A comparison of antimicrobial resistance in *Escherichia coli* among humans and bovines in Washington State

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Abstract

A comparison of antimicrobial resistance in *Escherichia coli* among humans and bovines in Washington State

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With antimicrobial resistance being one of the top global public health threats, integrated antimicrobial resistance surveillance systems are critical in gathering data, understanding resistance trends, creating stewardship plans and accurately quantifying resistance at national and local levels. We report on the Washington Integrated Surveillance for Antibiotic Resistance (WISAR) database that houses data from human and animal data from hospitals, laboratories, and clinics in Washington State, as well as human and animal data from the US National Antibiotic Resistance Monitoring System. This analysis used two datasets from the WISAR database to look at outpatient human antimicrobial susceptibility testing (AST) data for *E. coli* from October 2017 (n=1311) and bovine AST data for *E. coli* from 2002-2017 (n=253) in an attempt to analyze resistance trends between *E. coli* in humans and bovine in Washington state.

A panel of 5 antibiotics were used for this analysis to allow conclusions and resitotypes to be developed. We found the odds of resistance between humans and bovine for individual antibiotics as well as developed resistotype plots to compare resistotypes between humans and bovine isolates. Using Clinical Laboratory Standards Institute (CLSI) breakpoints, the data showed the odds of resistance for 3rd generation cephalosporins and aminoglycosides (OR: 2.90, $p<0.001$) to be greater for bovine than for humans. The odds of resistance to fluoroquinolones and trimethoprim sulfa were respectively 33% less (OR: 0.33, $p<0.001$) and 21% less (OR: 0.21, $p<0.001$) in bovines than for humans. We found the same statistically significant directionality of results using ECOFF breakpoints. This proof of concept analysis highlights the challenges in using local surveillance data and comparing human and animal strains for AMR as well as provides recommendations for moving forward with this type of data. Integrated antimicrobial susceptibility testing data creates an opportunity for a collaborative effort to discuss the next stages for local efforts in antimicrobial stewardship across human, animal, and environmental sectors as well as gaining an understanding of: 1) what conclusions can be made between data sets? 2) how valid are these conclusions? 3) what data is needed to make this type of comparison in resistance across sectors?

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Introduction

Antimicrobial resistance (AMR) is one of the major global public health threats of this century and has been identified as a priority by national and international agencies (Prevention, 2018) (Sharma et al., 2017) (World Health Organization, 2014). Modern medicine relies on the availability of effective antibiotics for organ transplants, surgeries, infections, and other common procedures. It is estimated that AMR will lead to increased mortality with an increase in death attributable to AMR and increased morbidity with an increased number of sick and prolonged sick individuals. It is important to tie in the economic burden of AMR by considering its future impact on health care costs, indirect costs and costs of action. By investing in surveillance and stewardship programs, we are investing in a healthier and less financial burdensome future (Taylor et al., 2014).

Antimicrobial resistance is the ability of a bacteria to resist the effects of a drug. The bacteria are not killed, so their ability to overtake and disrupt organ systems aren't prevented. The mechanisms behind resistance include 1) changes in permeability of the bacterial cell wall 2) active efflux of the antibiotic from the cell 3) enzymatic degradation/modification of the antibiotic 4) modification of antibiotic targets 5) acquiring alternative pathways to those inhibited by the antibiotic and 6) overproduction of a target enzyme (Dever & Dermody, 1991). These mechanisms can be intrinsic, or extrinsic and acquired through gene transfer.

Contributions to Growing Resistance

Human and veterinary medicine both use antibiotics and are in turn both contributing to emerging resistance, even when antibiotics are used appropriately. Human medicine antimicrobial use includes 1) treatment of sick individuals and 2) prophylactic treatment for

individuals after undergoing surgery or injurious trauma for prevention. In food production, antibiotics are used for 1) treatment of sick animals 2) metaphylactic treatment of sick and healthy animals within the same group and 3) prophylactic treatment of healthy animals to prevent disease in cases such as stressful conditions, before transporting animals and during surgical procedures, and growth promotion (Sharma et al, 2017). As of January 2017, the Veterinary Feed Direction (VFD), implemented by the Food and Drug Administration, removed production uses of medically important antimicrobials in animal feed resulting in U.S. livestock producers no longer being able to use medically important antibiotics growth promotion. The effectiveness of the VFD has not yet been accessed (M. Woolhouse, Ward, van Bunnik, & Farrar, 2015). Antibiotic use in humans also contributes to emerging AMR as inappropriate and over use of antibiotics in human medicine have been identified as areas in which AMR stewardship could intervene to minimize resistance (Rogers Van Katwyk, Grimshaw, Mendelson, Taljaard, & Hoffman, 2017).

Antimicrobial use in veterinary and human medicine are linked through the movement of resistant bacteria and resistant genes in the environment. This can be through direct contact with an infected human/animal, through contamination of food, water, and the environment or through waste or ground water (M. E. J. Woolhouse & Ward, 2013) (Kivits, Broers, Beeltje, van Vliet, & Griffioen, 2018). The complexity of resistant bacteria and resistance genes moving between populations and through the environment sparks debate on which sectors are contributing to the emerging resistance. Without quantitative AMR data such the WISAR database and NARMS surveillance, emerging AMR cannot be effectively addressed.

Significance of Integrated Antimicrobial Surveillance

Resistant genes and bacteria pass between people, animals and the environment and integrated surveillance systems are necessary to understand the complexity of AMR. In 2014 the World Health Organization (WHO) published *WHO Antimicrobial Resistance: Global Report on Surveillance*. This report focused on nine bacteria of international concern and identified two key findings: very high rates of resistance were observed in all WHO regions and that there are significant gaps in surveillance, methodology standards, data sharing and coordination (World Health Organization, 2014). The WHO has also established the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) that published the *Surveillance of Antimicrobial Resistance AGISAR* manual. This manual provides guidance on establishing integrated surveillance programs including surveillance and monitoring approaches, collection of standardized data at regional and national levels, and data management systems and risk communication (Organization, 2013). The AGISAR manual outlines the elements for an integrated antimicrobial resistance surveillance system. However, the elements are general and do not spell out many of the issues encountered in an integrated surveillance system at the local level. The AGISAR report also does not focus on environmental monitoring such as testing soil, wastewater, or wildlife samples.

At the national level, the National Antimicrobial Resistance Monitoring System (NARMS) was established in 1996 to track antimicrobial resistance in foodborne and other enteric bacteria. It nationally samples sick humans, retail meats, cecal ground products, and carcass swabs with a focus on *Salmonella*, *Capylobacter, E. coli* and *E. coli* O157, *Enterococcus,* and *Shigella*. The sample collection comes from health departments and institutions across the U.S. The testing methodology for all NARMS samples are standardized with specific

laboratories using a Sensititre machine for antimicrobial susceptibility testing and whole genome sequencing of *Salmonella, Campylobacter* and select *E. coli* (U.S. Department of Health and Human Services, 2017). NARMS collects a large amount of national data, but the data may be limited when localized to a particular region. Washington State joined NARMS in 2010 and since then has only contributed 841 human isolates and 254 cecal retail isolates. For Washington State, NARMS simply does not have enough data to understand specific bacteria-drug combinations to gain an understanding of local resistance. For AMR to be assessed at the local level in Washington State, there needs to be a robust collaboration of data from human, animal, and environmental sectors. This includes a vast number of samples from retail foods, food-producing animals, human health clinics and labs, animal hospitals, and environmental samples from water, soil, and wildlife.

Washington State antimicrobial susceptibility testing takes place in private and public labs and institutions for both human and veterinary medicine. Human and animal hospitals and clinics are able to perform antimicrobial susceptibility testing (AST) for their patients. For human medicine, AST can be performed in the inpatient or outpatient setting while AST for veterinary care is not as thorough for every patient. Whether AST is performed depends on the owner, the condition of the patient, as well as the owner's socioeconomic status and preference. For food-producing animals, AST is not consistently performed, making collection of robust data is difficult. Producers will not always send samples collected from a sick animal for AST as it may not be the most cost-effective way for a producer to treat a herd. By testing one animal in herd, the AST result may not apply to the entire herd and the results are not as important for treatment. Thus, antimicrobials can be used to treat a herd of animals without knowing the phenotypic resistance for each animal.

Antibiotic Use and Stewardship in Food Producing Animals

Bacterial infections on farms can result from two main factors: exogenous and endogenous. Exogenous risk factors cannot be controlled by farmers and include regional prevalence of pathogens, climate, and market changes associated with the baseline level of disease (Lhermie, Grohn, & Raboisson, 2017) (Raboisson et al., 2012). Endogenous risk factors are those that are influenced by the farmer's response to exogenous factors: animal nutrition, feed quality, animal housing, and other production factors. Animal disease is an economic loss to a production system, and effective use of antibiotics is a production factor that can increase a farm production return.

In order to understand antimicrobial usage on farms, the cost-benefit of healthy livestock and antimicrobial usage needs to be considered in conjunction with risk factors for infections on farms. A farmer's decision to use antibiotics on a farm is related to their expertise, their behavior and risk aversion, the availability of diagnostic tests, the institutional influences and availability, efficacy and price of antimicrobials (Lhermie et al., 2017).

High-Risk Populations for AMR Transmission

Transfer of AMR bacteria between humans and animals can occur through the food chain supply, direct contact, or environmental routes (Lhermie et al., 2017). This analysis helps understand the data limitations, but feels it is important to highlight the high-risk populations where human-bovine AMR transmission is likely to occur. Occupations at a high risk of exposure to resistant bacterial strains include farmers, food handlers, and veterinarians (Sharma et al., 2017). Yamaji et al. 2018 did a population-based study and found that poultry sold in in retail stores may be a source of uropathogenic *E. coli*. The results of this study demonstrate the ability for resistant bacteria to move between animals and humans outside the farm setting.

