inhibitory concentration (MIC) distribution (µg/ml) for *Streptococcus suis* isolates recovered from swine in 2020.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	>1	≤2	2	>2	≤4	4	≤8	8	>8	16	>16	32	>32	64	>64	≤256	>256
Aminocyclitol	Spectinomycin	167														38			76		23		9	21		
Aminoglycoside	Gentamicin	167						48				38			49		25		2	5						
	Neomycin	167												64			24		36		31	12				
Cephalosporin	Ceftiofur	167		144			11		3			2			3		2	2								
Fluoroquinolone	Danofloxacin	167	16		43		75		24	9																
	Enrofloxacin	167	26		60		61		14			2	4													
Folate pathway	Sulfadimethoxine	167																							62	105
antagonist	Trimethoprim/Sulfa	167									159		8													
Lincosamide	Clindamycin	167		32			3		2			4			4		5		5	112						
Macrolide	Gamithromycin	101						22				6			5		8	60								
	Tidipirosin	101						3				2			5		8		3	80						
	Tilmicosin	167									5			13	10		16		3	78	1			41		
	Tulathromycin	167						8				4			7	21	4		6		4		6	107		
	Tylosin	167				16			26			4			4		1				1	115				
Penicillin	Ampicillin	167		158			5		1			1			1		1									
	Penicillin	167	131		10		5		3			9			4		1	4								
Phenicol	Florfenicol	167					6		43			108			9			1								
Pleuromutilin	Tiamulin	167				27			39			36			11		5		10		9	30				
Tetracycline	Chlortetracycline	66				5			2			1			2		5	51								
	Oxytetracycline	66				4			3			2					2	55								
	Tetracycline	101				3			2			4			4		3	85								

1: Swine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Total number of isolates for each antibiotic reflect a combination of the BoPo6F and BoPo7F plates. Not all antibiotics in the table are present on both plates, leading to differences in total numbers of isolates. 3: Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentration of BoPo6F and BoPo7F plates = 2/38 µg/mL.

Table 12. Clinical signs/diagnostic indications associated with *Streptococcus suis* isolates recovered from swine in 2020.

Clinical signs/Indications	Counts in 2020	% of Counts	Clinical signs/Indications	Counts in 2020	% of Counts
Pneumonia/Respiratory infection	82	49.1	Diarrhea/Gastroenteric infection	4	2.4
Central nervous system infection	26	15.6	Arthritis/Joint/Bone infection	4	2.4
Sepsis/Septicemia	24	14.4	Abscess/Skin/Wound infection	3	1.8
Undetermined	7	4.2	Other*	2	1.2
Endocarditis/Epicarditis/Pericarditis	6	3.6	Liver/Kidney/Spleen infection	2	1.2
Mixed/Secondary infection	6	3.6	Peritonitis/Polyserositis	1	0.6
Total	167				

*Other diagnoses included infection (1) and a complex case of bronchopneumonia, chondritis, leptomeningoencephalitis, and osteomyelitis (1).

Figure 6. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in *Streptococcus suis* isolates recovered from swine in 2019 – 2020.

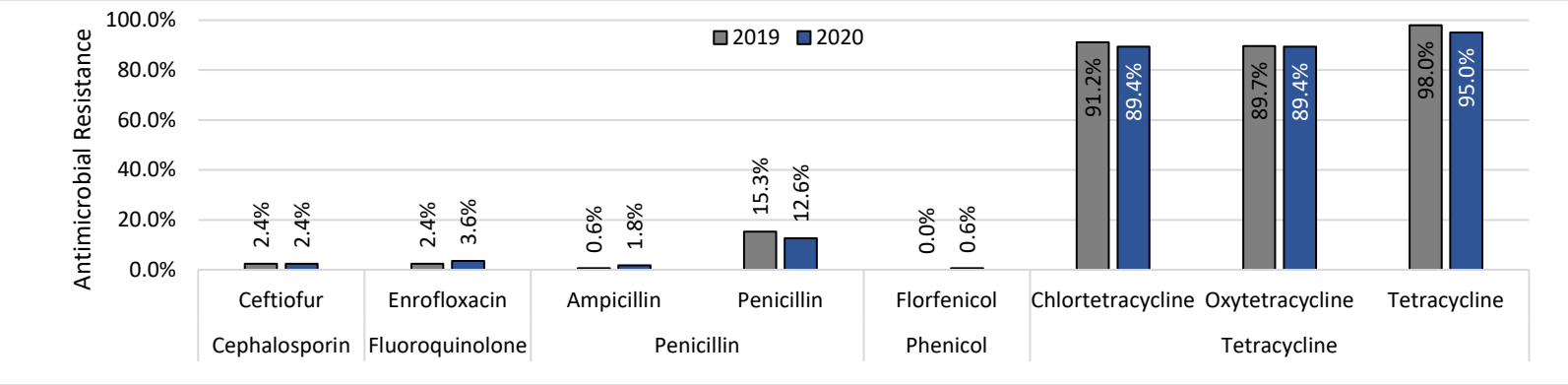


Table 13. Per-isolate and per-antibiotic resistance phenotypes for *Streptococcus* suis isolates recovered from swine in 2020.

Resistance phenotype (X antibiotics)		5	4	3	2	1	0
Isolates with resistance phenotype		2	5	7	56	86	11
cephalosporin	ceftiofur	1	3 (1)	0	0 (2)	0	0
fluoroquinolone	enrofloxacin	2	2	1	1 (5)	0 (9)	0
penicillin	ampicillin	2	1	0 (1)	0	0	0
	penicillin	2	5	7	7 (1)	0 (4)	0
phenicol	florfenicol	0	0	1	0 (3)	0 (6)	0
tetracycline	chlortetracycline	1	4	5	48	1 (1)	0 (1)
	oxytetracycline	1	4	5	48	1 (1)	0 (2)
	tetracycline	1	1	2	8	84	0 (2)

Values for each antimicrobial agent listed in rows 3 - 10 represent # isolates resistant to each antimicrobial (column 2) with an overall phenotype of resistance against X antimicrobials (row 1). Parenthetical values represent # isolates with intermediate susceptibility against each antimicrobial (column 2), where applicable. Interpretive values are based on the *Vet01S* (CLSI, 2020).

APPENDIX C: MIC Distributions and Clinical Signs for *E. coli* and *P. multocida* in Poultry

Table 14. Minimum inhibitory concentration (MIC) distributions in µg/ml for *Escherichia coli* isolates recovered from chickens and turkeys combined in 2020.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	≤2	2	>2	4	>4	≤8	8	>8	10	16	>16	>20	≤32	32	>32	64	>64	128	256	>256	512	1024	>1024
Aminocoumarin	Novobiocin	483											1	482																		
Aminocyclitol	Spectinomycin	483													53				316				29		3	82						
Aminoglycoside	Gentamicin	483				255			107		13		4			9	95															
	Neomycin	483								383			23			4			1				13	59								
	Streptomycin	483													302				22				22		61		46	15		3	2	10
Cephalosporin	Ceftiofur	483		113			297		16		4			33																		
Fluoroquinolone	Enrofloxacin	483	446		15		8		6		5	3																				
Folate pathway antagonist	Sulfadimethoxine	483																				82			123		88	20	170			
	Sulfathiazole	483																				304			12		3	1	163			
	Trimethoprim/Sulfa	483				426			4		2	51																				
Lincosamide	Clindamycin	483							1					482																		
Macrolide	Erythromycin	483							1				2	480																		
	Tylosin	483																1			482											
Penicillin	Amoxicillin	483		1					8		106		182			25			1	160												
	Penicillin	483							1		1					5	476															
Phenicol	Florfenicol	483						5			123		321			26	8															
Tetracycline	Oxytetracycline	483		1			14		155		82		4				227															
	Tetracycline	483		1			18		174		61		2			2	225															

1: Poultry-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Enrofloxacin is not approved for use in poultry in the U.S. as of 2005. 3: Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentration on Avian1F plate = 0.5/9.5 µg/mL, 1/19 µg/mL, and 2/38 µg/mL.

Table 15. Minimum inhibitory concentration (MIC) distributions in µg/ml for *Escherichia coli* isolates recovered from chickens in 2020.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	≤2	2	>2	4	>4	≤8	8	>8	10	16	>16	>20	≤32	32	>32	64	>64	128	256	>256	512	1024	>1024
Aminocoumarin	Novobiocin	326												326																		
Aminocyclitol	Spectinomycin	326													42				212				20		2	50						
Aminoglycoside	Gentamicin	326				189			68		10					2	57															
	Neomycin	326								286			14			3			1				4	18								
	Streptomycin	326													232				15				13		32		22	6		2	1	3
Cephalosporin	Ceftiofur	326		89			198		10		3			26																		
Fluoroquinolone	Enrofloxacin	326	298		12		5		5		4	2																				
Folate pathway antagonist	Sulfadimethoxine	326																				66			90		66	15	89			
	Sulfathiazole	326																				230			10		1	1	84			
	Trimethoprim/Sulfa	326				298			3		2	23																				
Lincosamide	Clindamycin	326							1					325																		
Macrolide	Erythromycin	326							1				2	323																		
	Tylosin	326																1			325											
Penicillin	Amoxicillin	326		1					5		91		130			17			1	81												
	Penicillin	326							1		1					5	319															
Phenicol	Florfenicol	326						5			98		204			14	5															
Tetracycline	Oxytetracycline	326		1			13		135		50		3				124															
	Tetracycline	326		1			17		142		40		2			2	122															

1: Poultry-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Enrofloxacin is not approved for use in poultry in the U.S. as of 2005. 3: Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentration on Avian1F plate = 0.5/9.5 µg/mL, 1/19 µg/mL, and 2/38 µg/mL.

Table 16. Minimum inhibitory concentration (MIC) distributions in µg/ml for *Escherichia coli* isolates recovered from turkeys in 2020.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	≤2	2	>2	4	>4	≤8	8	>8	10	16	>16	>20	≤32	32	>32	64	>64	128	256	>256	512	1024	>1024
Aminocoumarin	Novobiocin	157											1	156																		
Aminocyclitol	Spectinomycin	157													11				104				9		1	32						
Aminoglycoside	Gentamicin	157				66			39		3		4			7	38															
	Neomycin	157								97			9			1							9	41								
	Streptomycin	157													70				7				9		29		24	9		1	1	7
Cephalosporin	Ceftiofur	157		44			99		6		1			7																		
Fluoroquinolone	Enrofloxacin	157	148		3		3		1		1	1																				
Folate pathway antagonist	Sulfadimethoxine	157																				16			33		22	5	81			
	Sulfathiazole	157																				74			2		2		79			
	Trimethoprim/Sulfa	157				128			1			28																				
Lincosamide	Clindamycin	157												157																		
Macrolide	Erythromycin	157												157																		
	Tylosin	157																			157											
Penicillin	Amoxicillin	157							3		15		52			8				79												
	Penicillin	157															157															
Phenicol	Florfenicol	157									25		117			12	3															
Tetracycline	Oxytetracycline	157					1		20		32		1				103															
	Tetracycline	157					1		32		21						103															

1: Poultry-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Enrofloxacin is not approved for use in poultry in the U.S. as of 2005. 3: Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentration on Avian1F plate = 0.5/9.5 µg/mL, 1/19 µg/mL, and 2/38 µg/mL.

Table 17. Clinical signs and diagnoses associated with *Escherichia coli* infections recovered from chickens and turkeys in 2020.

Clinical signs/Indications	Chickens	Turkeys	Combined	% of Counts (Combined)	Clinical signs/Indications	Chickens	Turkeys	Combined	% of Counts (Combined)
Sepsis/Septicemia	65	18	83	17.2	Yolk Sac infection	13	0	13	2.7
Reproductive tract infection	59	2	61	12.6	Endocarditis/Epicarditis/Pericarditis	7	4	11	2.3
Pneumonia/Respiratory infection	13	48	61	12.6	Other*	7	2	9	1.9
General health	23	34	57	11.8	Airsacculitis	4	3	7	1.4
Liver/Kidney/Spleen infection	32	7	39	8.1	Abscess/Skin/Wound infection	6	1	7	1.4
Peritonitis	30	0	30	6.2	Mixed/Secondary infection	3	4	7	1.4
Colibacillosis	17	9	26	5.4	Polyserositis	0	2	2	0.4
Diarrhea/Gastroenteric infection	12	12	24	5.0	Eye infection	1	0	1	0.2
Undetermined	18	7	25	5.2	Neonatal infection	1	0	1	0.2
Arthritis/Joint/Bone infection	15	4	19	3.9	Total	326	157	483	

*Other diagnoses included fecal float (1), Marek's disease (5), and a combined yolk sac infection with polyserositis (1) in chickens. Other diagnoses in turkeys included malnutrition (1) and Newcastle's disease (1).

Table 18. Minimum inhibitory concentration (MIC) distributions in µg/ml for *Pasteurella multocida* isolates recovered from chickens and turkeys combined in 2020.

