Report on NARMS On-Farm Sampling Project 2011-2015

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Executive Summary

The National Antimicrobial Resistance Monitoring System (NARMS) was developed over 20 years ago to monitor enteric bacteria in humans, food producing animals and retail meats for antimicrobial resistant bacteria (AMR). Included in this program was assessing the quantities and levels of AMR bacteria in samples collected from food producing animals at slaughter plants. While this has provided valuable information, there has been a gap in our knowledge of the relationship between the bacterial AMR profiles identified by NARMS and the bacterial AMR profile on the farm. This project was developed to begin assessing the relationship between bacterial and AMR profiles on the farm and at the slaughterhouse and to determine the feasibility of testing on-farm samples and correlating them to the slaughter samples.

This was a large and complex study that incorporated multiple groups of scientists at both government and academic institutions collaborating to obtain data. Species assessed included swine, beef and dairy cattle and poultry (broilers and turkeys). While several of the projects began to collect antibiotic usage data, most of the emphasis of this study was collecting samples on farms and comparing them to cohort animals at slaughter. Included in the goal was evaluating the feasibility of sample collection and best practices for collection, culture and laboratory techniques.

It quickly became apparent that each of the commodities require different techniques and data collection strategies. Dairy cattle are challenging as they do not go to slaughter on a regular basis, but tend to be culled individually, often being sent to the market. With swine and poultry, it may be a challenge to go onto farm production sites due to biosecurity control concerns. Beef cattle appeared fairly easy to sample, but our researchers had some push back when they tried to collect antibiotic usage data. However, in each species the scientists were successful in collecting data.

Overall, the level of AMR bacteria were quite low in most of the species assessed. While there was often a high prevalence of the foodborne pathogens, *Salmonella* and *Campylobacter*, the majority of isolates in most cases were pan-susceptible. However, there were AMR bacteria identified in all studies indicating that more needs to be done to maintain susceptibility to antibiotics for animal and human health. Several studies looked for the presence of bacteria with β-lactamases or carbapenemases which are indicative of resistance to several of the medically important antibiotics of concern in humans. Little evidence of these bacteria was found in the limited studies conducted to date. In addition, one of the studies looked for the presence of extra intestinal *E. coli* which have been associated with human urinary tract infections and found a very low level present on beef cattle hides and none on meat samples. While all of these studies are limited in scope, this information provides evidence that food producing animals appear to have low levels of these AMR patterns in the foodborne pathogens;

however, more research needs to be done on other commensal bacteria that may harbor resistance elements.

Overall, the studies were successful in assessing and comparing the AMR profiles of foodborne bacteria in food producing animals on-farm and at slaughter and from these preliminary studies, multiple manuscripts have been written and published that will provide valuable information, direction and guidance as we move forward to address AMR bacteria in food producing animals.

Background

The National Antimicrobial Resistance Monitoring System (NARMS) was developed over 20 years ago to monitor antimicrobial susceptibility in enteric bacteria from humans, food producing animals, and retail meats. The goal was to measure and quantify antimicrobial resistance (AMR) in enteric bacteria and to provide scientific data to be used by stakeholders and to inform policy, agendas and research direction. It has become one of the primary measurement of the level of AMR associated with food and food producing animals.

One weakness which had been identified with NARMs in relation to policy and measuring AMR, is lack of knowledge on the farm and the role the slaughter house environment plays in product contamination. The goal of these studies was to assess the level and type of AMR in food producing animals on the farm and follow as possible through the slaughter process. Species included in the study were swine, poultry (broilers and turkeys), and dairy and beef cattle.

While the overall goal of the study was to assess AMR on farms and compare to AMR profiles at slaughter, there was variation between groups in their study design, goals, and research results. This report summarizes the results of each study. The Primary Investigators (PIs) have also published the results in greater detail, where applicable.

Dairy Cattle

The study was conducted by Drs. Jo Ann Van Kessel and Jeff Karns, ARS-Beltsville, MD. There were 3 studies conducted from 2011-2015. The studies were conducted in collaboration with Drs. Dave Wolfgang and Ernest Hovingh from Pennsylvania State University.