Escherichia coli is a gram negative bacteria normally found in the intestines of humans and animals (Prevention, 2018). It is considered a WHO bacterium of international concern and is a major player in community and hospital acquired urinary tract infections (UTIs), bloodstream, intra-abdominal, skin and soft tissue, and foodborne infections. *E. coli* can be move between humans and animal in a shared environment. Specifically, food animal production workers are at a higher risk of contact with microbes such as *E. coli*. Their work is in direct contact with animals and waste and can commonly be in an enclosed environment. Animal slaughtering and processing plants fall into this same risk category as these workers handle livestock; clean carcasses; cut, dress, and pack meat; and have a high risk of cuts and lacerations (Ho, O'donoghue, & Boost, 2014) (Sharma et al., 2017). The risk can also extend beyond these food animal production workers and into the communities that animal production takes place. The subsequent analysis will focus on *E. coli* as a pathogen due to its public health importance.

Within Washington State, dairy production is the second largest part of the agricultural sector. In January 2018, Washington State had 274,000 milk cows (USDA, 2017). These dairy farm operations can be found in 29 out of 39 Washington counties with the majority of the farms concentrating in the Yakima Valley (Press, 2017). The presence of beef cattle feed yards are also significant in Washington state with 24 beef cow operations with 500-999 head and 11 operations with 1,000 of more head (Service, 2014). At these facilities, workers have daily exposures to cows and are at a greater risk of AMR bacteria transmission. Using bovine and human data, this thesis will attempt to summarize resistance from two data sources with the idea that an integrated surveillance system could perhaps detect when changes in antimicrobial resistance occur and specifically focus on population at the highest risk, such as those workers and communities located in close proximity to large animal operations.

Antimicrobial Susceptibility Testing

When a human or animal has a suspected infection, a sample from the host will be collected such as blood, urine, feces, tissue, sinus, or wound swab. The sample is brought to a laboratory for antimicrobial susceptibility testing. This can be done using a Kirby-Bauer Disk Diffusion or Minimum Inhibitory Concentrations (MIC) Testing (CDC, 2014). MIC testing can be done with broth of agar media with broth micro dilutions being the most commonly used. In the lab, colonies from the collected bacteria sample are selected and prepared in a standardized inoculum suspension of 5×10^5 CFU/ml. The antibiotics and dilution scheme are prepared as per the panel instructions. Also used are breakpoint panels containing 2-3 dilutions based off of MIC breakpoints. These breakpoints panels are difficult to assess quality control of testing as well as difficulty for surveillance.

After dilution schemes are prepared, the antibiotic dilution panels and bacteria in the 96 well plate are then incubated for 16-24 hours at specific incubation conditions depending on the bacteria type (CDC, 2014). After incubation, the lowest concentration of antimicrobial agent needed to inhibit bacteria growth is indicated as the MIC value. Using published documents, the MIC interpretive criteria is used to interpret the MIC test results. MIC lab results are categorized into susceptible (S), intermediate (I) or resistant (R) categories based off of CLSI interpretative criteria (C. L. S. Institute, 2018).

In commercial testing systems such as the Vitek 2 System and Sensitire ARIS 2X, the laboratory tests are automated and have enhanced computers that interpret susceptibility results. Commercial MIC panels are manufactured by various companies that are cleared by the Food and Drug Administration (FDA) to ensure verification.

There are multiple research bodies that publish breakpoints to assist clinicians in interpreting MIC tests. Two main committees are the Clinical Lab Scientific Institute (CLSI) in the United States and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). In both committees, breakpoints are established through previous data on MIC distributions, pharmacokinetic-pharmacodynamic data, and clinical outcome data. Both CLSI and EUCAST include human and veterinary breakpoints. However, breakpoints are not as thorough for veterinary medicine due to lack of available data, lack of AST testing standards across labs, and differences between and within species that affects the route/duration/frequency of a drug. This lack of breakpoints makes comparing and analyzing human and animal AST data difficult. Veterinary breakpoints are generally lower than human breakpoints and in cases of no published breakpoint, human breakpoints are used. The appropriate way to handle the lack of a breakpoints for clinical and surveillance purposes is subject to debate.

Both CLSI and EUCAST have secondary "population" breakpoints called Epidemiologic Cut-Off Values (Called ECVs or ECOFFs). This ECV/ECOFF breakpoint is the highest MIC for organisms devoid of phenotypically detectable acquired resistance (Toutain et al., 2017). It separates microorganisms into two groups: wild-type organisms without acquired resistance (MICs less than or equal to the breakpoint) and non-wild type organisms with acquired resistance (MICs greater than the breakpoint value). Classifying an organism into wild-type does not mean that is it by default treatable. On the same note, classifying an organisms as non-wild type does not mean it is by default resistant.

ECV/ECOFFs are established by aggregating thousands of MIC distribution data to create a population distribution of MIC values. A breakpoint is established to distinguish between organisms with and without phenotypic resistance for a specific bug-drug combination

(Testing, 2017). It takes into consideration that MIC measurements have inherent variability, differences in who performs the lab test, how the results are read, the cell density in the inoculum, materials used, temperature and testing system. ECV/ECOFF are most appropriately used when clinical breakpoints are not sensitive enough, not determined, change over time, and differ between systems in species. Using ECV/ECOFF breakpoints in this analysis allows for a more comparable platform to look at resistance trends between species. Although the data is aggregated from multiple countries, time points, and host species, using ECV/ECOFF breakpoints allow for a population-level understanding of phenotypic resistance and takes into consideration the lack of correlation between MIC values, pharmacokinetics and clinical response/ outcomes. MIC values and their associated interpretations are important for understanding susceptibility testing. Interpretive criteria for two different antibiotics can indicate susceptible, but a higher MIC value within the susceptibility classification may indicate an antibiotic being a better agent over another.

This thesis was motivated by Washington State's need to utilize an integrated AST database that includes local veterinary and medical data for integrated analysis of AMR. This study will analyze the available AST data to summarize resistance as well as outline the barriers to integrate surveillance at the local level.

Methods

Data Sources

The data used in this analysis comes from the Washington State Integrated Surveillance for Antimicrobial Resistance (WISAR) database. This database is housed by the Center for One Health Research (COHR) at the University of Washington in collaboration with the Washington

State Department of Health (WADOH). The WISAR database includes human and animal data from hospitals, laboratories, and clinics in Washington State, as well as human and animal data from the US National Antibiotic Resistance Monitoring System (U.S. Department of Health and Human Services, 2017). For the purposes of this research, human AST data from a large human clinical laboratory, Quest Diagnostics, and bovine AST data from the Washington Animal Disease Diagnostics Laboratory (WADDL) were used. Quest Diagnostics is a clinical laboratory with outpatient clinics across the United States. The dataset used in the analysis includes one month of Quest Diagnostics outpatient data from October 2017 (Figure 1). WADDL data includes bovine *E. coli* isolates collected from 2002-2017. This data includes samples collected from Washington State University teaching hospital, veterinary clinics, and samples submitted in other states. For the purposes of this analysis we assume that the majority of the samples collected are from Washington and assume that animal isolates present in WADDL data were representative of those in Washington State.

Data were received with raw minimum inhibitory concentration (MIC) values as well as the corresponding interpretations for each bacteria-antibiotic combination. Quest Diagnostic interpretations were defined using the M100 Clinical Laboratory Standards Institute (CLSI defined above) guidelines (C. L. S. Institute, 2018). WADDL MIC value data and interpretations followed CLIS guidelines but has changed over the years in the dilutions of antibiotics, breakpoints, and the panel of antibiotics used. It is recommended that MIC test results should be reported using current breakpoints as they most accurately reflect the current understanding of antimicrobial resistance. Thus, raw MIC values had current CLSI breakpoints applied (C. a. L. S. Institute, 2011). The data structure necessary to analyze resistance included host species, date

of isolate testing, isolate source, bacteria species, MIC result, and MIC interpretations. Additional human metadata included state, zip code, and gender (Table 2).

WADDL uses Sensititre software for AST in conjunction with CLSI breakpoints to assign isolates as susceptible, intermediate or resistant. Our study found difficulties in WADDL data interpretations due to changes in panel dilutions and changes in testing procedures over the years resulting in incomparable data across years. Due to these differences in MIC testing and clinical interpretations as well as concerns over comparing human and veterinary datasets, subsequent analysis used both CLSI and ECV/ECOFF breakpoints.

Data Cleaning

Due to inconsistencies in the WADDL data and comparability of breakpoints between human and animal AST data, CLSI and ECOFF breakpoints were applied to all raw MIC data except for enrofloxacin. Enrofloxacin has not published a CLSI breakpoint, and for subsequent analysis, the CLSI breakpoint will be the interpretation WADDL had assigned to the raw data. It is important to acknowledge that the antibiotic panel was developed based on data availability and assumptions made about the usefulness of an antibiotic used to represent a drug class across human and veterinary medicine.

The structure of the data allows for one isolate to express the raw MIC and respective interpretation of the results across a panel of antibiotics. This AST panel differs between species, isolates and clinical cases so a representative drug class panel of interest was developed to compare antimicrobial resistance across species (Table 4).