Antibiotic class	Antibiotic	Total	≤0.06	≤0.125	0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	≤2	2	>2	4	>4	5	≤8	8	>8	10	16	>16	20	>20	≤32	32	64	128	256	>256	>1024
Aminocoumarin	Novobiocin	56						8			15		17		11	5																
Aminocyclitol	Spectinomycin	56																4				14					37	1					
Aminoglycoside	Gentamicin	56						3			5		32		16																	
	Neomycin	56										14			20				19			3										
	Streptomycin	56																21				31					3					1
Cephalosporin	Ceftiofur	56				50			1		1		2		1	1																
Fluoroquinolone	Enrofloxacin	56		52			2						1	1																		
Folate pathway antagonist	Sulfadimethoxine	56																								20		9	5	3	19	
	Sulfathiazole	56																								27		10	8	5	6	
	Trimethoprim/Sulfa	56						54						2																		
Lincosamide	Clindamycin	56						1			1					54																
Macrolide	Erythromycin	56		1					1		5		34		13	2																
	Tylosin	56															1				5			19	31							
Penicillin	Amoxicillin	56				44			7		1								2				2									
	Penicillin	56	27		13		9		1				3		1					2												
Phenicol	Florfenicol	56								53			1		2																	
Tetracycline	Oxytetracycline	56				26			11		11		5		2					1												
	Tetracycline	56				27			11		12		3		1				1	1												

1:No antimicrobial breakpoints have been established. 2: Enrofloxacin is not approved for use in poultry in the U.S. as of 2005. 3: Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentration on the Avian1F plate = 0.5/9.5 µg/mL, 1/19 µg/mL, and 2/38 µg/mL.

Table 19. Minimum inhibitory concentration (MIC) distributions in µg/ml for *Pasteurella multocida* isolates recovered from chickens in 2020.

Antibiotic class	Antibiotic	Total	≤0.06	≤0.125	0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	≤2	2	>2	4	>4	5	≤8	8	>8	10	16	>16	20	>20	≤32	32	64	128	256	>256	>1024
Aminocoumarin	Novobiocin	41						5			14		11		7	4																
Aminocyclitol	Spectinomycin	41																2				12					26	1					
Aminoglycoside	Gentamicin	41						3			4		24		10																	
	Neomycin	41										13			12				13			3										
	Streptomycin	41																20				20					1					
Cephalosporin	Ceftiofur	41				36					1		2		1	1																
Fluoroquinolone	Enrofloxacin	41		38			2						1																			
Folate pathway antagonist	Sulfadimethoxine	41																								12		9	4	3	13	
	Sulfathiazole	41																								20		8	5	4	4	
	Trimethoprim/Sulfa	41						39						2																		
Lincosamide	Clindamycin	41									1					40																
Macrolide	Erythromycin	41							1		5		25		9	1																
	Tylosin	41															1				2			17	21							
Penicillin	Amoxicillin	41				33			3		1								2				2									
	Penicillin	41	24		7		4						3		1					2												
Phenicol	Florfenicol	41								38			1		2																	
Tetracycline	Oxytetracycline	41				19			7		9		3		2					1												
	Tetracycline	41				20			6		10		2		1				1	1												

1:No antimicrobial breakpoints have been established. 2: Enrofloxacin is not approved for use in poultry in the U.S. as of 2005. 3: Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentration on the Avian1F plate = 0.5/9.5 µg/mL, 1/19 µg/mL, and 2/38 µg/mL.

Table 20. Minimum inhibitory concentration (MIC) distributions in µg/ml for *Pasteurella multocida* isolates recovered from turkeys in 2020.

Antibiotic class	Antibiotic	Total	≤0.06	≤0.125	0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	≤2	2	>2	4	>4	5	≤8	8	>8	10	16	>16	20	>20	≤32	32	64	128	256	>256	>1024
Aminocoumarin	Novobiocin	15						3			1		6		4	1																
Aminocyclitol	Spectinomycin	15																2				2					11					
Aminoglycoside	Gentamicin	15									1		8		6																	
	Neomycin	15										1			8				6													
	Streptomycin	15																1				11					2					1
Cephalosporin	Ceftiofur	15				14			1																							
Fluoroquinolone	Enrofloxacin	15		14										1																		
Folate pathway antagonist	Sulfadimethoxine	15																							8			1		6		
	Sulfathiazole	15																							7		2	3	1	2		
	Trimethoprim/Sulfa	15						15																								
Lincosamide	Clindamycin	15						1								14																
Macrolide	Erythromycin	15		1									9		4	1																
	Tylosin	15																			3			2	10							
Penicillin	Amoxicillin	15				11			4																							
	Penicillin	15	3		6		5		1																							
Phenicol	Florfenicol	15								15																						
Tetracycline	Oxytetracycline	15				7			4		2		2																			
	Tetracycline	15				7			5		2		1																			

1:No antimicrobial breakpoints have been established. 2: Enrofloxacin is not approved for use in poultry in the U.S. as of 2005. 3: Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentration on the Avian1F plate = 0.5/9.5 µg/mL, 1/19 µg/mL, and 2/38 µg/mL.

Table 21. Clinical signs and diagnoses associated with *Pasteurella multocida* infections recovered from chickens and turkeys in 2020.

Clinical signs/Indications	Chickens	Turkeys	Combined	% of Counts (Combined)
Fowl cholera	15	6	21	37.5
Sepsis/Septicemia	11	0	11	19.6
Reproductive tract infection	2	4	6	10.7
Arthritis/Joint/Bone infection	3	2	5	8.9
Other*	3	1	4	7.1
Undetermined	1	2	3	5.4
Polyserositis/Peritonitis	3	0	3	5.4
Eye infection	1	0	1	1.8
Mixed/Secondary infection	1	0	1	1.8
Liver/Kidney/Spleen infection	1	0	1	1.8
Total	41	15	56	

Other diagnoses included *E. coli* infection (1), cellulitis (1), and co-occurring *E. coli* and *P. multocida* infection (1) in chickens. Other diagnoses in turkeys included co-occurring *E. coli* and *P. multocida* infection (1).

APPENDIX D: MIC Distributions and Clinical Signs for *E. coli, S. equi* and *S. zooepidemicus* in Horses

Table 22. Minimum inhibitory concentration (MIC) distributions in µg/ml for *Escherichia coli* isolates recovered from horses in 2020.

Antibiotic class	Antibiotic	Total	≤0.06	≤0.25	0.25	≤0.5	0.5	≤1	1	>1	≤2	2	>2	≤4	4	>4	≤8	8	>8	16	>16	32	>32	64	>64
Aminoglycoside	Amikacin	267									28			215	6			8		1		4	5		
	Gentamicin	267						215				8			4				40						
Ansamycin	Rifampin	267						4				11			89	163									
Beta-lactam	Ticarcillin	232															167			8		1		2	54
	Ticarcillin/Clavulan	232															185			21		9		10	7
Carbapenem	Imipenem	267						266										1							
Cephalosporin	Cefazolin	267										20		192	5			5	9	2	34				
	Ceftazidime	267						231				5			3			8		6	2	8		1	3
	Ceftiofur	267		82			137		5			3			1	30		1	8						
Fluoroquinolone	Enrofloxacin	267	29	208			4		4	6		2	14												
Folate pathway antagonist	Trimethoprim/Sulfa	267				157			1							109									
Macrolide	Azithromycin	232		4					7			73			101	47									
	Clarithromycin	267						5											262						
	Erythromycin	267		4														2	261						
Penicillin	Ampicillin	267		4					11			95			74			14			11		58		
	Oxacillin	267		4												263									
	Penicillin	267	4															3	260						
Phenicol	Chloramphenicol	267												98				118		6		4	41		
Tetracycline	Doxycycline	267					7		12		163	6			11			7	9	10	42				
	Minocycline	35			6		9		11				9												
	Tetracycline	267							20		167	5			3			1	71						

1: Equine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Total number of isolates for each antibiotic and MIC range reflect a combination of data from the Equin1F and Equin2F plates. Not all antibiotics in the table are present on both plates, leading to differences in total numbers of isolates. Interpretations of Sensitive (S), Intermediate (I), or Resistant (R) may not be possible for certain antibiotics due to breakpoint values falling below the lowest MIC dilutions available on the plates. 3: Amikacin breakpoints for adult horses are shown. Foal breakpoints in µg/ml are as follows: S ≤ 2, I = 4, R ≥ 8. 4: Enrofloxacin and doxycycline dilutions on the Equin1F and Equin2F plates are above the S and I breakpoint values. Thus, interpretation of MIC data was restricted to only R values. Equine enrofloxacin breakpoints in µg/ml are: S ≤ 0.12, I = 0.25, R ≥ 0.5, and doxycycline breakpoints in µg/ml are: S ≤ 0.12, I = 0.25, R ≥ 0.5. 5: Ticarcillin/clavulanate (abbrev: Clavulan) concentrations on the Equin1F and Equin2F plates = 8/2 µg/mL, 16/2 µg/mL, 32/2 µg/mL and 64/2 µg/mL. Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentrations on the Equin1F and Equin2F plates = 0.5/9.5 µg/mL, 1/19 µg/mL, and 4/76 µg/mL.

Figure 7. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in *Escherichia coli* isolates recovered from horses in 2018 – 2020.

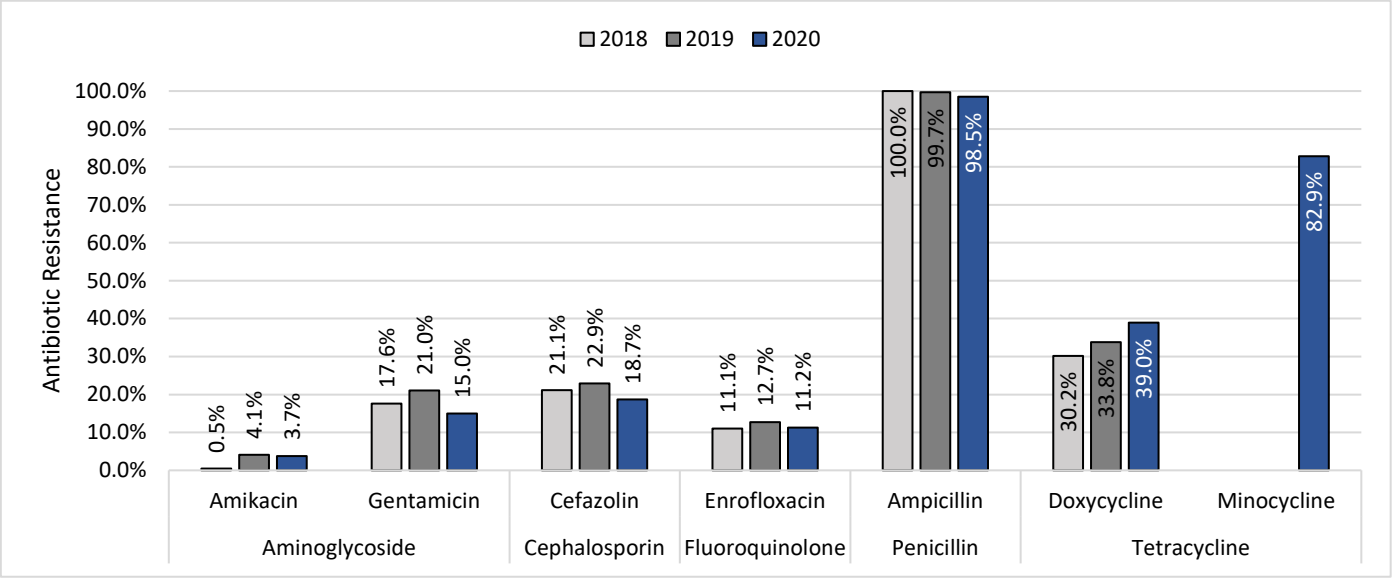


Table 23. Clinical signs and diagnoses associated with *Escherichia coli* infections recovered from horses in 2020.

Clinical signs/Indications	Counts in 2020	% of Counts	Clinical signs/Indications	Counts in 2020	% of Counts
Reproductive Tract infection	137	51.3	Abortion/Placental infection	3	1.1
Abscess/Skin/Wound infection	40	15.0	Other*	3	1.1
Pneumonia/Respiratory infection	20	7.5	Arthritis/Joint/Bone infection	2	0.7
Diarrhea/Gastroenteric infection	15	5.6	Peritonitis/Polyserositis	2	0.7
Undetermined	12	4.5	Liver/Kidney/Spleen infection	2	0.7
Sepsis/Septicemia	11	4.1	Mixed/Secondary infection	1	0.4
Neonatal infection	7	2.6	Central Nervous System infection	1	0.4
Breeding Management	6	2.2	Colibacillosis	1	0.4
Urinary Tract infection	4	1.5	Total	267	

*Other diagnoses included general infection (1), guttural pouch empyema (1), and visceral larval migrans (1).

Table 24. Per-isolate and per-antibiotic resistance phenotypes for *Escherichia coli* isolates recovered from horses in 2020.