Study 1:

The goal of the first study was to ascertain the optimal sample collection methods and to coordinate/define culture techniques for use in the study. Samples (440 fecal and manure samples) were collected from multiple dairy farms and two slaughter facilities in PA and tested for *Salmonella* and *E. coli.* A subset of samples were collected from cull animals on the farm and followed up by sample collection in the holding pens in the slaughter house.

Results: Salmonella was isolated from 49% of the samples and *E. coli* was isolated from all but 2 of the samples. Eleven *Salmonella* serotypes were identified: Agona, Anatum, Cerro, Infantis, Kentucky, Kiambu, Mbdanka, Meleagridis, Montevideo, Muenchen, and Typhimurium. Of these, Cerro, Kentucky, and Montevideo were the most predominant. One *Salmonella* and one *E. coli* isolate per positive sample was evaluated for antibiotic sensitivity using the NARMS panel of antimicrobials (by FDA-CVM-NARMS group). All of the *Salmonella* isolates were sensitive to all of the antimicrobials in the testing panel. However, 21.5% of the *E. coli* isolates were resistant to at least one antimicrobial. The most common resistance was to tetracycline (86 isolates), streptomycin (44 isolates), sulfisoxazole (41 isolates), and ampicillin (18 isolates). Resistance to 3 or more antimicrobials was observed in approximately 10% of the isolates. One isolate was resistant to 9 antimicrobials and another isolate was resistant to 10 antimicrobials. Due to the relatively low prevalence of resistance, no conclusions could be drawn from the paired animal portion of the pilot.

Two different media were used for parallel enrichment of *Salmonella* from the samples, RVS broth and tetrathionate broth. This is frequently done to ensure that isolation methods are not biased against certain serotypes. In this preliminary study, *Salmonella* was isolated from the tetrathionate enrichment

but not the RVS enrichment of 216 samples, *Salmonella* was isolated from both enrichment broths for 89 samples, and *Salmonella* was isolated from the RVS enrichment, but not the tetrathionate enrichment of one sample. The isolate from this latter enrichment was serotype Cerro, commonly isolated with tetrathionate enrichments. We have observed similar results in previous studies and determined that in future studies we would only enrich samples in tetrathionate.

Study 2:

The goal of Study 2 was to collect samples from soon-to-be culled cows from two commercial dairy farms (designated K and L) and a slaughter facility in PA. Fecal samples were collected at the farms and rectal and hide swabs were collected at the slaughterhouse. Additional samples were collected at the slaughterhouse that lacked matching farm cohorts.

Results: Farm K had a high prevalence of *Salmonella* (99% of fecal samples were positive) while all samples collected from Farm L were *Salmonella*-negative. The rectal (n=372) and hide swabs (n=379) collected from the slaughterhouse from cattle from farm K were 34% and 85% positive for *Salmonella*, respectively. Although *Salmonella* was never isolated from fecal samples collected directly from Farm L, 22% of the rectal swabs and 78% of the hide swabs from Farm L cull cows were *Salmonella*-positive at slaughter. The serogroup profile for Farm K isolates was different in slaughterhouse samples compared with farm samples. Serogroup C1 represented 92% of on-farm isolates while this serogroup only represented 75% and 49% of the isolates from rectal and hide swabs from these animals at the slaughterhouse, respectively. Serogroup C1 represented less than 20% of the isolates from animals at the slaughterhouse that did not originate at either of the two study farms. Some (n=100) of the *Salmonella* isolates have been characterized using Sensititre culture plates (NARMS panel) and all were pan-susceptible. Additional pre-screening supports the observation of limited antimicrobial resistance in these dairy cow-associated salmonellae, however one MDR (Ampicillin, Cefoxitin, Chloramphenicol, Tetracycline, Streptomycin) isolate was obtained. The serotype of this MDR isolate was Montevideo (C1).