Case Definitions

Resistance was defined as an intermediate or resistance interpretation of the MIC. This groups each isolate into a binary “Non-susceptible” and “Susceptible” category for each

antibiotic in the panel. Percent resistance was defined by the percentage of isolates resistant to the antibiotic of interest divided by the total number of isolates tested for each antibiotic. It was assumed that if an isolate was found to be resistant to at least one drug in a specified drug class it would be resistant to the entire antimicrobial class. Outcomes of interest include multi drug resistance (MDR), resistance to individual antibiotics and phenotypic "resistotype" patterns across isolates. MDR is defined in the 2015 NARMS reports as an isolate having resistance to 3 or more antimicrobial classes (U.S. Department of Health and Human Services, 2017) . Interpretations from the 5 drug class panel (Using CLSI and ECOFF breakpoints) were used to create a 5 character "resistotype". If an isolate was resistant to all 5 antibiotics in the panel, it would have a resistotype of "RRRRR". Alternatively, if an isolate was susceptible to any of the antibiotics it would be given the letter S in its place in the panel. This "resistotype" is used in subsequent analysis to gain an in depth understanding of resistance trends across the 5 antibiotics classes of interest.

Data Analysis

Data cleaning and statistical analysis were performed using R Software version 3.4.3 (R v3.4.3, R Core Team (2016), R Foundation for Statistical Computing, Vienna, Austria). CLSI and ECOFF breakpoints were established and applied to raw MIC values in the bovine and human data (Table 4). The outcome of MDR and proportional resistance to antibiotics in the panel were analyzed. Using CLSI and ECOFF breakpoints, a logistic regression model on the outcome of resistance was done using pooled human and bovine data. The response variable was the source of the isolate: human or bovine with no variables being controlled for.

Results

The raw MIC results from human and bovine data are summarized in Table 3. For ampicillin, the MIC distributions between humans and bovines were similar with bovine MIC values ranging from 1-64 mg/L and human MIC values ranging from 2-64 mg/L. Fluoroquinolone MIC distributions were more spread out for bovine (0.03-4 mg/L) than for humans (0.25-4 mg/L). Gentamicin had similar distributions between the bovine and human isolates (1-16 mg/L). Trimethoprim-sulfa MIC results for bovine were distributed across 0.5-8 mg/L, but the MIC results for the humans did not use a standardized range of MIC dilutions with cutoffs only at <20 mg/L, <40 mg/L and >256 mg/L which makes comparisons between data difficult.

Antibiotic panel

Counts of the five antibiotics of interest from bovines were plotted from 2002-2017 (Figure 2). In 2002 and 2006, all five antibiotics of interest were tested. In subsequent years, there was not the same testing done across antibiotics with trimethoprim-sulfa only being tested in 2002, 2003 and 2006. The years 2003, 2005 and 2010 only had 2/5 panel antibiotics tested. The greatest coverage was found in ampicillin with isolates tested for all years except 2002 and 2015-2017. Ceftiofur had the second-best coverage with only missing two extra years (2003 and 2005) compared to ampicillin. Plotting proportion of isolates resistant to an individual across 2002-2017, Figure 3 shows changes in trends across antibiotics with a peak in proportion resistant to ampicillin and gentamicin from 2007-2011 followed by a decrease in resistance in 2014. It is hard to draw conclusions of resistance trends across the years due to data gaps and inconsistencies in tests across years. No antibiotics of interest were tested in 2004. For all human data, all isolates were tested against all antibiotics of interest.

After applying CLSI and ECOFF breakpoints to the bovine and human data from Table 4, the outcome of MDR was summarized. Using CLSI breakpoints, 11.1% bovine isolates were MDR with the greatest proportion of MDR seen in 2013 with 37.5% of isolates with MDR. None of isolates were susceptible to all 5 antibiotics of interest using CLSI breakpoints. ECOFF breakpoints showed a more conservative categorization of wild-type and non-wild type. With this classification, 21.3% of bovine isolates were MDR with the highest proportion of MDR found in 2009 with 57.9% of isolates showing MDR 1.98% of isolates were susceptible to all 5 antibiotics of interested using the ECOFF breakpoint. Using CLSI breakpoints, 10.5% of human isolates were MDR while ECOFF breakpoints classified 100% of isolates as MDR. 0.78% and 5.23% of human isolates were susceptible to all 5 antibiotics using CLSI and ECOFF breakpoints, respectively.

Logistic Regression

The analysis used CLSI and ECOFF breakpoints to look at differences in resistance between humans and bovine across breakpoint classification systems. A logistic regression model using CLSI breakpoints found the odds of resistance for 3rd generation cephalosporins and aminoglycosides (OR: 2.90, $p<0.001$) to be greater in bovines than in humans. The odds of resistance to fluoroquinolones and trimethoprim-sulfa were respectively 33% less (OR: 0.33, $p<0.001$) and 21% less (OR: 0.21, $p<0.001$) in bovines than in humans (Table 8). We found the same statistically significant directionality of results using ECOFF breakpoints. Logistic regression using ECOFF breakpoints found similar statistical findings with an odds of resistance to 3rd generation cephalosporins and aminoglycosides being greater for bovine than human isolates (OR 1.53E-03, $p<0.001$ and OR 3.23, $p<0.001$) and odds of resistance in fluoroquinolones and trimethoprim-sulfa being less for bovine than in humans (Table 9).

Resistotype Plots

Using the combinations of breakpoint interpretations for each antibiotic of interest, five-character resistotypes for each isolate were created. Figures 6a and 6b show the human and bovine *E. coli* resistotype plots for all possible combinations in the data and the associated proportion of isolates with that resistotype using CLSI breakpoints. The most common resistotype in humans and bovine was an isolate susceptible to all five antibiotics (56.8% "SSSSS"). Human resistance to penicillin emerges as the next most common resistotype (13% "RSSSS") followed by trimethoprim-sulfa (9.7% "RSSSR") and fluoroquinolones (2.4% "RSRSR") resistance. In the human isolates, trimethoprim-sulfa is a driving the resistance for all resistotypes. Only 10 human *E. coli* isolates from the data were not tested against any of the antibiotics of interest (0.8% "NNNNN"). The bovine isolates were not all tested against the five antibiotics of interest, so many resistotype had missing data indicated by an "N" in the plots (Figure 6b and 7b). For the purposes of analysis, a "full" panel of resistotypes was used for comparison to humans (n=145) (Figure 8). A "full" panel of bovine isolates only includes data from 2004 and 2006 in which all 5 antibiotics were tested. In the full panel of bovine resistotypes, the most common resistotype is susceptible to all five antibiotics (44.1% "SSSSS") followed by resistance to ampicillins (20.7% "RSSSS") and 3rd generation cephalosporins and aminoglycosides (10.3% "RRSRS").

Removing trimethoprim-sulfa as an antibiotic of interest due to a large amount of human resistance (CLSI 21.4%; ECOFF 100%) and limited sampling in bovine isolate from years 2002, 2003 and 2006, full resistotype plots were created using four antibiotic classes: penicillins, 3rd generation cephalosporins, fluoroquinolones and aminoglycosides (Figure 9). With only four antibiotics of interest, the most common bovine resistotype was fully susceptible to all four

(44.1% “SSSS”) followed by resistance to 3rd generation cephalosporins (20.7% “RSSS”) and additional resistance to aminoglycosides (10.3% “RRSR”). Resistance to all four antibiotics (“RRRR”) was found in 2.8% of bovine isolates. The human resistotype plots showed 59.5% isolates susceptible to the four antibiotic panel (“SSSS”), 23.4% of isolates resistant to only ampicillins (“RSSS”), followed by 4.9% of isolates additionally resistance to fluoroquinolones (“SSRS”). 1.1% of human *E. coli* isolates were resistant to all four antibiotics.

Discussion

This analysis used human and bovine AST data to compare trends in resistance. Our analysis found multi drug resistance in 11.1% of bovine isolates and 10.5% of human isolates using CLSI breakpoints. 12.3% of bovine isolates and 100% of human isolates were classified as MDR using ECOFF breakpoints. Logistic regression showed bovine isolate to have greater odds of resistance to 3rd generation cephalosporins and aminoglycosides for both CLSI and ECOFF breakpoints. We also found the odds resistance to fluoroquinolones and trimethoprim-sulfa to be lower in bovines than in humans. Using resistotype plots, the bovine and human resistotypes were aligned to pull out differences in resistotype proportions. Humans and bovines had the same top two resistotypes: susceptible to all four antibiotics and only resistant to ampicillins (“SSSS” and “RSSS”). We cannot statistically conclude differences between proportions using the two datasets but feel that future studies with more robust time series data can utilize this type of visualization with resistotplots to inform hypothesis and drive analysis.

Understanding antimicrobial resistance in humans, animals and the environment includes quantification of the problem, needs identification, resource administration and educational outreach. Studies have emphasized the need to accurately quantify AMR to design

an effective control plan divided into stewardship, surveillance, infection prevention and control and research (Lhermie et al., 2017). Using regional data to understand local resistance in a more granular way is needed for AMR surveillance outside of national programs such as NARMS.

The data used for this thesis is not robust enough to draw significant conclusion on the status of *E. coli* in bovines and humans in Washington State. Rather, this thesis serves as a pilot study to address the barriers to integrated surveillance at the regional level as well as what analysis is possible with the current data in order to inform future data collection and sector involvement and to improve stewardship in both human and animal medical care.

MIC Distribution Issues

By placing the raw MIC values for bovine and human isolates side-by-side, we hope to draw attention to similarities and differences to understand the phenotypic characteristics *of E. coli* from each species. Higher MIC values, even if within the susceptible category can indicate a bacterium's tendency of being more resistance than that of a lower MIC. However, comparing MIC distributions from bacteria taken from two different species for two different disease manifestations makes drawing a conclusion complicated. We cannot say that *E. coli* from a human isolate and *E. coli* from a bovine isolate are related. The concept of environmental sharing of resistant genes and bacteria is the biological plausibility for transmission, but the data used in this thesis cannot confirm this type of transmission.