Resistance phenotype (X antibiotics)		7	6	5	4	3	2	1	0
Isolates with resistance phenotype		1	7	11	25	30	41	150	10
aminoglycoside	amikacin	1	2	4 (1)	1 (2)	0 (1)	2	0 (2)	0 (2)
	gentamicin	1	7	11	17 (1)	3 (1)	1 (1)	0 (1)	0
cephalosporin	cefazolin	1	7	11	21	7 (3)	3 (2)	0	0
fluoroquinolone	enrofloxacin	1	7	5	10	3	4	0	0
penicillin	ampicillin	1	7	11	25	30	39	150	0
tetracycline	doxycycline	1	7	11	24	28	33	0	0
	minocycline	1	5	2	2	19	0 (6)	0	0

Values for each antimicrobial agent listed in rows 3 - 9 represent # isolates resistant to each antimicrobial (column 2) with an overall phenotype of resistance against X antimicrobials (row 1). Parenthetical values represent # isolates with intermediate susceptibility against each antimicrobial (column 2), where applicable. Interpretive values are based on the *Vet01S* (CLSI, 2020).

Table 25. Minimum inhibitory concentration (MIC) distributions in µg/ml for *Streptococcus equi* isolates recovered from horses in 2020.

Antibiotic class	Antibiotic	Total	≤0.06	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	≤2	2	≤4	4	>4	≤8	8	>8	16	32	>32
Aminoglycoside	Amikacin	75											10				6		9	11	39
	Gentamicin	75							14			8		11			22	20			
Ansamycin	Rifampin	75							71					1	3						
Beta-lactams	Ticarcillin	71														71					
	Ticarcillin/Clavulan	71														71					
Carbapenem	Imipenem	75							75												
Cephalosporin	Cefazolin	75							4				71								
	Ceftazidime	75							73			1		1							
	Ceftiofur	75			70			5													
Fluoroquinolone	Enrofloxacin	75			6			3		50		16									
Folate pathway antagonist	Trimethoprim/Sulfa	75					73			1		1									
Macrolide	Azithromycin	71			66			1		1		1			2						
	Clarithromycin	75			4				66			1						4			
	Erythromycin	75			70												1	4			
Penicillin	Ampicillin	75			71							3		1							
	Oxacillin	75			71										4						
	Penicillin	75	66	4		1												4			
Phenicol	Chloramphenicol	75											71				4				
Tetracycline	Doxycycline	75		3		1					69						2				
	Minocycline	4		4																	
	Tetracycline	75			3			1			62			6			1	2			

1: Equine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Total number of isolates for each antibiotic and MIC range reflect a combination of data from the Equin1F and Equin2F plates. Not all antibiotics in the table are present on both plates, leading to differences in total numbers of isolates. Interpretations of Sensitive (S), Intermediate (I), or Resistant (R) may not be possible for certain antibiotics due to breakpoint values falling below the lowest MIC dilutions available on the plates. 3: Cefazolin, enrofloxacin and doxycycline dilutions on the EQUIN1F antimicrobial sensitivity plate are above the breakpoint values for sensitive and intermediate. Thus interpretation of MIC data was restricted to only resistant values for this plate. Cefazolin breakpoints in µg/ml are: S ≤ 2, I = 4, R ≥ 8, and breakpoints for doxycycline and minocycline in µg/ml are: S ≤ 0.12, I = 0.25, R ≥ 0.5, and enrofloxacin breakpoints for horses are: S ≤0.12; I = 0.25; R ≥0.5. 4: Breakpoints for intermediate and resistant values for ampicillin have not been established for horses. 5: Ticarcillin/clavulanate concentrations on EQUIN1F plate = 8/2 µg/mL, 16/2 µg/mL, 32/2 µg/mL and 64/2 µg/mL. Trimethoprim/sulfamethoxazole concentrations on both EQUIN1F and EQUIN2F plates = 0.5/9.5 µg/mL, 1/19 µg/mL, 2/38, and 4/76 µg/mL.

Figure 8. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in *Streptococcus equi* isolates recovered from horses in 2019 – 2020.

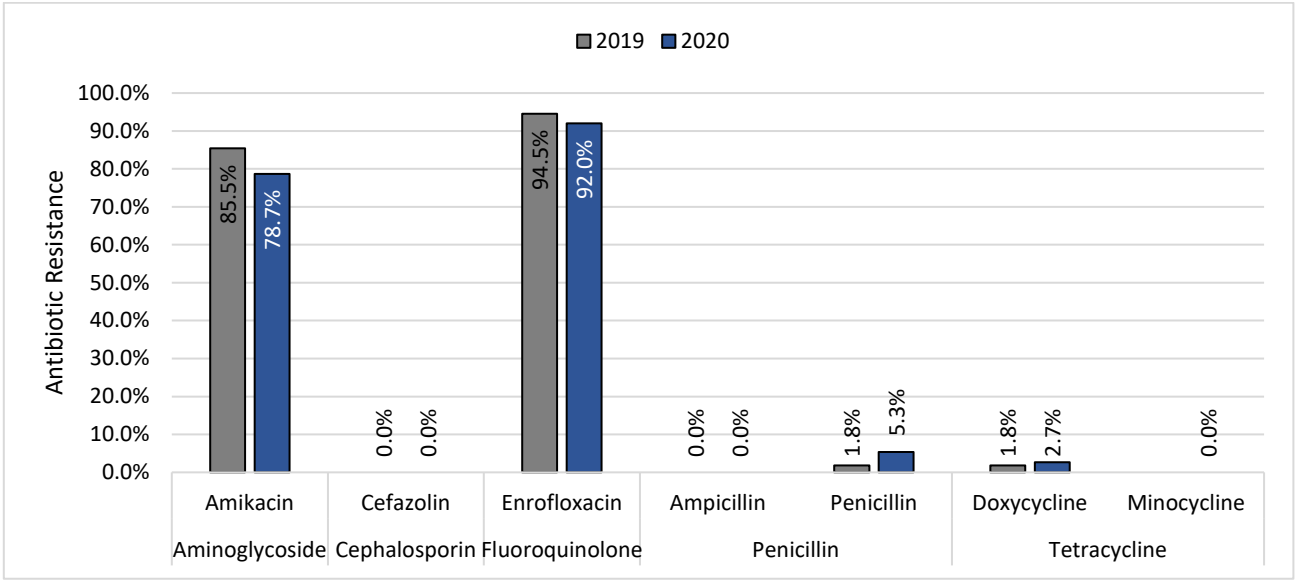


Table 26. Clinical signs and diagnoses associated with *Streptococcus equi* infections recovered from horses in 2020.

Clinical signs/Indications	Counts in 2020	% of Counts
Pneumonia/Respiratory infection	49	65.3
Abscess/Skin/Wound infection	18	24.0
Undetermined	5	6.7
Reproductive Tract infection	2	2.7
Mixed/Secondary infection	1	1.3
Total	75	

Table 27. Minimum inhibitory concentration (MIC) distributions in µg/ml for *Streptococcus equi* subspecies *zooepidemicus* isolates recovered from horses in 2020.

Antibiotic class	Antibiotic	Total	≤0.06	≤0.125	0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	>1	≤2	2	>2	≤4	4	>4	≤8	8	>8	16	>16	32	>32	64	>64
Aminoglycoside	Amikacin	369											2			32				24		71		37	203		
	Gentamicin	369								56				51			49			51	162						
Ansamycin	Rifampin	369								361				3			2	3									
Beta-lactams	Ticarcillin	333																	326			4				1	2
	Ticarcillin/Clavulan	333																	326			5		1		1	
Carbapenem	Imipenem	369								368				1													
Cephalosporin	Cefazolin	369								35				1		326				2			5				
	Ceftazidime	369								348				13			1			4		1				1	1
	Ceftiofur	369				351			8		3			1				6									
Fluoroquinolone	Enrofloxacin	369	1		1	11			77		249	2		22	6												
Folate pathway antagonist	Trimethoprim/Sulfa	369						349			4			2			4	10									
Macrolide	Azithromycin	333				318			6		4			2				3									
	Clarithromycin	369				35				324				2			1			2	5						
	Erythromycin	369				353			3		2						5				6						
Penicillin	Ampicillin	369				355			3		2			3						1			1	1	3		
	Oxacillin	369				349			6		1			1			1	11									
	Penicillin	369	313	35	7		1		4					1						1	7						
Phenicol	Chloramphenicol	369														348				14		2		2	3		
Tetracycline	Doxycycline	369		25			2				2		274				18			41	1	4	2				
	Minocycline	36		27					1					1	7												
	Tetracycline	369				2					1		135				102			50	79						

1: Equine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Total number of isolates for each antibiotic and MIC range reflect a combination of data from the Equin1F and Equin2F plates. Not all antibiotics in the table are present on both plates, leading to differences in total numbers of isolates. Interpretations of Sensitive (S), Intermediate (I), or Resistant (R) may not be possible for certain antibiotics due to breakpoint values falling below the lowest MIC dilutions available on the plates. 3:Cefazolin, enrofloxacin and doxycycline dilutions on the EQUIN1F antimicrobial sensitivity plate are above the breakpoint values for sensitive and intermediate. Thus interpretation of MIC data was restricted to only resistant values for this plate. Cefazolin breakpoints in µg/ml are: S ≤ 2, I = 4, R ≥ 8, and breakpoints for doxycycline and minocycline in µg/ml are: S ≤ 0.12, I = 0.25, R ≥ 0.5, and enrofloxacin breakpoints for horses are: S ≤0.12; I = 0.25; R ≥0.5. 4: Breakpoints for intermediate and resistant values for ampicillin have not been established for horses. 5: Ticarcillin/clavulanate (abbrev: Clavulan) concentrations on EQUIN1F plate = 8/2 µg/mL, 16/2 µg/mL, 32/2 µg/mL and 64/2 µg/mL. Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentrations on both EQUIN1F and EQUIN2F plates = 0.5/9.5 µg/mL, 1/19 µg/mL, 2/38, and 4/76 µg/mL.

Figure 9. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in *Streptococcus equi* subspecies *zooepidemicus* isolates recovered from horses in 2019 – 2020.

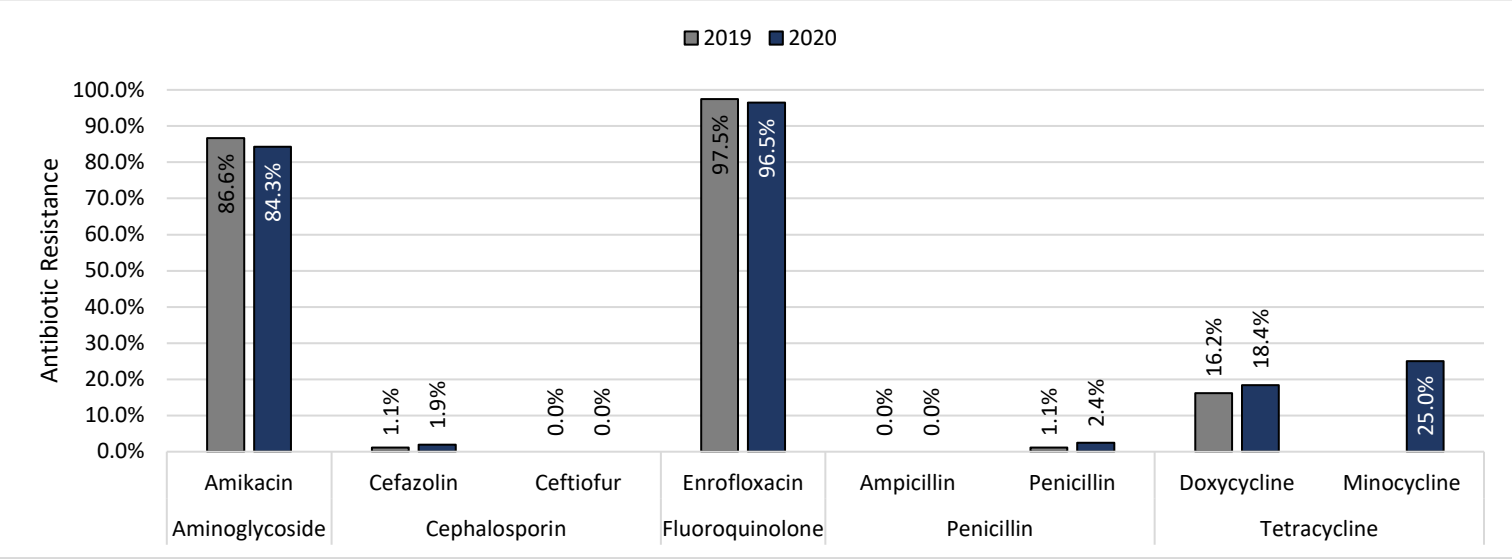


Table 28. Clinical signs and diagnoses associated with *Streptococcus equi* subspecies *zooepidemicus* infections recovered from horses in 2020.