Non type-specific *E. coli* were also isolated from each of the samples, which were assayed using the Sensititre NARMS panel on the first 300 isolates (from 100 samples). Resistance to at least one antimicrobial was observed in 23% of the isolates and 5.3% were resistant to 4 or more antimicrobials. To reduce the number of isolates for which Sensititre analysis is needed, we pre-screened 3 to 5 isolates per sample for resistance to 8 antimicrobials (Ampicillin, Cefoxitin, Chloramphenicol, Tetracycline, Streptomycin, Kanamycin, Ciprofloxacin) using antimicrobial-supplemented Mueller Hinton agar plates. Prescreening showed that 18% of all samples yielded at least one isolate with resistance to at least one antimicrobial. Resistance to tetracycline was most common followed by streptomycin resistance. A few ampicillin resistant isolates were obtained but most were sensitive to cefoxitin. Approximately 12% of all samples yielded some isolates with resistance to 3 or more antimicrobials. When comparing isolates from fecal samples collected at the farm with isolates collected from cohort animals at the slaughter house more isolates were resistant at slaughter (resistance to 3 or more antimicrobials: Farm K: 4.6% vs. 9.1%; Farm L: 4.3% vs. 16.7%).

In addition to isolating non type-specific *E. coli*, each sample was cultured on cefotaxime-supplemented MacConkey agar (1 µg/ml) to screen for β-lactamase producing bacteria. Approximately 70% of the samples yielded resistant isolates at this concentration. Further screening for resistance to an 8 antimicrobial panel (Ampicillin, Cefotaxime, Chloramphenicol, Tetracycline, Streptomycin, Kanamycin,

Ciprofloxacin) was also conducted via replica plating using antimicrobial-supplemented Mueller Hinton agar plates. The results of the pre-screenings were used to select isolates for Sensititre analysis.

One of the topics of discussion during the planning stages of this project was how well slaughterhouse sampling represented individual farms. The non-specific *E. coli* resistance data and the *Salmonella* prevalence and serogroup data together support previous observations of the transfer and mixing of bacteria as the cows are moved from the farm into the slaughter process.

Study 3:

The goal of this study was to determine the AMR profile across a cross-section of dairy herds in Pennsylvania. Six composite manure samples were collected from each farm to represent specific age groups of animals including pre- and post-weaned calves, and dry cows and lactating cows. Additionally, a brief survey on antibiotic usage was included.

Results: Samples from 80 farms have been collected and cultured for *Salmonella* and for non type-specific *E. coli*. At least 5 isolates of *E. coli* and 5 isolates of *Salmonella*, when present, were tested for antimicrobial resistance via replica plating with Mueller Hinton agar plates that are supplemented with 'breakpoint concentrations' of Ampicillin, Cefoxitin, Chloramphenicol, Tetracycline, Streptomycin, Kanamycin, Ciprofloxacin, or Cefotaxime. In addition, each sample was cultured directly on MacConkey agar plates that are supplemented with cefotaxime (4 µg/ml), cefepime (4 µg/ml), or imipenem (4 µg/ml) to screen for β-lactamase producing bacteria.

All tested *Salmonella* isolates (>1000) were pan-susceptible with the exception of several isolates from lactating cows on one farm that were tetracycline-resistant. A PCR method was used to place *Salmonella* isolates into a serogroup and the dominant serogroups are Group Unknown (50% of farms), C1 (31% of farms), and C2 (19% of farms). Group B was isolated from one farm and Group E from another. Representative isolates from each farm have been serogrouped and, as anticipated, the predominant serogroups are Cerro (50%), Montevideo (31%), and Kentucky (18%).

For non-type-specific *E. coli*, with all antimicrobials tested, resistance was more prevalent in isolates from the pre- and post-weaned calves than in isolates from the adult cows (dry and lactating). Resistant isolates were obtained from 88% of the pre-weaned calf samples, 81% of the post-weaned calf samples, 46% of the dry cow samples, and 64% of the lactating cow group samples. None of the Imipenem-supplemented plates yielded isolates. Isolates from the Cefepime- and the Cefotaxime-supplemented plates were screened for resistance using the replica plating method followed by NARMS panel testing. Based on a comparison of the selective isolation results, the dry and lactating animals appeared to harbor similar resistant phenotypes as the younger animals; however, the resistant *E. coli* represented a smaller percentage of the total *E. coli* population in the older animals than in the younger animals. Continued efforts are being made to compare and contrast resistant isolates isolated from the older and younger animal groups.