The human isolates MIC values for trimethoprim-sulfa were difficult to compare to bovine as the human *E. coli* MIC dilutions only included <20, <40 or >256 mg/L. One of the issues when integrating data from different laboratories is inconsistencies in dilution tests and data reporting. A human *E. coli* isolate may have a true MIC value of 4 mg/L but Quest's lab

test only collects MIC data <20 mg/L. The accuracy that a human isolate classified with <20 mg/L and how it compares to a bovine isolate with and MIC of 0.5 mg/L is difficult to answer with this data and brings up the issue of across lab reporting standards that make integrating AST datasets difficult to draw conclusions on resistance trends with fragmented data and analysis results. Future studies should consider the data source, species, barriers to MIC testing and interpretation as well as the antibiotics panel class representatives and intrinsic resistance for bacteria-antibiotic combinations.

Data Limitations

Changes in breakpoints and AST panels resulted in data gaps across years in the bovine data, making comparisons across years difficult. Quest diagnostics had provided the WISAR database with one month of human outpatient data. With a future dataset from Quest of outpatient AST for five years, the dataset can pull out important resistance trends in human *E. coli* that can contribute to AMR surveillance that a month of human AST data is not able to do.

Sampling bias in the study comes from both human and veterinary samples. Animals admitted to WADDL could be biased by health status of the animal and perceived value of the animal to the owner. This limitation has been acknowledged in other studies using veterinary diagnostic data for AMR surveillance studies (Davidson, Byrne, Pires, Magdesian, & Pereira, 2018). The data may not accurately represent the prevalence of *E. coli* resistance patterns in cattle in Washington State but the prevalence of *E. coli* resistance in samples submitted to WADDL. Private labs are often used for bovine testing over state labs such as WADDL due to differences in costs and concerns over the results from AST being held at a state lab.

Human data sampling bias comes from individuals who had their samples sent to a Quest Diagnostics for testing. The data could be biased by including individuals with higher

socioeconomic status and/or with an insurance plan more willing to cover the testing costs or by the severity infectious cases being treated in the outpatient setting. In some cases, UTI diagnoses will be made without AST and empiric therapy would be given. In these instances, AST testing is not collected through a private clinic or through Quest and isolates from these individuals would not be represented in the data. In these instances, AST testing is not collected through a private clinic and isolates from these individuals would not represented in the data.

Limitations in the data occur from variables that can occur within a single lab during MIC test results. This includes intrinsic issues such as skipped wells, poor growth, mixed cultures, improper dilutions, and differences in persons running tests. Across labs, inconsistencies include different testing methods and different reporting designs. Breakpoint interpretations also come with inconsistencies from differences between species and data sources, incomplete veterinary breakpoints, and differences in breakpoints classifications in analysis.

CLSI and ECOFF Breakpoints

Using CLSI and ECOFF breakpoints for logistic regression of resistance to individual antibiotics resulted in the similar statistical directionality. This provides support that the most appropriate way to analyze and integrate AST data is to compare with choosing one classification system to use and compare.

Multi-drug Resistance

The outcome of MDR has limitations on its importance and relevance in the context of this study. Looking at antibiotic resistance for each individual antibiotic provides part of the

resistance picture, but more importantly it is the combination of drugs a certain isolate is resistant to and when phenotypes are being seen in the population.

Analysis Limitations

Many of the medically important drugs in the panel are not used to treat mastitis or respiratory disease in cows bringing in the question of how relevant the results are to veterinary medicine. Penicillins and 3rd generation cephalosporins are included in the panel as both are Beta-lactam drugs with medical importance. Ceftiofur and ceftriaxone are exclusively used in veterinary and human medicine, respectively. Although increases in resistance to 3rd generation cephalosporins is concerning among the bovine isolates, ceftriaxone and ceftiofur were used as proxies and may not accurately reflect true resistance class of 3rd generation cephalosporins. The idea of one drug representing a drug class is an inherent limitation due to the differences in antibiotics that make up the drug class. Resistance to one antibiotic in the class doesn't infer resistance to all within the class.

Fluoroquinolone resistance has been an increasing concern because of its important in treatment of bronchitis, UTIs, pneumonia, skin and soft tissue infections, sinusitis and bacteremia (Micromedex, 2002). This study found resistance to ciprofloxacin in 13.1% of human isolates using CLIS breakpoints. The bovine fluoroquinolone representative, enrofloxacin, is exclusively used in veterinary medicine. This study found bovine 9.9% of bovine isolates to be resistant to enrofloxacin. Ideally, all major drug classes would be available in the data, and the same AST drug panel would be used to allow for appropriate comparisons to avoid using drug class representatives.

The main barriers to veterinary integration of AMR data is the lack of AST standardization across labs. There is no program for monitoring AMR in veterinary clinics for

companion animals or veterinary diagnostic labs for sick animals in agriculture. A survey distributed to veterinary diagnostic labs in the United States found the AST methods most commonly used by veterinary diagnostic labs to be disk diffusion and broth microdilution in conjunction with CLSI standards for result interpretation (Dargatz, Erdman, & Harris, 2017). A different study mailed a questionnaire to veterinary diagnostic labs in the U.S to identify laboratories that were conducing AST and found a lot of variability in the testing methods and data storage across veterinary diagnostic labs (Brooks et al., 2003). If AST data from veterinary diagnostic laboratories across the U.S. were utilized for regional AMR surveillance, there could be significant analysis and surveillance programs put into place.

The ideal integrated surveillance system would use a central reference laboratory to collect all samples from human, animal and environmental sources in Washington State. There would be a single lab methodology using MIC testing with a broad panel of antibiotics with extended antibiotic dilutions so data on MIC changes over time could be analyzed. As in the analysis from this thesis, resistotype plots would be used to identify resistance patterns of concern with who genome sequencing accompanying the phenotypic testing to understand resistance trends in Washington. This type of a surveillance program would require a considerable amount of funding, time and resources to establish. With antimicrobial susceptibility testing being done at private and public human and veterinary health facilities, surveillance could leverage this testing and combine testing results from multiple sources into an integrated database. Through this thesis and AMR data integration, four main barriers to integrated AMR at the local level have been established:

1. Data gathering

Human hospitals and clinics collect AST results from patients. Accessing data on isolates taken from patients is linked to private health information. Human and veterinary hospitals and clinics may not be willing to distribute AST data freely because of this. Veterinary diagnostic labs may be hesitant to share AST data as it is linked to producers in specific locations and may cause privacy concerns. Even with shared AST data, there is difficulty when datasets have limited metadata due to privacy issues that prevent specific analysis to be done.

2. Integrating data and comparing antibiotics

Once data is received, the antibiotic panels used in human AST and veterinary AST are not always equivalent. In order to look at specific drug classes, drug class representative need to be established. In this analysis, ceftiofur was used as a veterinary drug class representative for 3rd generation cephalosporins while ceftriaxone was used as the human drug class representative. For fluoroquinolones, enrofloxacin was used as the veterinary drug class representative and ciprofloxacin was used as the human drug class representative. Ideally, the same drug would be tested across both species so assumptions on drug class representatives could be avoided.

3. Interpreting MIC results

Humans and animals have different pharmacokinetic-pharmacodynamics (PK-PD) with drugs and MIC dilutions used for humans are not always appropriate for clinical relevance in animals. Differences in PK-PD are seen in differing animal species and animal breeds. (Toutain et al., 2017) CLSI or ECOFF breakpoints can be used to compare AST data with limitations from both classification systems. CLSI breakpoints are clinically relevant but are not available for all veterinary testing and may not be sensitive enough to analyze. ECOFF are recommended to use

when CLSI breakpoints differ between laboratories and species and when CLSI breakpoints change over time. Limitations of using ECOFF breakpoints are that they cannot represent or compare rates of resistance between agents because the ECOFF breakpoints include organisms over geographical areas and time points (Toutain et al., 2017).

4. Appropriately understanding the data and presenting results

After collecting, integrating and analyzing MIC and interpretations based on established cut point classifications, there is the difficult task of appropriately presenting the data. Veterinary and human practitioners are interested in the clinical cut points and their clinical relevance while public health practitioners and groups interested in surveillance are more interested in the bird's eye view of the data and its population health meaning. It is important to be transparent about how the data was integrated, manipulated and planned to be presented to groups to avoid miscommunications and interpretation.

Future steps to integrated AMR surveillance should considers the limitations and barriers identified. This report hopes to bring attentions to these limitation as well as encourage groups including human and veterinary hospitals, human and veterinary clinics, and diagnostic labs as well as encourage farmers to push for AST in their animals so the WISAR database to can become a robust tool for regional surveillance in Washington State.

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The author would like to thank the WISAR working group for their purport of this research as well as Quest Diagnostics and WADDL for allowing the use of their data. This research was supported by the 5T42OH008433-14 Grant funded by CDC under the Northwest Center for Occupational Health & Safety and the Department of Environmental & Occupational Health Sciences as well as the Epidemiology and Laboratory Capacity (ELC) Cooperative Agreement grant. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Center for Disease Control and Prevention of the Department of Health and Human Services or the Washington State Department of Health. The author would also like to thank Brian High through the University of Washington IT with assistant in streamlining of R code and in database management consulting.

Figures and Tables

Figure 1. Map of Washington State: red dot indicates zip code location of Quest Diagnostic isolate sampling.