Clinical signs/Indications	Counts in 2020	% of Counts	Clinical signs/Indications	Counts in 2020	% of Counts
Reproductive Tract infection	115	31.2	Diarrhea/Gastroenteric infection	4	1.1
Pneumonia/Respiratory infection	107	29.0	Eye infection	3	0.8
Abscess/Skin/Wound infection	54	14.6	Neonatal infection	2	0.5
Undetermined	32	8.7	Urinary Tract infection	2	0.5
Abortion/Placental infection	16	4.3	Mastitis	2	0.5
Arthritis/Joint/Bone infection	13	3.5	Liver/Kidney/Spleen infection	1	0.3
Breeding Management	10	2.7	Other*	1	0.3
Sepsis/Septicemia	6	1.6	Peritonitis/Polyserositis	1	0.3
*Other diagnoses inlude multifactorial cause of death (1).			Total	369	

Table 29. Per-isolate and per-antibiotic resistance phenotypes for *Streptococcus equi* subspecies *zooepidemicus* isolates recovered from horses in 2020.

Resistance phenotype (X antibiotics)		5	4	3	2	1	0
Isolates with resistance phenotype		2	9	50	260	44	4
aminoglycoside	amikacin	2	9	48	249 (4)	3 (19)	0 (1)
cephalosporin	cefazolin	2	2	2	1	0	0
fluoroquinolone	enrofloxacin	2	9	48	257	40	0
penicillin	penicillin	2	1	4	1	1	0
tetracycline	doxycycline	2	8	47	11 (2)	0	0
	minocycline	0	7	1	1	0	0

Values for each antimicrobial agent listed in rows 3 - 8 represent # isolates resistant to each antimicrobial (column 2) with an overall phenotype of resistance against X

antimicrobials (row 1). Parenthetical values represent # isolates with intermediate susceptibility against each antimicrobial (column 2), where applicable. Interpretive values are based on the *Vet01S* (CLSI, 2020).

APPENDIX E: MIC Distributions and Clinical Signs for *E. coli* and *S. intermedius* group in Dogs

Table 30. Minimum inhibitory concentration (MIC) distribution in µg/ml for *Escherichia coli* isolates recovered from dogs with urinary tract infections (UTIs) in 2020.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	≤2	2	>2	≤4	4	>4	≤8	8	>8	16	>16	32	>32	64	>64
Aminoglycoside	Amikacin	590											571				14		3		2			
	Gentamicin	590		33			371		145		16			4			2	19						
Beta-lactams	Amoxicillin/Clavul	590							3		51			345			109	82						
	Piperacillin/Tazo	590														572			11		3		1	3
Carbapenem	Imipenem	590						586			2			2										
Cephalosporin	Cefazolin	590						78			323			82			16		10		5	76		
	Cefovecin	590		31			265		178		29			10			2	75						
	Cefpodoxime	590						491			11			4			9	75						
	Ceftazidime	590											530				10		21	29				
	Cephalexin	590									2			164			306		24	94				
Fluoroquinolone	Enrofloxacin	590	492		16		17		7		2			1	55									
	Marbofloxacin	590	492		13		21		8		1			1	54									
	Orbifloxacin	590						515			10			8			1	56						
	Pradofloxacin	590		528			5		2		6	49												
Folate pathway antagonist	Trimethoprim/Sulfa	590				524			4		1			1	60									
Penicillin	Ampicillin	590							12		177			226			21	154						
Phenicol	Chloramphenicol	590								14				141			348		57		5	25		
Tetracycline	Doxycycline	590		1			26		161		261			61			23	57						
	Tetracycline	590											508				4		2	76				

1: Canine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Cefazolin, cephalexin, cefovecin, amoxicillin/clavulanic acid (abbrev: Clavul), and ampicillin have separate breakpoints for *E. coli* isolates recovered from canine urinary tract infections (UTIs). 3: Extended spectrum beta-lactamase (ESBL) testing is indicated for isolates with MIC values ≥ 8 µg/ml for cefpodoxime, or values >2 µg/ml for ceftazidime. 4: Intermediate and resistant breakpoint values for amoxicillin/clavul and ampicillin have not been established for canine UTIs. 5: Pradofloxacin is not approved for use in dogs in the U.S. 6: Amoxicillin/Clavul concentrations on the CompGN1F plate = 0.25/0.12 µg/mL, 0.5/0.25 µg/mL, 1/0.5 µg/mL, 2/1 µg/mL, 4/2 µg/mL, and 8/4 µg/mL. Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentrations on the CompGN1F plate = 0.12/2.38 µg/mL, 0.25/4.75 µg/mL, 0.5/9.5 µg/mL, 1/19 µg/mL, 2/38 µg/mL, and 4/76 µg/mL. 7: Piperacillin/Tazobactam (abbrev: Tazo).

Figure 10. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in *Escherichia coli* isolates recovered from dogs with urinary tract infections (UTIs) in 2018 – 2020.

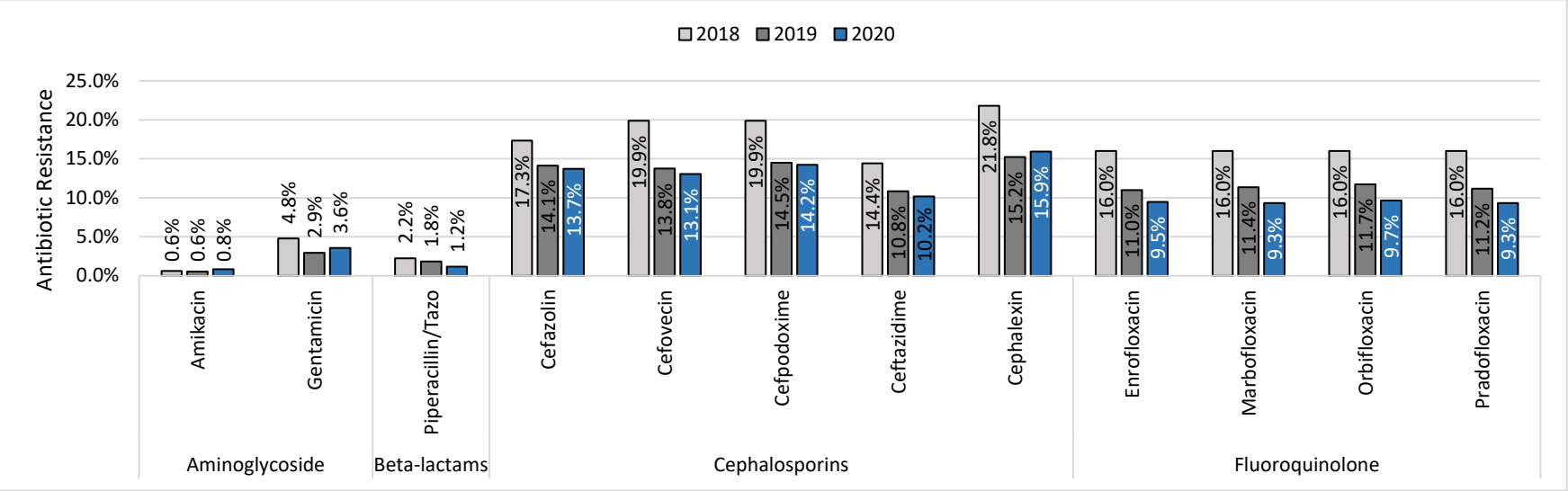


Table 31. Per-isolate and per-antibiotic resistance phenotypes for *Escherichia coli* isolates recovered from dogs with urinary tract infections (UTIs) in 2020.

Resistance phenotype (X antibiotics)		10	9	8	7	6	5	4	3	2	1	0
Isolates with resistance phenotype		1	8	25	1	2	8	50	6	10	13	466
Aminoglycoside	Amikacin	1	2 (1)	0 (1)	0 (1)	0	0 (1)	0 (2)	0 (1)	1 (1)	0 (1)	0 (6)
	Gentamicin	1	5 (3)	0	1	2	5	0	0	1	6	0 (1)
Beta-lactams	Amoxicillin/Clavul	0	0	0	0	0	0	0	0	0	0	0
	Piperacillin/Tazo	0 (1)	1	0 (1)	0 (1)	1	3	1 (5)	0	1	0	0 (3)
Cephalosporins	Cefazolin	1	8	25	1	2	5	31	6	2	0	0
	Cefovecin	1	8	25	1	2	5	33	1 (5)	1 (1)	0 (1)	0 (3)
	Cefpodoxime	1	8	25	1	2	5	32 (1)	5 (1)	5 (1)	0 (1)	0
	Cephalexin	1	8	25	1	2	5	32	6	8	6	0
Fluoroquinolone	Enrofloxacin	1	8	25	1	0 (1)	3	18 (4)	0	0 (1)	0 (2)	0 (1)
	Marbofloxacin	1	8	25	0	0	3	18 (1)	0	0	0	0
	Orbifloxacin	1	8	25	1	0 (1)	3 (2)	18 (6)	0	1	0 (2)	0 (7)
	Pradofloxacin	1	8	25	0 (1)	1	3	17 (4)	0	0 (1)	0 (1)	0
Penicillin	Ampicillin	0	0	0	0	0	0	0	0	0	0	0

Values for each antimicrobial agent listed in rows 3 - 15 represent # isolates resistant to each antimicrobial (column 2) with an overall phenotype of resistance against X antimicrobials (row 1). Parenthetical values represent # isolates with intermediate susceptibility against each antimicrobial (column 2), where applicable. Interpretive values are based on the *Vet01S* (CLSI, 2020).

Table 32. Minimum inhibitory concentration (MIC) distribution in µg/ml for *Escherichia coli* isolates recovered from dogs without urinary tract infections (non-UTIs) in 2020.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	≤2	2	>2	≤4	4	>4	≤8	8	>8	16	>16	32	>32	64	>64
Aminoglycoside	Amikacin	323											314				7		1			1		
	Gentamicin	323		18			203		79		5			1			1	16						
Beta-lactams	Amoxicillin/Clavul	323		1			2		4		22			154			62	78						
	Piperacillin/Tazo	323														314			4		1		1	3
Carbapenem	Imipenem	323						322			1													
Cephalosporin	Cefazolin	323						26			165			46			11		8		5	62		
	Cefovecin	323		14			108		114		14			10			2	61						
	Cefpodoxime	323						247			3			1			7	65						
	Ceftazidime	323											268				10		23	22				
	Cephalexin	323												79			151		16	77				
Fluoroquinolone	Enrofloxacin	323	270		7		11		3		1			1	30									
	Marbofloxacin	323	268		5		14		3		2			1	30									
	Orbifloxacin	323						279			9			3			1	31						
	Pradofloxacin	323		290			1		1		2	29												
Folate pathway antagonist	Trimethoprim/Sulfa	323				288			4						31									
Penicillin	Ampicillin	323		2					9		71			113			8	120						
Phenicol	Chloramphenicol	323								5				82			188		26		4	18		
Tetracycline	Doxycycline	323					12		90		150			27			15	29						
	Tetracycline	323											275						2	46				

1: Canine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Extended spectrum beta-lactamase (ESBL) testing is indicated for isolates with MIC values ≥ 8 mg/ml for cefpodoxime, or values >2 mg/ml for ceftazidime. 3: Pradofloxacin is not approved for use in dogs in the U.S. 4: Amoxicillin/Clavul concentrations on the CompGN1F plate = 0.25/0.12 µg/mL, 0.5/0.25 µg/mL, 1/0.5 µg/mL, 2/1 µg/mL, 4/2 µg/mL, and 8/4 µg/mL. Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentrations on the CompGN1F plate = 0.12/2.38 µg/mL, 0.25/4.75 µg/mL, 0.5/9.5 µg/mL, 1/19 µg/mL, 2/38 µg/mL, and 4/76 µg/mL. 5: Piperacillin/Tazobactam (abbrev: Tazo).

Figure 11. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in *Escherichia coli* isolates recovered from dogs without urinary tract infections (non-UTIs) in 2018 – 2020.