A brief survey was conducted on most of the study farms that addressed antimicrobial use. The data are currently being collated and compared with results of AMR bacterial results.

Conclusions:

One of the primary challenges of testing matching cohorts of dairy cattle between on-farm and slaughter sites is that many of the cull animals pass through a buying station and/or an auction house prior to arrival at a slaughter house. As a result, tracking the animals can be challenging and their exposure to other bacteria at each site may alter the results of a direct comparison between on-farm and slaughter results.

Based on the preliminary results from these initial studies, it can be questioned how well slaughterhouse sampling represent individual farms. The non-specific *E. coli* resistance data and the *Salmonella* prevalence and serogroup data support previous observations of the transfer and mixing of bacteria as the dairy cows are moved from the farm to slaughter.

This was a successful collaboration between ARS scientists and academic veterinarians from Pennsylvania State University. Some antibiotic usage data has been collected which is being analyzed. There is interest in maintaining a relationship with FDA to discuss ongoing ARS research and assist in identifying knowledge gaps or trends or as consultants on special projects as needed.

Publications:

Submitted Abstract

American Society for Microbiology General Meeting 2015: Huilin Cao, Jeffrey S. Karns, Abani K. Pradhan, Dave R. Wolfgang, Ernest Hovingh, Jo Ann S. Van Kessel. Antimicrobial Resistance of *Salmonella* and *E. coli* from Pennsylvania Dairy Herds. Submitted.

Swine

The swine study was conducted by Drs. T Frana, C Logue, N. da Silva, J. Beary, and A. O'Connor from Iowa State University and Dr. P. Cray at North Carolina State University. Dr. Jim McKean was the original PI, but passed away during the course of the study.

The *overall goal* of this study was to compare incidence and the AMR profile of foodborne bacteria pre- and post-slaughter to determine if bacteria collected from swine at the slaughter plant could be used for on-farm prevalence AMR bacteria surveillance. A major concern with the use of slaughter house samples is that *Salmonella* serotypes obtained after animals have passed into and through the slaughter plant are not consistent and do not provide an accurate profile of *Salmonella* profiles on the farm. At the time of these studies, there was an outbreak of Porcine Epidemic Diarrhea Virus (PEDV) in US swine, making on-farm sample collection impossible. Therefore, the study was changed from "on-farm" to initial arrival at the slaughter house using clean trucking techniques and comparing those samples to samples obtained from the same cohort of pigs after slaughter. In addition to *Salmonella*, data were also collected on *Campylobacter* and *E. coli.*

The samples were collected at a large, Midwestern abattoir, which processes approximately 18,000 finishing pigs daily. For each collection time period, truckload lots (150+ pigs) were selected upon arrival at the abattoir from 1 of 2 farms. After selecting the group of pigs, they were tracked to the pens where they would rest in the lairage. Samples were collected from the lairage pen prior to the pigs entering the pen. Also included were samples from surrounding pens. After the pigs were placed in pens, samples were collected as quickly as possible from the pigs. Following lairage rest of a minimum of 2 hours, each

lot of animals were processed as a single group with a gap between lots for differentiation. In addition, cecal samples were collected as the pigs were processed.

Samples were collected from 359 pigs from 14 truck lots over eight dates between September 3 and November 19, 2013.

The primary outcomes of interest were *Salmonella* serotype and AMR patterns within serotypes. In addition, the prevalence and AMR of *Campylobacter* and *E. coli* were secondary outcomes of interest.