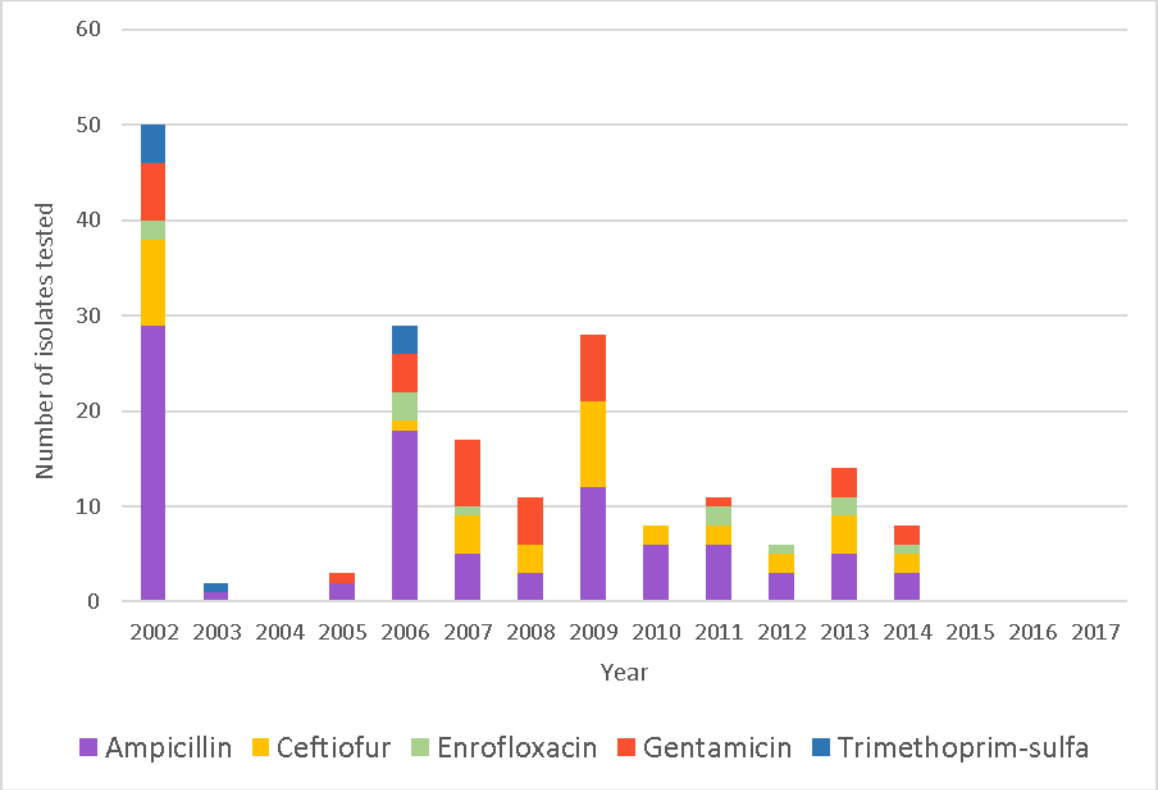

Figure 2. Number of bovine isolates tested against antibiotic panel of interest, 2002-2017.

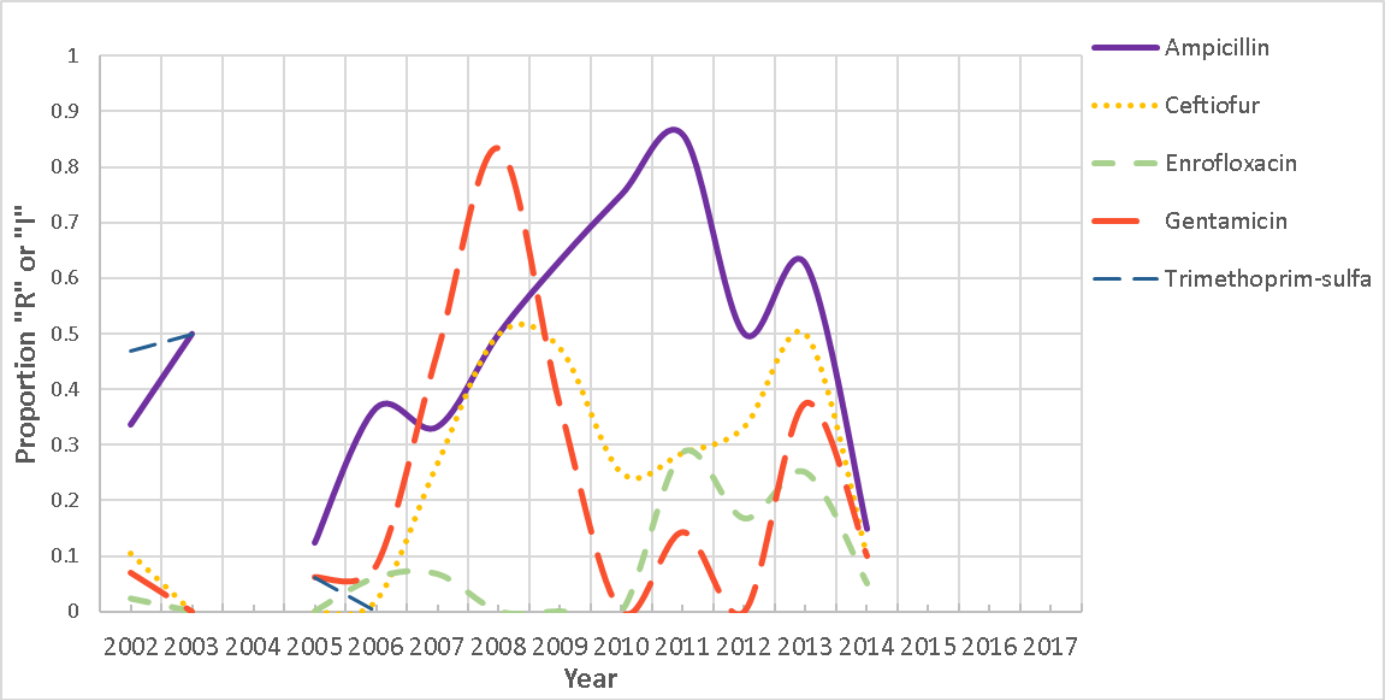

Figure 3. Proportion of bovine isolates classified by CLSI as "I" or "R" to antibiotics of interest, 2002-2017.

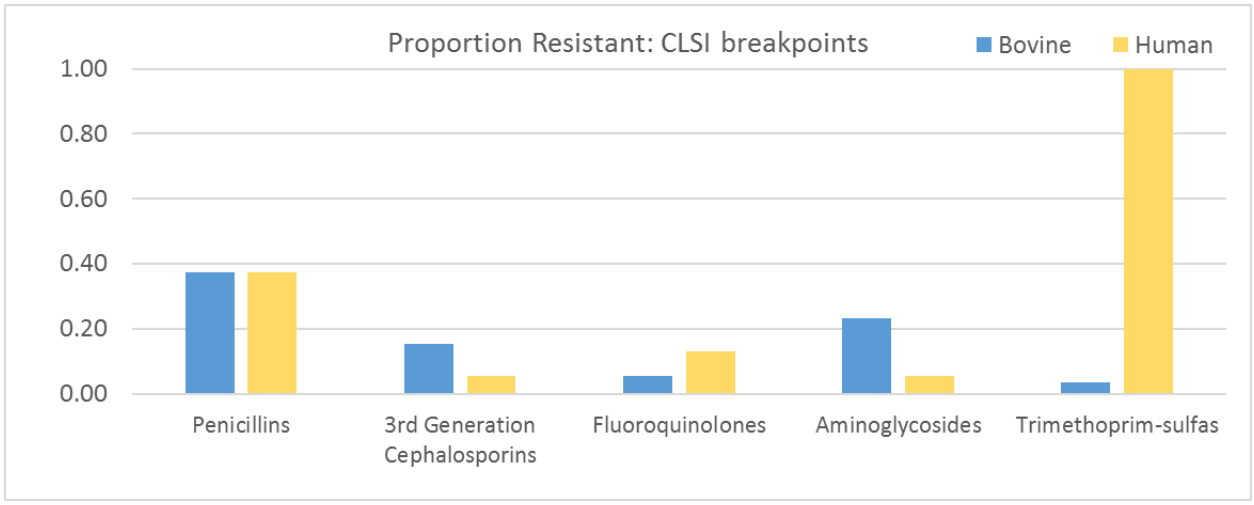

Figure 4. Proportion of bovine and human isolates resistant to antibiotic class of interest using CLSI breakpoints.