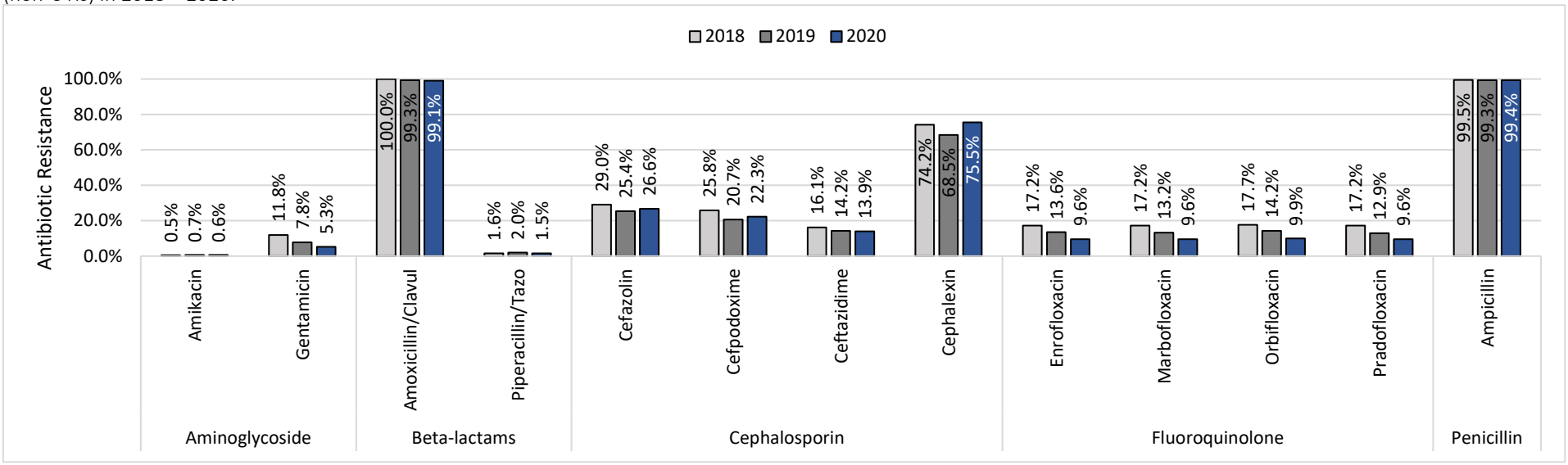


Table 33. Clinical signs and diagnoses associated with *Escherichia coli* infections recovered from dogs without urinary tract infections (non-UTIs) in 2020.

Clinical signs/Indications	Counts in 2020	% of Counts	Clinical signs/Indications	Counts in 2020	% of Counts
Abscess/Skin/Wound infection	94	29.1%	Sepsis/Septicemia	10	3.1%
Reproductive tract infection	58	18.0%	Peritonitis/Polyserositis	9	2.8%
Otitis/Ear infection	42	13.0%	Mastitis	6	1.9%
Pneumonia/Respiratory infection	41	12.7%	Other*	4	1.2%
Diarrhea/Gastroenteric infection	30	9.3%	Eye infection	2	0.6%
Liver/Kidney/Spleen infection	13	4.0%	Mixed/secondary infection	1	0.3%
Undetermined	13	4.0%	Total	323	

*Other diagnoses included congenital disorder (1), *E. coli* co-infection with *S. canis* and *S. pseudintermedius* (1), thoracic cavity infection (1), and mast cell tumor (1).

Table 34. Per-isolate and per-antibiotic resistance phenotypes for *Escherichia coli* isolates recovered from dogs without urinary tract infections (non-UTIs) in 2020.

Resistance phenotype (X antibiotics)		12	11	10	9	8	7	6	5	4	3	2	1	0
Isolates with resistance phenotype		1	4	14	6	1	8	28	16	18	152	73	1	1
Aminoglycoside	Amikacin	0	0	0	0 (1)	0	1 (1)	0 (1)	0 (1)	1	0 (3)	0	0	0
	Gentamicin	1	4	4	0	1	4	0	0	3 (1)	0	0	0	0
Beta-lactams	Amoxicillin/Clavul	1	4	14	6	1	7	28	16	18	152	72 (1)	1	0 (1)
	Piperacillin/Tazo	1	0 (2)	1	0	0	2	0	0 (2)	1	0	0	0	0
Cephalosporin	Cefazolin	1	4	14	6	0 (1)	6 (2)	25	16	12 (3)	2 (34)	0 (6)	0	0
	Cefpodoxime	1	4	14	6	0	6	25	16	0 (1)	0	0	0	0
	Ceftazidime	1	4	9 (4)	0 (1)	0	6	25	0 (4)	0	0 (1)	0	0	0
	Cephalexin	1	4	14	6	1	8	25 (3)	16	18	150 (2)	1 (72)	0 (1)	0 (1)
Fluoroquinolone	Enrofloxacin	1	4	14	6	1	2	3	0 (1)	0 (2)	0 (1)	0	0	0

	Marbofloxacin	1	4	14	6	1	2	3	0 (1)	0 (1)	0	0	0	0
	Orbifloxacin	1	4	14	6	1	2 (1)	3	0 (2)	1 (1)	0 (7)	0 (1)	0	0
	Pradofloxacin	1	4	14	6	1	2	3	0	0 (1)	0 (1)	0	0	0
Penicillin	Ampicillin	1	4	14	6	1	8	28	16	18	152	73	0	0

Values for each antimicrobial agent listed in rows 3 - 15 represent # isolates resistant to each antimicrobial (column 2) with an overall phenotype of resistance against X antimicrobials (row 1). Parenthetical values represent # isolates with intermediate susceptibility against each antimicrobial (column 2), where applicable. Interpretive values are based on the *Vet01S* (CLSI, 2020).

Table 35. Minimum inhibitory concentration (MIC) distribution in µg/ml for oxacillin sensitive (OxS) *Staphylococcus intermedius* group isolates recovered from dogs with urinary tract infections (UTIs).

Antibiotic class	Antibiotic	Total	≤0.06	≤0.125	0.125	≤0.25	0.25	≤0.5	0.5	>0.5	≤1	1	>1	≤2	2	>2	≤4	4	>4	≤8	8	>8	≤16	16	>16	32	>32
Aminoglycoside	Amikacin	148																					148				
	Gentamicin	148															134				3			10	1		
Ansamycin	Rifampin	148									147				1												
Beta-lactams	Amoxicillin/Clavul	148				146			1						1												
Carbapenem	Imipenem	148									147				1												
Cephalosporin	Cefazolin	148												147					1								
	Cefovecin	148	2		71		63		11						1												
	Cefpodoxime	148												148													
	Cephalothin	148												147				1									
Fluoroquinolone	Enrofloxacin	148				116			13			4						1	14								
	Marbofloxacin	148									131				3			1	13								
	Pradofloxacin	148				132			1			3			7	5											
Folate pathway antagonist	Trimethoprim/Sulfa	148												132				6	10								
Glycopeptide	Vancomycin	148									146				2												
Lincosamide	Clindamycin	148						129											19								
Macrolide	Erythromycin	148				92			36			1							19								
Nitrofuran	Nitrofurantoin	148																					148				
Penicillin	Ampicillin	148				109			16			7			7			4			3	2					
	Oxacillin	148				148																					
	Penicillin	148	62		11		18		14			5			3			9			9	17					
Phenicol	Chloramphenicol	148																		134				2		3	9
Tetracycline	Doxycycline	148		101			5		2	40																	
	Minocycline	148						108				1			10	29											
	Tetracycline	148				100			6			2	40														

1: Canine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: I and R breakpoints have not been established for amoxicillin/clavulanic acid (abbrev: Clavul) in canine urinary tract infections (UTIs). 3: Pradofloxacin is not approved for use in dogs in the U.S. 4: Human-derived breakpoints for oxacillin (S ≤ 0.25 µg/mL, R ≥ 0.5 µg/mL) were used to categorize isolates as oxacillin-sensitive (OxS) or oxacillin-resistant (OxR). 5: Amoxicillin/Clavul concentrations on the CompGN1F plate = 0.25/0.12 µg/mL, 0.5/0.25 µg/mL, 1/0.5 µg/mL, 2/1 µg/mL, 4/2 µg/mL, and 8/4 µg/mL. Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentrations on the CompGN1F plate = 0.12/2.38 µg/mL, 0.25/4.75 µg/mL, 0.5/9.5 µg/mL, 1/19 µg/mL, 2/38 µg/mL, and 4/76 µg/mL.

Figure 12. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in oxacillin sensitive (OxS) *Staphylococcus intermedius* group isolates recovered from dogs with urinary tract infections (UTIs) in 2018 – 2020.

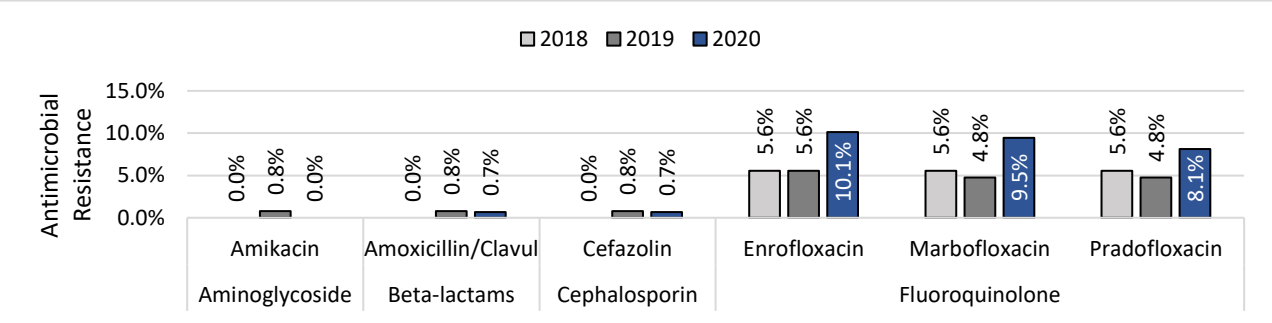


Table 36. Minimum inhibitory concentration (MIC) distribution in µg/ml for oxacillin resistant (OxR) *Staphylococcus intermedius* group isolates recovered from dogs with urinary tract infections (UTIs) in 2020.

Antibiotic class	Antibiotic	Total	≤0.06	≤0.125	0.125	≤0.25	0.25	≤0.5	0.5	>0.5	≤1	1	>1	≤2	2	>2	≤4	4	>4	≤8	8	>8	≤16	16	>16	32	>32
Aminoglycoside	Amikacin	58																					57				1
	Gentamicin	58															30				10			15	3		
Ansamycin	Rifampin	58									55					3											
Beta-lactams	Amoxicillin/Clavul	58				11			18			10			3			6			6	4					
Carbapenem	Imipenem	58									58																
Cephalosporin	Cefazolin	58												43				2	13								
	Cefovecin	58			1		2		2			8			5			6			9	25					
	Cefpodoxime	58												18				9			8	23					
	Cephalothin	58												49				2	7								
Fluoroquinolone	Enrofloxacin	58				10			1			3						1	43								
	Marbofloxacin	58									13				2			1	42								
	Pradofloxacin	58				14						2			15	27											
Folate pathway antagonist	Trimethoprim/Sulfa	58												21				4	33								
Glycopeptide	Vancomycin	58									56				2												
Lincosamide	Clindamycin	58						18											40								
Macrolide	Erythromycin	58				13			6										39								
Nitrofuran	Nitrofurantoin	58																					58				
Penicillin	Ampicillin	58				1			3			3			5			5			9	32					
	Oxacillin	58							9			12			7	30											
	Penicillin	58	1				1		1			1			3			1			2	48					
Phenicol	Chloramphenicol	58																		45						1	12
Tetracycline	Doxycycline	58		12						46																	
	Minocycline	58						13							4	41											
	Tetracycline	58				12							46														

1: Canine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Cefazolin, cephalothin, cefovecin, cefpoxodime, amoxicillin/clavulanic acid (abbrev: Clavul), and penicillin would be reported as Resistant (R) based on oxacillin resistance. 3: Pradofloxacin is not approved for use in dogs in the U.S. 4: Human-derived breakpoints for oxacillin (S ≤ 0.25 µg/mL, R ≥ 0.5 µg/mL) were used to categorize isolates as oxacillin-sensitive (OxS) or oxacillin-resistant (OxR). 5: Amoxicillin/Clavul concentrations on the CompGP1F plate = 0.25/0.12 µg/mL, 0.5/0.25 µg/mL, 1/0.5 µg/mL, 2/1 µg/mL, 4/2 µg/mL, and 8/4 µg/mL. Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentrations on the CompGP1F plate = 2/38 µg/mL and 4/76 µg/mL.

Figure 13. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in oxacillin resistant (OxR) *Staphylococcus intermedius* group isolates recovered from dogs with urinary tract infections (UTIs) in 2018 – 2020.

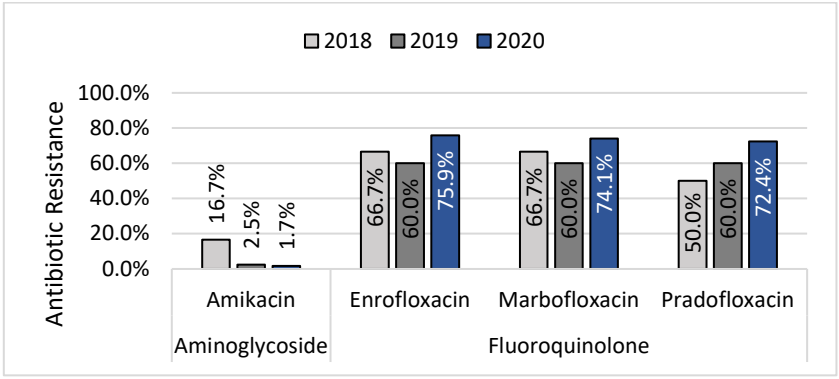


Table 37. Minimum inhibitory concentration (MIC) distribution in µg/ml for oxacillin sensitive (OxS) *Staphylococcus intermedius* group isolates recovered from dogs without urinary tract infections (non-UTIs).