Results: Of 359 samples, 307 samples were positive for *Salmonella* and 51 samples were negative. *Salmonella* was isolated from samples each day of sampling. From the 307 positive samples, 1163 isolates were obtained, with isolation of 4 *Salmonella* from a sample the most common. The most common serotype isolated was Derby, followed by I 4,{5},12:i. The third most common isolate varied based on sampling point with Typhimurium most commonly isolated upon arrival, however post slaughter Agona was more commonly isolated. Some isolates were identified only on a single days. Of the 1163 isolates, 898 (77%) were resistant to at least one antimicrobial tested.

Of the 359 samples collected, *Campylobacter* was detected in 342 samples and all were identified as *C. coli*. Most of the isolates appeared to be relatively unique when analyzed by PFGE analysis. There was no difference between isolates at arrival compared to those isolated at the end of slaughter. Of the 122 isolates obtained at arrival, AMR testing indicated resistance to at least one antimicrobial in all but 1 of the isolates. Similar results were found post slaughter with only 6 pan-susceptible out of 115 isolates.

Conclusions

The results of this study suggests that samples collected at the slaughter house or post-slaughter are not reflective of the AMR *Salmonella* in animals on the farm or at arrival at the abattoir. This indicates that other methods must be used to determine the on-farm prevalence and AMR patterns of *Salmonella* in swine. There was a great diversity of *Salmonella* serotypes and AMR patterns found in this study. Linking to antimicrobial use on the farm with samples collected at slaughter will be challenging. There appeared to be no difference in the genetic profiles of the *Campylobacter* at any stage of the study.

The researchers in this study found that collecting samples upon arrival and from post-slaughter cecum samples was relatively easy, although there may be concerns with getting fresh samples from the pigs upon arrival. This technique provides a potential mechanism for on-farm sample collection without going onto the farm due to the tight biosecurity concerns for many swine farms.

Publications:

Manuscript in preparation:

A quasi-experiment evaluating changes in antimicrobial resistance patterns in *Salmonella*, *Campylobacter* and *E. coli* collected from swine at different points of slaughter T. Frana, C. Logue, N. da Silva, J. Beary, P. Cray, A. M. O'Connor

Submitted Abstracts:

- Submitted to International Association for Food Protection: PJ Fedorka-Cray, T. Frana, C. Logue, N. da Silva, J. Beary and AM O'Connor. Isolation and Characterization of Escherichia coli Isolated from Swine at the Farm, Lairage and Slaughter
- Submitted to International Society for Veterinary Epidemiology and Economics: T. Frana, C. Logue[1], N. da Silva, J. Beary, P. Cray, A. M. O'Connor. A quasi-experiment evaluating changes in antimicrobial resistance patterns in *Salmonella*, *Campylobacter* and *E. coli* collected from swine at different points of slaughter
- Submitted to SAFEPORK 2015: T. Frana, C. Logue, N. da Silva, J. Beary, P. Cray, A. M. O'Connor. A quasi-experiment evaluating changes in antimicrobial resistance patterns in *Salmonella*, *Campylobacter* and *E. coli* collected from swine at different points of slaughter

Beef Cattle

Three groups of researchers investigated AMR on-farm and at slaughter in beef cattle. Within each group, there were several studies conducted resulting in a fairly comprehensive study of AMR in beef cattle.

Texas Tech University

The project was led by Dr. Guy Lonergan, with collaborators: S. A. Ison, J.J. Ison,H.E. Webb, K.K. Nightingale, M. Bugarel, H.C. den Bakker from Texas Tech University; H. Morgan Scott from Texas A&M University; P. McDermott and S. Ayers from FDA; and S. Granier and A. Brisabois from France. This project consisted of 5 studies.

Study 1:

Four feedlots in the Texas high plains were sampled every 14-28 days from September to December, 2012. Two pens of cattle were sampled shortly after arrival. The same cohort of cattle were sampled at 3 subsequent visits. In addition, two pens within one week of slaughter were sampled at each feedlot. Within each cohort, 20 well defined fecal pats were collected. Samples were tested for non-type *E. coli* and *Salmonella*.

Results: Non-type specific *E. coli* were recovered from all but 3 samples (99.8% prevalence – 1,261 positive out of 1264). The AMR profile varied with 70% pan-susceptible. Of the remaining isolates, 15.5%, 5.7%, 3.4% were resistant to two, four, or five or more drugs respectively.