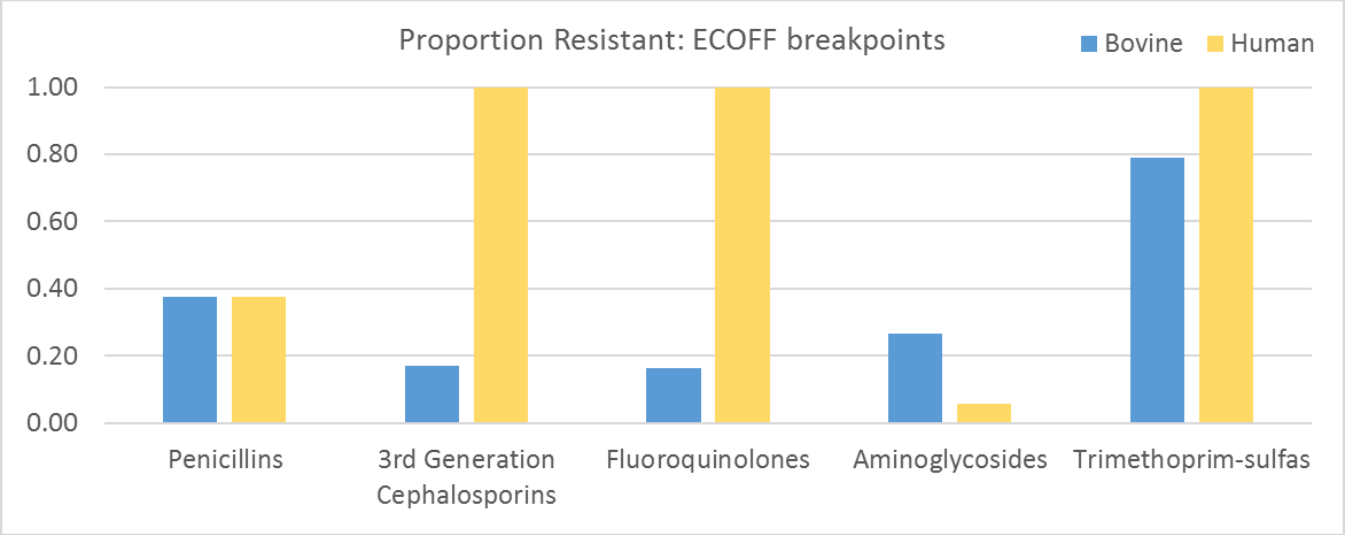

Figure 5. Proportion of bovine and human isolates resistant to antibiotic class of interest using ECOFF breakpoints.

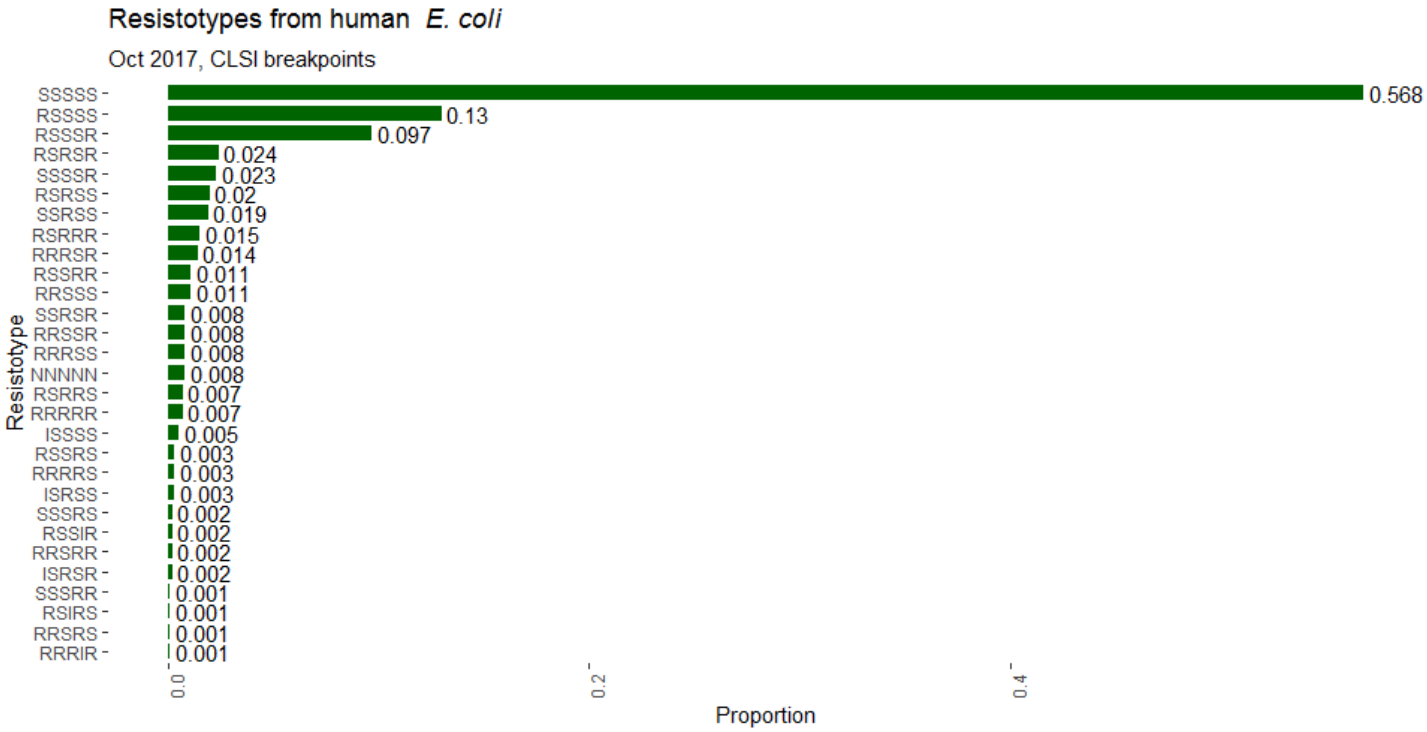

Figure 6a. Resistotype plot of human E. coli (n=1301) isolates using CLSI breakpoints. R=Resistant, I=Intermediate, S=Susceptible, N=No testing done for antibiotic class.

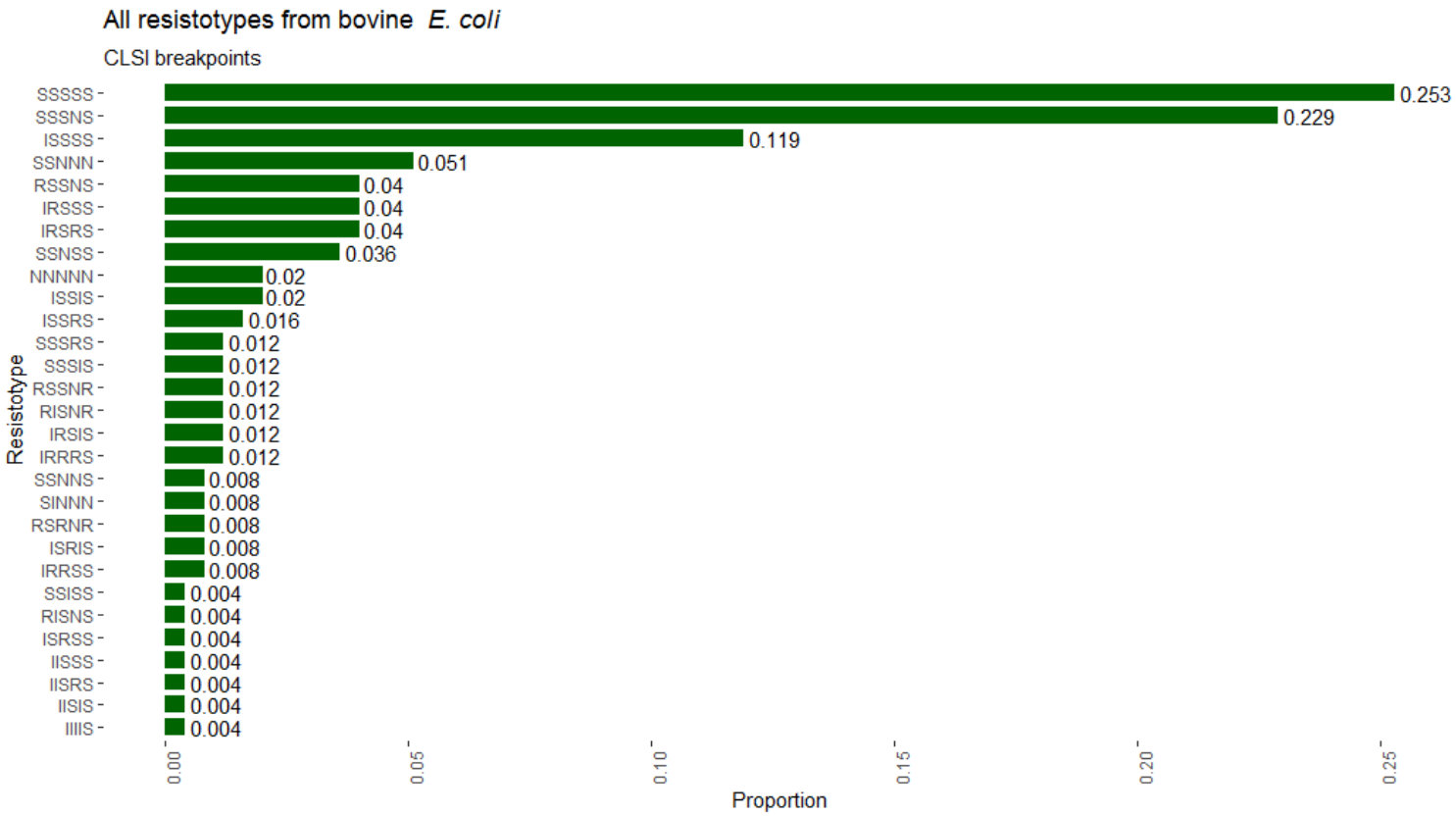

Figure 6b. Resistotype plot of bovine E. coli isolates (n=253) using ECOFF breakpoints. R=Resistant, I=Intermediate, S=Susceptible, N=No testing done for antibiotic class.