Antibiotic class	Antibiotic	Total	≤0.06	≤0.125	0.125	≤0.25	0.25	≤0.5	0.5	>0.5	≤1	1	>1	≤2	2	>2	≤4	4	>4	≤8	8	>8	≤16	16	>16	32	>32	64
Aminoglycoside	Amikacin	467																					461			2	4	
	Gentamicin	467															424				13			19	11			
Ansamycin	Rifampin	467									465					2												
Beta-lactams	Amoxicillin/Clavul	467				463			3			1																
Carbapenem	Imipenem	467									466								1									
Cephalosporin	Cefazolin	467												466				1										
	Cefovecin	467	5		205		234		20			3																
	Cefpodoxime	467												465				1			1							
	Cephalothin	467												466				1										
Fluoroquinolone	Enrofloxacin	467				395			15			35			4			1	17									
	Marbofloxacin	467									444				4			4	15									
	Pradofloxacin	467				441			4			5			6	11												
Folate pathway antagonist	Trimethoprim/Sulfa	467												419				15	33									
Glycopeptide	Vancomycin	467									461				5			1										
Lincosamide	Clindamycin	467						399				5			1			1	61									
Macrolide	Erythromycin	467				271			125			1			3			2	65									
Nitrofuran	Nitrofurantoin	467																					466					1
Penicillin	Ampicillin	467				321			44			33			27			19				10	13						
	Oxacillin	467				467																						
	Penicillin	467	182		23		39		43			22			27			27				33	71						
Phenicol	Chloramphenicol	467																		424				4		4	35	
Tetracycline	Doxycycline	467		319			27		6	115																		
	Minocycline	467						348				9			20	90												
	Tetracycline	467				322			29			1	115															

1: Canine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Cefpoxodime breakpoints are established for wounds, abscesses, and urinatry tract infections only in dogs. 3: Antibiotic sensitivity plate dilutions for amikacin = 16 µg/mLand 32 µg/mL. Canine amikacin breakpoints are as follows: S ≤ 4 µg/mL, I = 8 µg/mL, R ≥ 16 µg/mL.4: Human-derived breakpoints for oxacillin (S ≤ 0.25 µg/mL, R ≥ 0.5 µg/mL) were used to categorize isolates as oxacillin-sensitive (OxS) or oxacillin-resistant (OxR). 5: Amoxicillin/Clavul concentrations on the CompGP1F plate = 0.25/0.12 µg/mL, 0.5/0.25 µg/mL, 1/0.5 µg/mL, 2/1 µg/mL, 4/2 µg/mL, and 8/4 µg/mL. Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentrations on the CompGN1F plate = 0.12/2.38 µg/mL, 0.25/4.75 µg/mL, 0.5/9.5 µg/mL, 1/19 µg/mL, 2/38 µg/mL, and 4/76 µg/mL.

Figure 14. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in oxacillin sensitive (OxS) *Staphylococcus intermedius* group isolates recovered from dogs without urinary tract infections (non-UTIs) in 2018 – 2020.

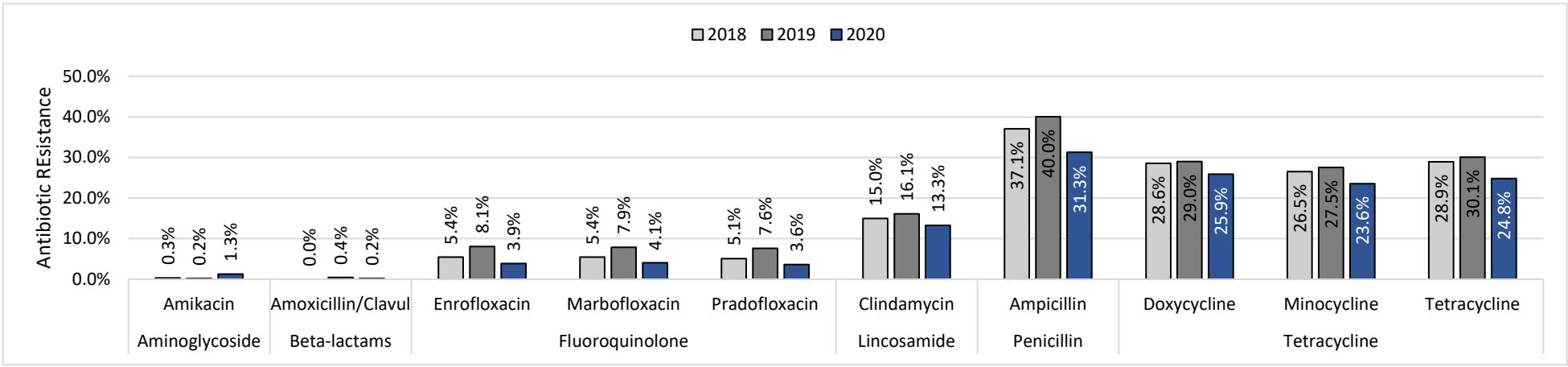


Table 38. Per-isolate and per-antibiotic resistance phenotypes for oxacillin sensitive (OxS) *Staphylococcus intermedius* group isolates recovered from dogs without urinary tract infections (non-UTIs) in 2020.

Resistance phenotype (X antibiotics)		8	7	6	5	4	3	2	1	0
Isolates with resistance phenotype		5	7	2	16	43	42	14	110	228
Aminoglycoside	Amikacin	1	0	0	0	1	0	1	3	0
Beta-lactams	Amoxicillin/Clavul	0	0	0	0	0	0	0 (1)	1 (1)	0 (1)
Cephalosporins	Cefazolin	0	0	0	0	0	0	0 (1)	0	0
	Cefovecin	0	0	0	0	0 (1)	0	0 (1)	0	0 (1)
	Cefpodoxime	0	0	0	0	0	0	1	0 (1)	0
	Cephalothin	0	0	0	0	0	0	0 (1)	0	0
Fluoroquinolone	Enrofloxacin	5	7	2	1 (3)	1 (9)	2 (3)	0 (1)	0 (7)	0 (16)
	Marbofloxacin	5	7	2	2	1	2 (1)	0 (1)	0 (2)	0
	Pradofloxacin	5	7	2	1 (2)	1	1 (1)	0 (1)	0 (2)	0 (3)
Lincosamide	Clindamycin	5	5	0	14	18	1 (1)	7 (1)	12 (1)	0 (3)
Penicillin	Ampicillin	4	3	0	14	24	2	10	89	0
Tetracycline	Doxycycline	5	7	2	16	42	40 (1)	5 (1)	4 (19)	0 (6)
	Minocycline	5	6 (1)	2	16	42	38 (1)	1 (2)	0 (3)	0 (2)
	Tetracycline	5	7	2	16	42	40	3 (2)	1 (19)	0 (8)

Values for each antimicrobial agent listed in rows 3 - 16 represent # isolates resistant to each antimicrobial (column 2) with an overall phenotype of resistance against X antimicrobials (row 1). Parenthetical values represent # isolates with intermediate susceptibility against each antimicrobial (column 2), where applicable. Interpretive values are based on the *Vet01S* (CLSI, 2020).

Table 39. Clinical signs and diagnoses associated with oxacillin sensitive (OxS) *Staphylococcus intermedius* group isolates recovered from dogs without urinary tract infections (non-UTIs).

Clinical signs/Indications	Counts in 2020	% of Counts	Clinical signs/Indications	Counts in 2020	% of Counts
Abscess/Skin/Wound infection	254	54.4%	Peritonitis/Polyserositis	5	1.1%

Otitis/Ear infection	113	24.2%	Arthritis/Joint/Bone infection	3	0.6%
Pneumonia/Respiratory infection	27	5.8%	Mastitis	2	0.4%
Undetermined	20	4.3%	Abortion/Placental infection	2	0.4%
Reproductive tract infection	13	2.8%	Liver/Kidney/Spleen infection	2	0.4%
Eye infection	13	2.8%	Mixed/secondary infection	1	0.2%
Sepsis/Septicemia	6	1.3%	Diarrhea/Gastroenteric infection	1	0.2%
Other*	5	1.1%	Total	467	

*Other diagnoses included lymphadenitis (1), infection (2), paraplegia (1), and stomatitis (1).

Table 40. Minimum inhibitory concentration (MIC) distribution in µg/ml for oxacillin resistant (OxR) *Staphylococcus intermedius* group isolates recovered from dogs without urinary tract infections (non-UTIs) in 2020.

Antibiotic class	Antibiotic	Total	≤0.06	≤0.125	0.125	≤0.25	0.25	≤0.5	0.5	>0.5	≤1	1	>1	≤2	2	>2	≤4	4	>4	≤8	8	>8	≤16	16	>16	32	>32	64	>64
Aminoglycoside	Amikacin	289																					283			2	4		
	Gentamicin	289															119				75			59	36				
Ansamycin	Rifampin	289									271				6	12													
Beta-lactams	Amoxicillin/Clavul	289				39			71			42			31			40			40	26							
Carbapenem	Imipenem	289									282				4				3										
Cephalosporin	Cefazolin	289												184				21	84										
	Cefovecin	289			1		4		7			24			35			26			30	162							
	Cefpodoxime	289												71				24			29	165							
	Cephalothin	289												213				14	62										
Fluoroquinolone	Enrofloxacin	289				35			8			20			4			5	217										
	Marbofloxacin	289									59				4			9	217										
	Pradofloxacin	289				62			6			6			86	129													
Folate pathway antagonist	Trimethoprim/Sulfa	289												75				37	177										
Glycopeptide	Vancomycin	289									279				5						1				4				
Lincosamide	Clindamycin	289						44				2			1			1	241										
Macrolide	Erythromycin	289				23			22			1			1			1	241										
Nitrofuran	Nitrofurantoin	289																					283					3	3
Penicillin	Ampicillin	289				6			7			16			14			12			33	201							
	Oxacillin	289							31			50			36	172													
	Penicillin	289	2				1		5			5			9			10			10	247							
Phenicol	Chloramphenicol	289																		183				40		3	63		
Tetracycline	Doxycycline	289		40			5		2	242																			
	Minocycline	289						48				4			24	213													
	Tetracycline	289				40			6			2	241																

1: Canine-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Cefazolin, cephalothin, cefovecin, cefpoxodime, amoxicillin/clavulanic acid (abbrev: Clavul), and penicillin would be reported as Resistant (R) based on oxacillin resistance. 3: Antibiotic sensitivity plate dilutions for amikacin = 16 µg/mL and 32 µg/mL. Amikacin breakpoints are as follows: S ≤ 4 µg/mL, I = 8 µg/mL, R ≥ 16 µg/mL. 4: Pradofloxacin is not approved for use in dogs in the U.S. 5: Human-derived breakpoints for oxacillin (S ≤ 0.25 µg/mL, R ≥ 0.5 µg/mL) were used to categorize isolates as oxacillin-sensitive (OxS) or oxacillin-resistant (OxR). 6: Amoxicillin/Clavul concentrations on the CompGP1F plate = 0.25/0.12 µg/mL, 0.5/0.25 µg/mL, 1/0.5 µg/mL, 2/1 µg/mL, 4/2 µg/mL, and 8/4 µg/mL. Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentrations on the CompGP1F plate = 2/38 µg/mL and 4/76 µg/mL.

Figure 15. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in oxacillin resistant (OxR) *Staphylococcus intermedius* group isolates recovered from dogs without urinary tract infections (non-UTIs) in 2018 – 2020.

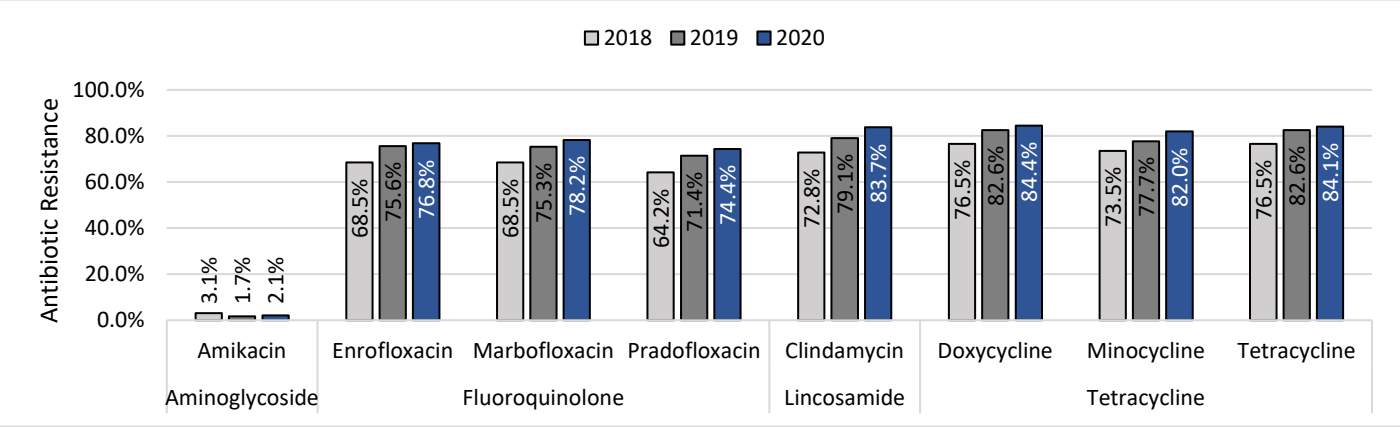


Table 41. Per-isolate and per-antibiotic resistance phenotypes for oxacillin resistant (OxR) *Staphylococcus intermedius* group isolates recovered from dogs without urinary tract infections (non-UTIs) in 2020.