Salmonella was recovered from 60.5% (n=795) of samples. Several culture techniques were used to isolate *Salmonella* including tetrathionate (TT) broth and Rappaport-Vassiliadis (RV) broth. Variation in isolation prevalence was observed with 20.0% isolated from both broths, 40.2% were cultured from only TT and 0.3% only from RV.

Despite the proximity of the feedlots, variation in prevalence occurred across feedlots. As an example, one isolate of *Salmonella* was recovered from 97.2% of the samples recovered in one feedlot whereas another regionally co-located feedlot had only 30.6% of that particular isolate. Prevalence across pens varied from 0 to 100% and varied by visit from 0 to 90% in one lot to 100 to 90% in another.

The most common AMR pattern for *Salmonella* was pan-susceptible with resistance to tetracycline observed in 25.1% of the samples. Ceftiofur resistance occurred in 4.7% of the isolates and co-resistance

patterns were observed with ACSSuT and MDR-AmpC phenotypes accounting for 2.37% and 2.7% of isolates, respectively.

The most frequent serotype isolated was Montevideo at 36.25% followed by Anatum, Kentucky, and Meleagridis. These serotypes were isolated most frequently in all feedlots, while the less common serotypes clustered by serotype in individual feedlots.

Conclusions reached included that when monitoring for common phenotypes of *E. coil* or *Salmonella,* fewer feedlots are needed and more pens within feedlots are required. Monitoring rare *E. coli* or *Salmonella* phenotypes requires more feedlots and fewer pens.

Study 2:

The goal of this study was to generate data on non-type specific ceftiofur- and tetracycline-resistant *E. coli in* samples collected on-farm and in slaughter plants. A feedlot in Texas was visited on 10 occasions and at each visit, 3 pens of cattle within 2 weeks of scheduled slaughter had 25 pen-floor fecal samples collected per pen. Pens of cattle were then sampled at slaughter with hide and rectal swabs sampled immediately after exsanguination and prior to the initial hide wash. Individual animals were not followed, but cohorts of animals in the same pens were sampled.

Results: Overall, the average concentrations for positive samples of non-type specific *E. coli* on for fecal pat, fecal swabs and hides as determined by direct-plating onto MAC media were 5.93, 6.52, and 5.51 $\log_{10}$ cfu per unit substrate respectively, MAC containing ceftiofur were 0.94, 0.2, 2.9 $\log_{10}$ cfu per unit substrate and MAC containing tetracycline were 4.49, 5.2, 4.68 $\log_{10}$ cfu per unit substrate, respectively. Observed prevalence of ceftiofur-resistant samples were 4.0, 8.0, and 12.0% among fecal pats, rectal swabs, and hide swab samples, respectively.

Conclusions in general found that in-plant samples provided similar information to samples collected on the farm from the same cohort approximately 2 weeks earlier.

Study 3:

The *goal* of this study was to assess different culture methods for their ability to estimate pen-level prevalence of β-lactamase-producing (ESBL) *E. coli.* The *results* of this study demonstrated that the observed prevalence of ESBL was greater when methods that incorporate both a selective-enrichment and selective-agar were used. Use of less selective methods results in underestimating the true prevalence of resistant organisms. While multi-drug resistance was not uncommon, none were resistance was to 4th generation cephalosporins, carbapenems, ciprofloxacin, or piperacillin/taxobactam combination and only 40 isolates demonstrated resistance to gentamicin.

Study 4:

The *goal* of this study was to investigate the potential for colistin resistance in *Salmonella* recovered from beef cattle. Of the 95 *Salmonella* strains phenotypically tested, none were found to harbor colistin resistance.