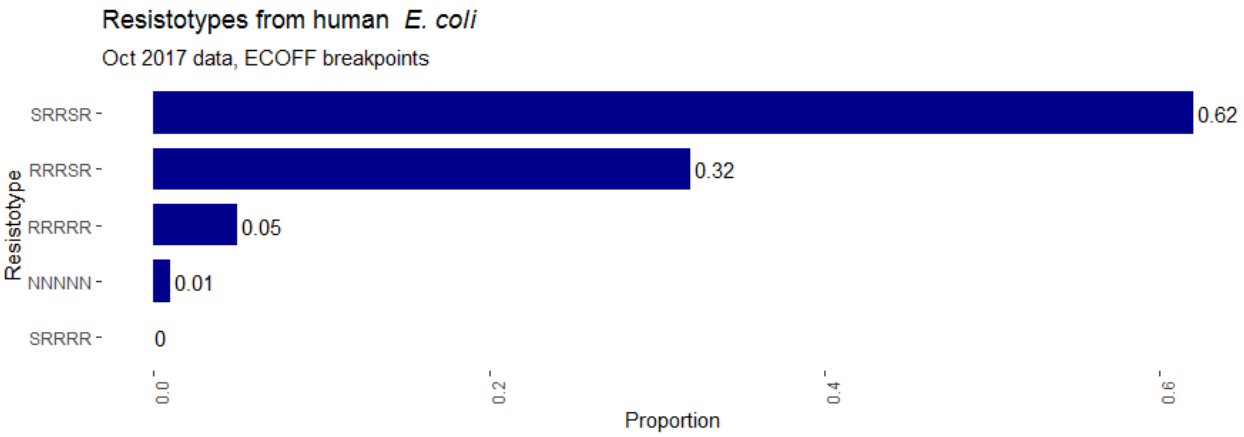

Figure 7a. Resistotype plot of human E. coli isolates (n=1301) using CLSI breakpoints. R=Non-wild type, S=wild-type, N=No testing done for antibiotic class.

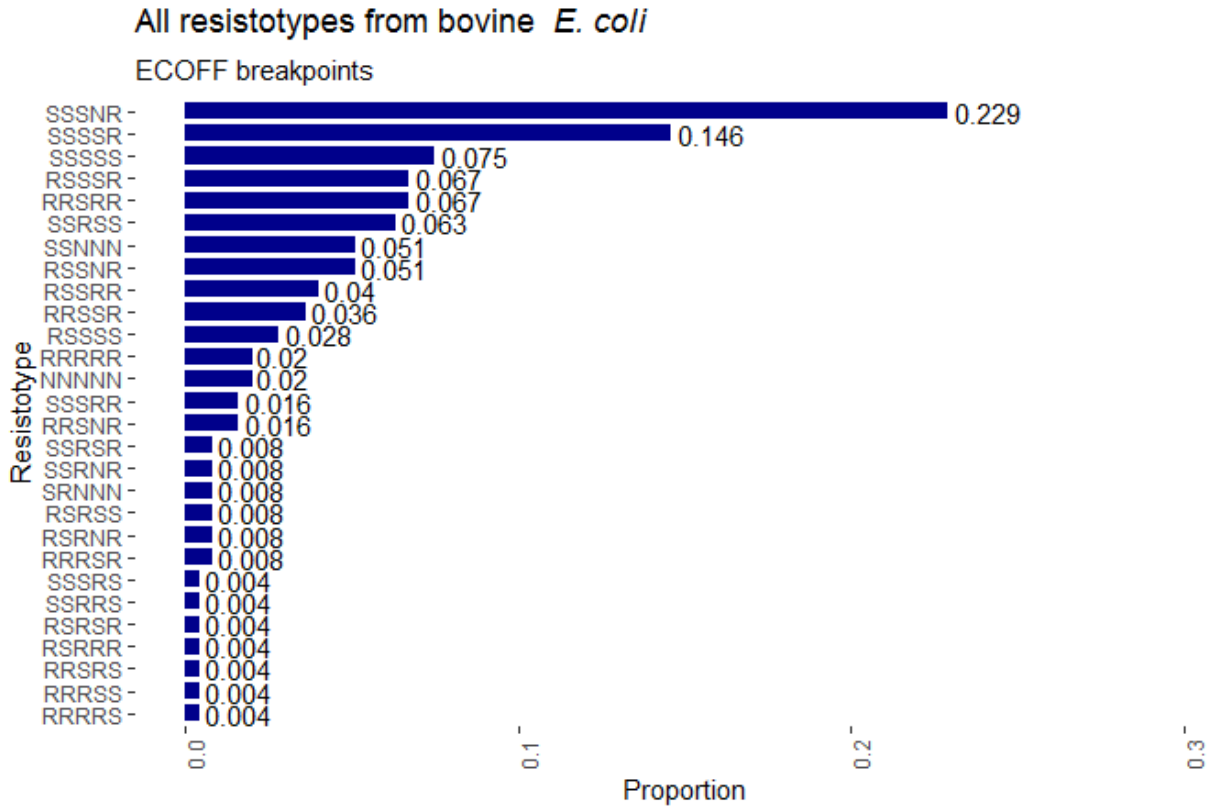

Figure 7b. Resistotype plot of bovine E. coli isolates (n=253) using ECOFF breakpoints. R=Non-wild type, S=wild-type, N=No testing done for antibiotic class.

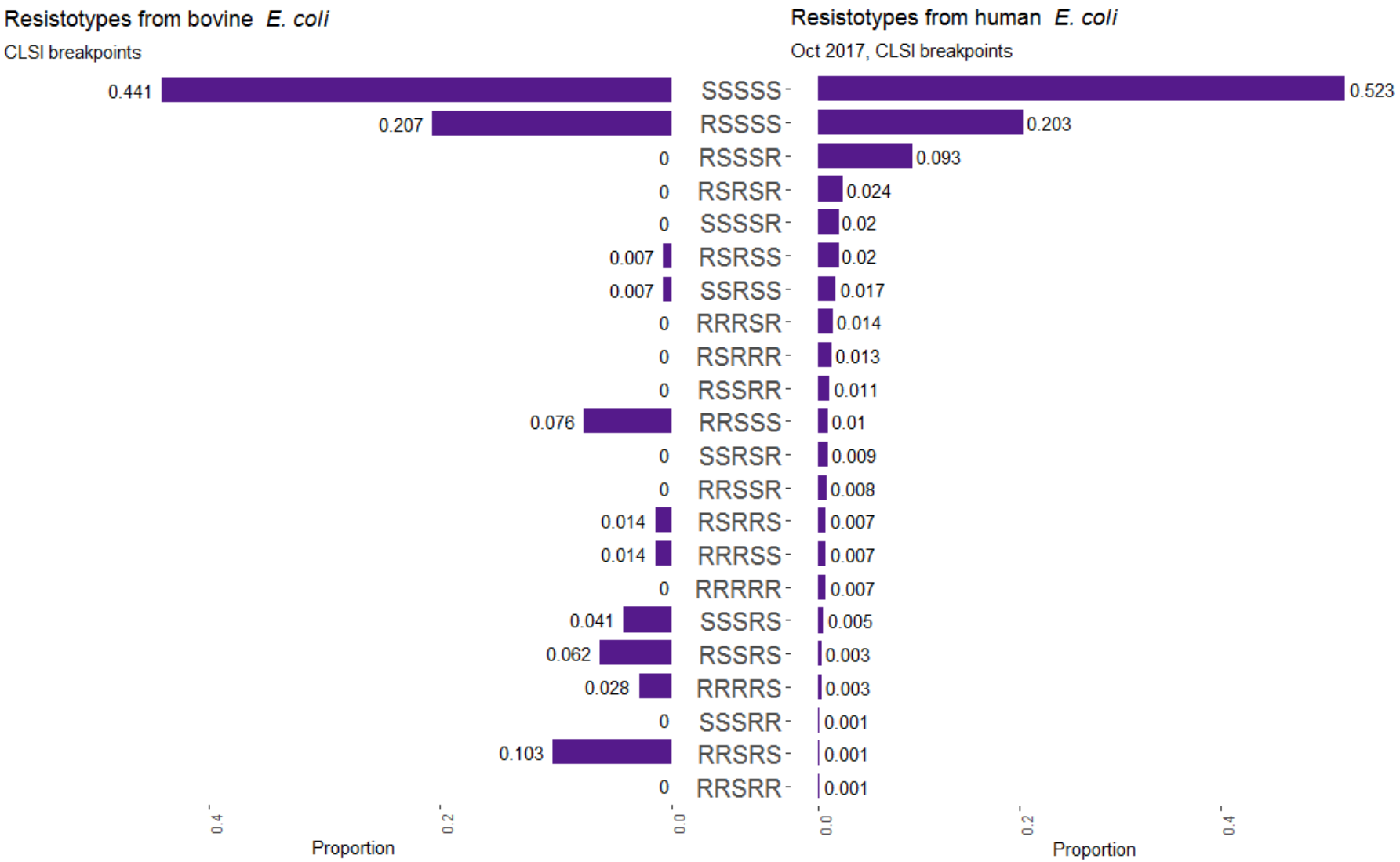

Figure 8. Full resistotype plots for all 5 antibiotic classes for human (n=1301) and bovine (n=145) E. coli isolates. Antibiotic classes in order: ampicillin, 3rd generation cephalosporins, fluoroquinolones, aminoglycosides, and trimethoprim-sulfa.