Resistance phenotype (X antibiotics)		8	7	6	5	4	3	2	1	0
Isolates with resistance phenotype		2	187	16	4	29	18	8	8	17
Aminoglycoside	Amikacin	2	0	0	1	0	0	2	1	0
Fluoroquinolone	Enrofloxacin	2	187	16	0 (4)	7 (7)	5 (3)	5 (1)	0 (3)	0 (6)
	Marbofloxacin	2	187	15	3	8	5 (1)	6 (2)	0 (1)	0
	Pradofloxacin	2	187	13 (3)	1 (2)	7 (1)	5	0 (5)	0 (1)	0
Lincosamide	Clindamycin	2	187	8	3 (1)	29	3 (1)	3	7 (1)	0
Tetracycline	Doxycycline	2	187	16	4	22 (1)	13	0 (3)	0	0 (1)
	Minocycline	2	187	13 (3)	4	21	10 (1)	0	0	0
	Tetracycline	2	187	15	4	22 (2)	13	0 (3)	0	0 (1)

Values for each antimicrobial agent listed in rows 3 - 10 represent # isolates resistant to each antimicrobial (column 2) with an overall phenotype of resistance against X antimicrobials (row 1). Parenthetical values represent # isolates with intermediate susceptibility against each antimicrobial (column 2), where applicable. Interpretive values are based on the *Vet01S* (CLSI, 2020).

Table 42. Clinical signs and diagnoses associated with oxacillin resistant (OxR) *Staphylococcus intermedius* group isolates recovered from dogs without urinary tract infections (non-UTIs) in 2020.

Clinical signs/Indications	Counts in 2020	% of Counts	Clinical signs/Indications	Counts in 2020	% of Counts
Abscess/Skin/Wound infection	191	66.1%	Reproductive tract infection	6	2.1%
Otitis/Ear infection	50	17.3%	Otitis/Ear infection	4	1.4%
Eye infection	10	3.5%	Sepsis/Septicemia	3	1.0%
Undetermined	8	2.8%	Mixed/Secondary infection	1	0.3%
Arthritis/Joint/Bone infection	7	2.4%	Diarrhea/Gastroenteric infection	1	0.3%
Pneumonia/Respiratory infection	7	2.4%	Liver/Kidney/Spleen infection	1	0.3%
			Total	289	

APPENDIX F: MIC Distributions and Clinical Signs for *E. coli* and *S. intermedius* group in Cats

Table 43. Minimum inhibitory concentration (MIC) distributions in µg/ml for *Escherichia coli* isolates recovered from cats with urinary tract infections (UTIs) in 2020.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	≤2	2	>2	≤4	4	>4	≤8	8	>8	16	>16	32	>32	64	>64
Aminoglycoside	Amikacin	322											313				9							
	Gentamicin	322		27			190		85		9						1	10						
Beta-lactams	Amoxicillin/Clavul	322							8		51			140			72	51						
	Piperacillin/Tazo	322														313			3		1		2	3
Carbapenem	Imipenem	322						320			1			1										
Cephalosporin	Cefazolin	322						68			161			32			20		7		4	30		
	Cefovecin	322		42			154		72		17			4			2	31						
	Cefpodoxime	322						277			4			3			6	32						
	Ceftazidime	322											299				6		9	8				
	Cephalexin	322									5			136			129		9	43				
Fluoroquinolone	Enrofloxacin	322	297		7				1						17									
	Marbofloxacin	322	296		5		5								16									
	Orbifloxacin	322						303			1			1				17						
	Pradofloxacin	322		303			1		1		2	15												
Folate pathway antagonist	Trimethoprim/Sulfa	322				306			1		2				13									
Penicillin	Ampicillin	322		1			1		14		127			58			11	110						
Phenicol	Chloramphenicol	322								5				114			163		33			7		
Tetracycline	Doxycycline	322		2			32		152		96			17			4	19						
	Tetracycline	322											297				1		2	22				

1: Feline-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Cefovecin only has feline E. coli breakpoints for urinary tract infections. 3: Extended spectrum beta-lactamase (ESBL) testing is indicated for isolates with MIC values ≥ 8 µg/ml for cefpoxodime or >2 µg/ml for ceftazidime. 3: Amoxicillin/clavulanic acid concentrations on COMPGN1F plate are 0.25/0.12 µg/mL, 0.5/0.25 µg/mL, 1/0.5 µg/mL, 2/1 µg/mL, 4/2 µg/mL and 8/4 µg/mL. Trimethoprim/sulfamethoxazole concentrations are 0.12/2.38 µg/mL, 0.25/4.75 µg/mL, 0.5/9.5 µg/mL, 1/19 µg/mL, 2/38 µg/mL, and 4/76 µg/mL.

Table 44. Minimum inhibitory concentration (MIC) distributions in µg/ml for *Escherichia coli* non-urinary tract infection (non-UTIs) isolates recovered from cats.

Antibiotic class	Antibiotic	Total	≤0.125	≤0.25	0.25	≤0.5	0.5	≤1	1	≤2	2	>2	≤4	4	>4	≤8	8	>8	16	>16	32	>32
Aminoglycoside	Amikacin	119											111				5		1			2
	Gentamicin	119		4			66		38		5							6				
Beta-lactams	Amoxicillin/Clavul	119					1		1		6			53			29	29				
	Piperacillin/Tazo	119														117			2			
Carbapenem	Imipenem	119						119														
Cephalosporin	Cefazolin	119						18			57			23			5		1		2	13
	Cefovecin	119		12			60		27		3			1			1	15				
	Cefpodoxime	119						101			1			1			1	15				
	Ceftazidime	119											105				3		7	4		
	Cephalexin	119									2			48			45		6	18		
Fluoroquinolone	Enrofloxacin	119	101		5		2		4						7							
	Marbofloxacin	119	99		1		5		5		2				7							
	Orbifloxacin	119						105			6			1				7				
	Pradofloxacin	119		110			2				1	6										
Folate pathway antagonist	Trimethoprim/Sulfa	119				110					1				8							
Penicillin	Ampicillin	119					1		2		37			19			2	58				
Phenicol	Chloramphenicol	119								2				42			64		7		1	3
Tetracycline	Doxycycline	119					17		49		35			4			4	10				
	Tetracycline	119											103						3	13		

1: Feline-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Extended spectrum beta-lactamase (ESBL) testing is indicated for isolates with MIC values ≥ 8 µg/ml for cefpodoxime or >2 µg/ml for ceftazidime. 3: Amoxicillin/clavulanic acid (abbrev: Clavul) concentrations on the CompGN1F plate are 0.25/0.12 µg/mL, 0.5/0.25 µg/mL, 1/0.5 µg/mL, 2/1 µg/mL, 4/2 µg/mL and 8/4 µg/mL. Trimethoprim/sulfamethoxazole (abbrev: Sulfa) concentrations are 0.12/2.38 µg/mL, 0.25/4.75 µg/mL, 0.5/9.5 µg/mL, 1/19 µg/mL, 2/38 µg/mL, and 4/76 µg/mL.

Figure 16. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in *Escherichia coli* isolates recovered from cats without urinary tract infections (non-UTIs) in 2018 – 2020.

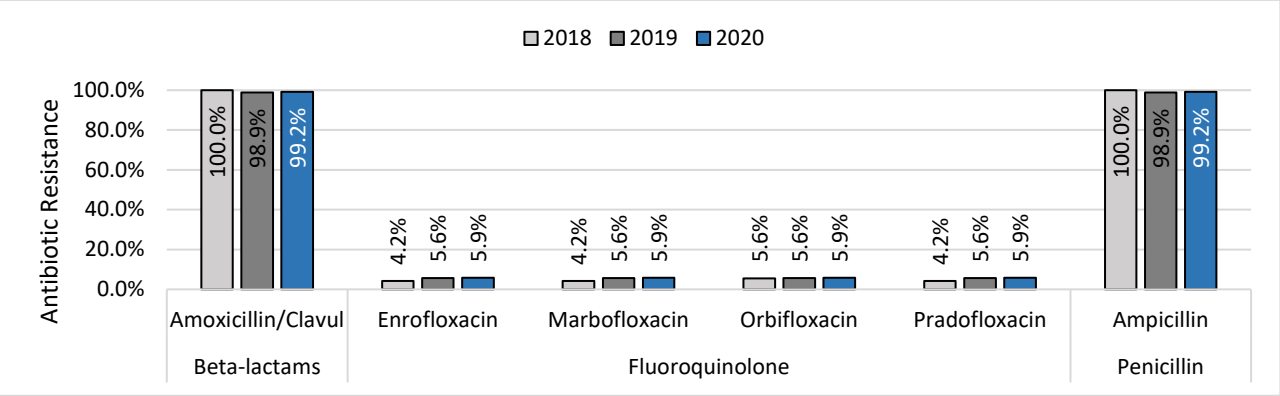


Table 45. Clinical signs and diagnoses associated with *Escherichia coli* isolates recovered from cats without urinary tract infections (non-UTIs) in 2020.

Clinical signs/Indications	Counts in 2020	% of Counts	Clinical signs/Indications	Counts in 2020	% of Counts
Diarrhea/Gastroenteric infection	27	22.7%	Sepsis/Septicemia	5	4.2%
Abscess/Skin/Wound infection	26	21.8%	Undetermined	5	4.2%
Reproductive tract infection	14	11.8%	Peritonitis/Polyserositis	3	2.5%
Pneumonia/Respiratory infection	13	10.9%	Other*	2	1.7%
Liver/Kidney/Spleen infection	12	10.1%	Abortion/Placental infection	1	0.8%
Otitis/Ear infection	11	9.2%	Total	119	

Table 46. Per-isolate and per-antibiotic resistance phenotypes for *Escherichia coli* isolates recovered from cats without urinary tract infections (non-UTIs) in 2020.

Resistance phenotype (X antibiotics)		6	5	4	3	2	1	0
Isolates with resistance phenotype		7	0	0	0	111	0	1
Beta-lactams	Amoxicillin/Clavul	7	0	0	0	111	0	0 (1)
Fluoroquinolone	Enrofloxacin	7	0	0	0	0 (3)	0	0 (1)
	Marbofloxacin	7	0	0	0	0 (1)	0	0 (1)
	Orbifloxacin	7	0	0	0	0 (6)	0	0 (1)
	Pradofloxacin	7	0	0	0	0 (1)	0	0 (1)
Penicillin	Ampicillin	7	0	0	0	111	0	0 (1)

Values for each antimicrobial agent listed in rows 3 - 8 represent # isolates resistant to each antimicrobial (column 2) with an overall phenotype of resistance against X antimicrobials (row 1). Parenthetical values represent # isolates with intermediate susceptibility against each antimicrobial (column 2), where applicable. Interpretive values are based on the *Vet01S* (CLSI, 2020).

Table 47. Minimum inhibitory concentration (MIC) distributions in µg/ml for oxacillin sensitive (OxS) *Staphylococcus intermedius* group isolates recovered from cats with urinary tract infections (UTIs) in 2020.

Antibiotic class	Antibiotic	Total	≤0.06	≤0.125	0.125	≤0.25	0.25	≤0.5	0.5	>0.5	≤1	1	>1	≤2	2	>2	≤4	4	>4	≤8	8	≤16	16
Aminoglycoside	Amikacin	14																				14	
	Gentamicin	14															13						1
Ansamycin	Rifampin	14									14												
Beta-lactams	Amoxicillin/Clavul	14				14																	
Carbapenem	Imipenem	14									14												
Cephalosporin	Cefazolin	14												14									
	Cefovecin	14			4		9		1														
	Cefpodoxime	14												14									
	Cephalothin	14												14									
Fluoroquinolone	Enrofloxacin	14				12						1							1				
	Marbofloxacin	14									13								1				
	Pradofloxacin	14				12			1							1							
Folate pathway antagonist	Trimethoprim/Sulfa	14												12				1	1				
Glycopeptide	Vancomycin	14									14												
Lincosamide	Clindamycin	14						14															
Macrolide	Erythromycin	14				11			3														
Nitrofuran	Nitrofurantoin	14																				14	
Penicillin	Ampicillin	14				8			3			2			1								
	Oxacillin	14				14																	
	Penicillin	14	4		1				1			1			2			1			4		
Phenicol	Chloramphenicol	14																		14			
Tetracycline	Doxycycline	14		10						4													
	Minocycline	14						10				1				3							
	Tetracycline	14				10							4										

1: Feline-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Human-derived breakpoints for oxacillin (S ≤0.25 µg/ml, R ≥0.5 µg/ml) were used to categorize isolates as oxacillin-sensitive (OxS) or oxacillin-resistant (OxR).