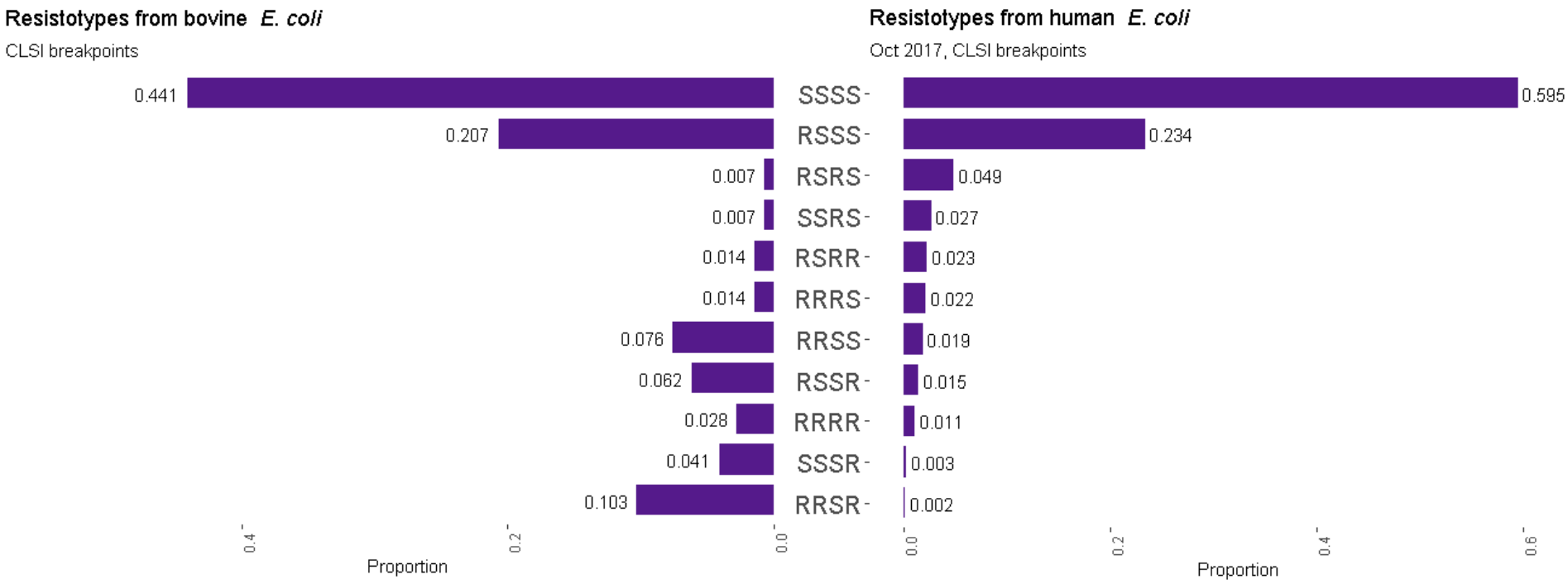

Figure 9. Full bovine resistotype plot (n=145) and human resistotype plot (n=1301) for four antibiotic classes using CLSI breakpoints: ampicillins, 3rd generation cephalosporins, fluoroquinolones, and aminoglycosides.

Table 1. Data sources and year. Bovine WADDL data collected from 2002-2017 and human Quest data collected from October 2017. Future data from Quest Diagnostics will include 5 years' worth of data for an estimated 78,660 E. coli isolates.

Year	***WADDL*** *Bovine*	***QUEST*** *Human*
2002	86	-
2003	2	-
2004	0	-
2005	16	-
2006	49	-
2007	15	-
2008	6	-
2008	19	-
2010	8	-
2011	7	-
2012	6	-
2013	8	-
2014	20	-
2015	3	-
2016	7	-
2017	1	1311
Total	**253**	**1311**

Table 2. Data structure for human and bovine isolates.

Variable	***Data source***
Date isolate was tested	Human and bovine
Isolate source	Human and bovine
Bacteria species	Human and bovine
Host species	Human and bovine
Antibiotic panel MIC result	Human and bovine
Antibiotic MIC interpretation	Human and bovine
State	Human
Zip code	Human
Host age	Human
Host gender	Human

Table 3. Quantitative MIC distribution for human and bovine isolates of E. coli with CLSI breakpoints indicated by bold mark.

Antibiotic class	Antibiotic	Origin	Number	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>256
Penicillin	Ampicillin	Bovine	253						8	79	59	9	74	2	17		
		Human	1301							453	203	157	13	474	1		
3rd Generation Cephalosporins	Ceftiofur	Bovine	253				29	161	16	4	10	28					
	Ceftriaxone	Human	1301						1231	4	9	11	46				
Fluoroquinolones	Enrofloxacin	Bovine	233	70	5	122	3	21	2	6	4						
	Ciprofloxacin	Human	1301				1072	34	24	1	170						
Aminoglycosides	Gentamicin	Bovine	154						110	3	5	15	21				
		Human	1301						1225	2	3	3	68				

Antibiotic class	Antibiotic	Origin	Number	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	*<20*	32	*<40*	>256
Trimethoprim/ sulfamethoxazole	Trimethoprim-sulfa	Bovine	233					49		176	1	7					
		Human	1301											1019		3	279

* Quest MIC data included values of <20 and <40. All isolates were classified as resistant to trimethoprim-sulfa as per Quest interpretation using CLSI breakpoints

Table 4. Minimum inhibitory concentration breakpoints (mg/L) for CLSI and ECOFF. No breakpoints are published for enrofloxacin and results used the interpretations of MIC as per WADDL

Drug class	***Species***	***Antibiotic***	***CLSI breakpoint***			***ECOFF breakpoint***	
			≤ S	**= I**	**≥ R**	**≤ S**	**> R**
Penicillin	Human and bovine	Ampicillin	8	16	32	8	8
3rd Generation Cephalosporins	Human only	Ceftriaxone	1	2	4	0.125	0.125
	Bovine only	Ceftiofur	2	4	8	1	1
Fluoroquinolones	Human only	Ciprofloxacin	1	2	4	0.064	0.064
	Bovine only	Enrofloxacin	NA	NA	NA	0.125	0.125
Aminoglycosides	Human and bovine	Gentamicin	4	8	16	2	2
Trimethoprim/sulfas	Human and bovine	Trimethoprim-sulfa	2/38	NA	4/76	1	1

Table 5a. MDR in bovine E. coli isolates with CLSI and ECOFF breakpoints, 2002-2017

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	All years
CLSI MDR	7	0	0	0	3	4	3	5	0	1	1	3	1	0	0	0	28
Prop. CLSI MDR	0.081	0	0	0	0.061	0.267	0.500	0.263	0	0.143	0.167	0.375	0.050	0	0	0	0.111
ECOFF MDR	11	0	0	2	9	4	3	11	2	4	2	4	2	0	0	0	54
Prop. ECOFF MDR	0.128	0	0	0.125	0.184	0.267	0.500	0.579	0.250	0.571	0.333	0.500	0.100	0	0	0	0.213
Total tested	86	2	0	16	49	15	6	19	8	7	6	8	20	3	7	1	253

Table 5b. MDR in human E. coli isolates with CLSI and ECOFF breakpoints, October 2017.

	Count
CLSI MDR	136
Prop. CLSI MDR	0.105
ECOFF MDR	1301
Prop. ECOFF MDR	1.0
Total tested	1301

Table 6. Proportion resistant (n) of bovine Escherichia coli to antibiotics in the drug panel from 2002-2017 using CLSI and ECOFF breakpoints. Resistance defined as breakpoint interpretation as "I" or "R".

	2002-2017	
Antibiotic:	**CLSI breakpoint**	**ECOFF breakpoint**
Ampicillin	0.375 (93/248)	0.375 (93/248)
Ceftiofur	0.153 (38/248)	0.169 (42/248)
Enrofloxacin	0.099 (23/233)	0.155 (36/233)
Gentamicin	0.055 (71/1301)	0.057 (74/1301)
Trimethoprim-sulfa	0.034 (8/233)	0.790 (184/233)

Table 7. Proportion resistant (n) human Escherichia coli to antibiotics in the drug panel from October 2017. Resistance defined as breakpoint interpretation as "I" or "R".

	October 2017	
Antibiotic:	**CLSI breakpoint**	**ECOFF breakpoint**
Ampicillin	0.375 (488/1301)	0.375 (488/1301)
Ceftriaxone	0.054 (70/1301)	1.0 (1301/1301)
Ciprofloxacin	0.131 (171/1301)	1.0 (1301/1301)
Gentamicin	0.055 (71/1301)	0.057 (74/1301)
Trimethoprim-sulfa	0.214 (279/1301)	1.0 (1301/1301)

Table 8. Summary of logistic regression analysis for human and bovine E. coli isolates; CLSI breakpoints

Drug	Species	No resistance	Resistance	OR	95% CI		p-value
Ampicillin	Bovine (1)	160	93	0.98	0.74	1.29	0.889
	Human (0)	823	488				
Ceftriaxone/ Ceftiofur	Bovine (1)	215	38	3.13	2.04	4.74	1.03E-07
	Human (0)	1241	70				
Ciprofloxacin/ Enrofloxacin	Bovine (1)	241	12	0.33	0.17	0.58	0.000327
	Human (0)	1140	171				
Gentamicin	Bovine (1)	217	36	2.9	1.88	4.41	9.95E-07
	Human (0)	1240	71				
Trimethoprim-sulfa	Bovine (1)	245	8	0.12	0.05	0.23	7.37E-09
	Human (0)	1032	279				

Table 9. Summary of logistic regression analysis for human and bovine E. coli isolates; ECOFF breakpoints

Drug	Species	No resistance	Resistance	OR	95% CI		p-value
Ampicillin	Bovine (1)	160	93	0.98	0.74	1.29	0.889
	Human (0)	823	488				
Ceftriaxone/ Ceftiofur	Bovine (1)	211	42	1.53E-03	7.13E-04	2.95E-03	2.00E-16
	Human (0)	10	1301				
Ciprofloxacin/ Enrofloxacin	Bovine (1)	217	36	1.28E-03	5.88E-04	2.49E-03	2.00E-16
	Human (0)	10	1301				
Gentamicin	Bovine (1)	212	41	3.23	2.13	4.84	1.18E-08
	Human (0)	1237	74				
Trimethoprim-sulfa	Bovine (1)	69	184	0.02	0.01	0.04	2.00E-16
	Human (0)	10	1301				

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