Table 48. Minimum inhibitory concentration (MIC) distributions in µg/ml for oxacillin resistant (OxR) *Staphylococcus intermedius* group isolates recovered from cats with urinary tract infections (UTIs) in 2020.

Antibiotic class	Antibiotic	Total	≤0.25	≤0.5	0.5	>0.5	≤1	1	>1	≤2	2	>2	≤4	4	>4	≤8	8	>8	≤16	>16	32	>32
Aminoglycoside	Amikacin	7																	7			
	Gentamicin	7											5				1			1		
Ansamycin	Rifampin	7					6					1										
Beta-lactams	Amoxicillin/Clavul	7	3		1						1							2				
Carbapenem	Imipenem	7					7															
Cephalosporin	Cefazolin	7								5					2							
	Cefovecin	7			1			2						1			1	2				
	Cefpodoxime	7								3				1			1	2				
	Cephalothin	7								5					2							
Fluoroquinolone	Enrofloxacin	7	1												6							
	Marbofloxacin	7					1								6							
	Pradofloxacin	7			1						5	1										
Folate pathway antagonist	Trimethoprim/Sulfa	7								4					3							
Glycopeptide	Vancomycin	7					6													1		
Lincosamide	Clindamycin	7		1											6							
Macrolide	Erythromycin	7													7							
Nitrofuran	Nitrofurantoin	7																	7			
Penicillin	Ampicillin	7	1					1						1			1	3				
	Oxacillin	7			2							5										
	Penicillin	7			1													6				
Phenicol	Chloramphenicol	7														2					2	3
Tetracycline	Doxycycline	7				7																
	Minocycline	7		1							4	2										
	Tetracycline	7						7														

1: Feline-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Cefazolin, cephalothin, cefovecin, cefpodoxime, amoxicillin/clavulanic acid (abbrev: Clavul), imipenem, ampicillin and penicillin would be reported as resistant (R) based on oxacillin resistance. 3: Human-derived breakpoints for oxacillin (S ≤ 0.25 µg/ml, R ≥ 0.5 µg/ml) were used to categorize isolates as oxacillin-sensitive (OxS) or oxacillin-resistant (OxR).

Table 49. Minimum inhibitory concentration (MIC) distributions in µg/ml for oxacillin sensitive (OxS) *Staphylococcus intermedius* group isolates recovered from cats without urinary tract infections (non-UTIs).

Antibiotic class	Antibiotic	Total	≤0.06	≤0.125	0.125	≤0.25	0.25	≤0.5	0.5	>0.5	≤1	1	>1	≤2	2	>2	≤4	4	>4	≤8	8	>8	≤16	16	>32
Aminoglycoside	Amikacin	30																					30		
	Gentamicin	30															27				1			2	
Ansamycin	Rifampin	30									30														
Beta-lactams	Amoxicillin/Clavul	30				30																			
Carbapenem	Imipenem	30									30														
Cephalosporin	Cefazolin	30												30											
	Cefovecin	30			13		17																		
	Cefpodoxime	30												30											
	Cephalothin	30												30											
Fluoroquinolone	Enrofloxacin	30				26									1				3						
	Marbofloxacin	30									27								3						
	Pradofloxacin	30				27									2	1									
Folate pathway antagonist	Trimethoprim/Sulfa	30												25					5						
Glycopeptide	Vancomycin	30									30														
Lincosamide	Clindamycin	30						25											5						
Macrolide	Erythromycin	30				20			5										5						
Nitrofuran	Nitrofurantoin	30																					30		
Penicillin	Ampicillin	30				20			4			3			2			1							
	Oxacillin	30				30																			
	Penicillin	30	15				3		1			1						4			3	3			
Phenicol	Chloramphenicol	30																		29					1
Tetracycline	Doxycycline	30		23			1			6															
	Minocycline	30						24								6									
	Tetracycline	30				22			2			6													

1: Feline-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Human-derived breakpoints for oxacillin (S ≤ 0.25 µg/ml, R ≥ 0.5 µg/ml] were used to categorize isolates as oxacillin-sensitive (OxS) or oxacillin-resistant (OxR).

Figure 17. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in oxacillin sensitive (OxS) *Staphylococcus intermedius* group isolates recovered from cats without urinary tract infections (non-UTIs) in 2018 – 2020.

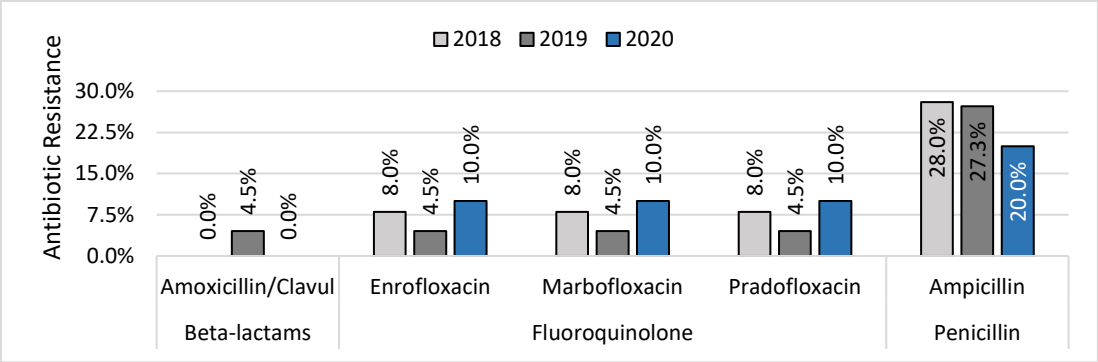


Table 50. Clinical signs and diagnoses associated with oxacillin sensitive (OxS) *Staphylococcus intermedius* group isolates recovered from cats without urinary tract infections (non-UTIs) in 2020.

Clinical signs/Indications	Counts in 2020	% of Counts
Abscess/Skin/Wound infection	17	56.7%
Otitis/Ear infection	8	26.7%
Pneumonia/Respiratory infection	4	13.3%
Undetermined	1	3.3%
Total	30	

Table 51. Minimum inhibitory concentration (MIC) distributions in µg/ml for oxacillin resistant (OxR) *Staphylococcus intermedius* group isolates recovered from cats without urinary tract infections (non-UTIs) in 2020.

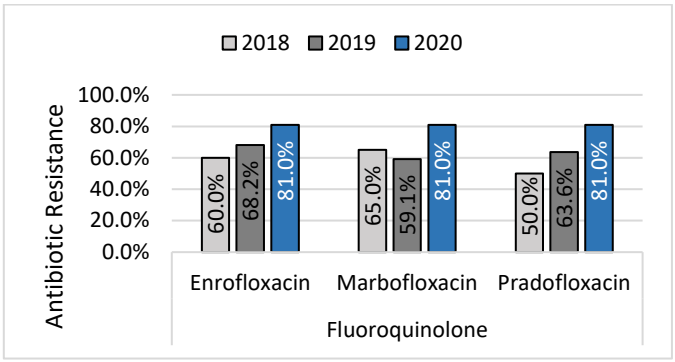
Antibiotic class	Antibiotic	Total	≤0.125	0.125	≤0.25	0.25	≤0.5	0.5	>0.5	≤1	1	>1	≤2	2	>2	≤4	4	>4	≤8	8	>8	≤16	16	>16	>32
Aminoglycoside	Amikacin	21																				21			
	Gentamicin	21														7				3			9	2	
Ansamycin	Rifampin	21								20					1										
Beta-lactams	Amoxicillin/Clavul	21			4			8			1			4						1	3				
Carbapenem	Imipenem	21								21															
Cephalosporin	Cefazolin	21											16				1	4							
	Cefovecin	21				1					1			3			2			2	12				
	Cefpodoxime	21											4				2			6	9				
	Cephalothin	21											17				1	3							
Fluoroquinolone	Enrofloxacin	21			1			1						2				17							
	Marbofloxacin	21								3				1				17							
	Pradofloxacin	21			4									11	6										
Folate pathway antagonist	Trimethoprim/Sulfa	21											4				5	12							
Glycopeptide	Vancomycin	21								21															
Lincosamide	Clindamycin	21					6											15							
Macrolide	Erythromycin	21			4			1			1							15							
Nitrofuran	Nitrofurantoin	21																				21			
Penicillin	Ampicillin	21			1						1			2			2			2	13				
	Oxacillin	21						1			7			2	11										
	Penicillin	21		1							1									2	17				
Phenicol	Chloramphenicol	21																	18				1		2
Tetracycline	Doxycycline	21	2						19																
	Minocycline	21					5								16										
	Tetracycline	21			2							19													

1: Feline-specific interpretive criteria are indicated for selected antibiotics. Interpretive values are based on the *Vet01S* (CLSI, 2020). 2: Cefazolin, cephalothin, cefovecin, cefpodoxime, amoxicillin/clavulanic acid (abbrev: Clavul), imipenem, ampicillin and penicillin would be reported as Resistant (R) based on oxacillin resistance. 3: Human-derived breakpoints for oxacillin (S ≤0.25 µg/ml, R ≥0.5 µg/ml) were used to categorize isolates as oxacillin-sensitive (OxS) or oxacillin-resistant (OxR).

Table 52. Clinical signs and diagnoses associated with oxacillin resistant (OxR) *Staphylococcus intermedius* group isolates recovered from cats without urinary tract infections (non-UTIs) in 2020.

Clinical signs/Indications	Counts in 2020	% of Counts
Abscess/Skin/Wound infection	16	76.2%
Pneumonia/Respiratory infection	2	9.5%
Mixed/Secondary infection	1	4.8%
Otitis/Ear infection	1	4.8%
Undetermined	1	4.8%
Total	21	

Figure 18. Antimicrobial resistance (% of isolates tested for each antimicrobial agent per year) in oxacillin resistant (OxR) *Staphylococcus intermedius* group isolates recovered from cats without urinary tract infections (non-UTIs) in 2018 – 2020.



APPENDIX G. Acknowledgments

The following laboratories contributed data and isolates to the 2020 Year 3 NAHLN AMR Pilot Project:

AL - Bacteriology & Mycology Diagnostic Laboratory; Auburn, AL
CA - California Animal Health & Food Safety Laboratory System; Davis, CA
CO - Colorado State University Veterinary Diagnostic Laboratory; Fort Collins, CO
FL - Bronson Animal Disease Diagnostic Laboratory; Kissimmee, FL
GA - Athens Veterinary Diagnostic Laboratory; Athens, GA
IA - Iowa State University Veterinary Diagnostic Laboratory; Ames, IA
IN - Indiana Animal Disease Diagnostic Laboratory; West Lafayette, IN
KS - Kansas State Veterinary Diagnostic Lab; Manhattan, KS
KY - University of Kentucky, Veterinary Diagnostic Laboratory; Lexington, KY
KY - Breathitt Veterinary Center; Hopkinsville, KY
LA - Louisiana Animal Disease Diagnostic Laboratory (LADDL); Baton Rouge, LA
MI - Michigan State University Veterinary Diagnostic Laboratory; Lansing, MI
MN - University of Minnesota Veterinary Diagnostic Laboratory; St. Paul, MN
MO - Columbia, University of Missouri Veterinary Medical Diagnostic Laboratory; Columbia, MO
MS - Mississippi State University Veterinary Research & Diagnostic Laboratory System; Pearl, MS
ND - North Dakota Veterinary Diagnostic Lab; Fargo, ND
NE - Nebraska Veterinary Diagnostic Center; Lincoln, NE
NY - Cornell University Animal Health Diagnostic Center; Ithaca, NY
OH - Ohio Animal Disease Diagnostic Laboratory; Reynoldsburg, OH
PA - University of Pennsylvania PADLS Harrisburg Veterinary Laboratory; Harrisburg, PA
PA - Pennsylvania State University, Animal Diagnostic Laboratory; University Park, PA
PA - University of Pennsylvania PADLS New Bolton Center Veterinary Laboratory; Kennett Square, PA
SD - South Dakota Animal Disease Research & Diagnostics Laboratory; Brookings, SD
TN - Kord Animal Health Diagnostic Laboratory; Nashville, TN
TX - Texas A&M Veterinary Medical Diagnostic Laboratory; College Station, TX
WA - Washington Animal Disease Diagnostic Laboratory; Pullman, WA
WI - Wisconsin Veterinary Diagnostic Laboratory; Madison, WI