Study 5:

The *goal* of this study was to assess the prevalence of carbapenem-resistant Enterobacteriaceae (CRE) resistance in cattle from herds with a history of 3rd generation cephalosporin use. *Preliminary results*

indicate that only 1 isolate from approximately 160 samples recovered was a CRE with a carbapenemase-positive phenotype. Additionally, fewer than 10 CRE isolates with a carbapenemase-negative phenotype were recovered. In addition, 19 Aeromonads/Psuedomonads (preliminary identification) with carbapenemase-positive phenotypes were recovered. All carbapenemase-positive phenotype isolates and approximately 15 CRE have been sequenced. A publication will be developed and genomes deposited in appropriate online libraries.

An additional project is working with APHIS to develop a survey instrument to capture antimicrobial use in cattle.

Conclusions

The projects performed by this group found a number of important data points. It determined a sampling scheme to detect and describe the prevalence of *E. coli* or *Salmonella* in cow pens based on whether the AMR phenotype is common or infrequent. In addition, in contrast to the results found with swine or dairy, it appears that in-plant samples were fairly consistent with the samples found in 2 weeks earlier in the feedlot pens.

Of importance, based on the research performed here, the prevalance of ESBL producing bacteria is low as is colistan and carbapenemase-positive bacteria indicating that beef cattle are not currently a likely source of bacteria with this AMR profile.

The research determined that sampling schemes and laboratory techniques to recover rare phenotypes were successful and will help with future on-farm studies. A challenge was the laboratory capacity to perform the work in a timely and efficient manner. As a result, additional laboratories were enlisted for assistance resulting in the determination of different biases which required harmonization of techniques and results. Also, the overall goal of the project changed over time.

The results of this study indicate that it is not going to be easy to successfully collect on-farm data in the short-term. Different sampling scenarios are going to be required depending on the frequency of serotype of interest within the system and some genetic profiles may need to be collected for different bacteria than *E. coli* and *Salmonella.* Further refinement/development of laboratory methods are needed to explore rare phenotypes and to ensure all data is harmonized and consistent between laboratories. Finally, outreach is needed for industry buy-in, especially if the government is going to collect the data.

Publications:

Peer-reviewed manuscripts

GH. Tyson, PF. McDermott, C. Li, Y. Chen, DA. Tadesse, S. Mukherjee, S. Bodeis-Jones, C. Kabera, SA. Gaines, GH. Loneragan, TS. Edrington, M. Torrence, DM. Harhay and S. Zhao. Whole-genome sequencing effectively predicts antimicrobial resistance phenotypes of Escherichia coli. J Antimicrob Chemother. 2015 Oct;70(10):2763-9.

HE Webb, M Bugarel, HC den Bakker, KK Nightingale, SA Granier, HM Scott, Loneragan GW. Carbapenem - Resistant Bacteria Recovered from Faeces of Dairy Cattle in the High Plains Region of the USA. PLoS One. 2016 Jan 29; 11 (1).

Webb HE, Granier SA, Marault M, Millemann Y, den Bakker HC, Nightingale KK, Bugarel M, Ison SA, Scott HM, Loneragan GH. Dissemination of the mcr-1 colistin resistance gene. Lancet Infect Dis. 2016 Feb; 16(2):144-5.

Submitted Abstracts

CRWAD

- S.A. Ison, J.J. Ison, H.M. Scott, P. McDermott, S. Ayers, M. Torrence G.H. Loneragan. An assessment of on-farm surveillance systems ability to accurately represent the burden of non-type specific Escherichia coli in beef cattle at harvest: a NARMS paired-match study.
- R.M. McCarthy, S.A. Ison, H.M. Scott, G.H. Loneragan. Evaluation of methods for culture detection of extended-spectrum beta-lactamase producing (ESBL) Escherichia coli.

BIFSCo

- Hattie Webb, Guy Loneragan, Sophie Granier, Kendra Nightingale, Marie Bugarel, Anne Brisabois, Renaud Lailler, Sarah Ison, Byron Chaves. Colistin Resistance in Salmonella of Bovine-origin.
- Marie Bugarel, Sarah Ison, Kendra Nightingale, Guy Loneragan. Characterization of the mechanisms of resistance to β-lactams in various bacterial strains isolated from bovine fecal samples in the United States

ARAE

- Webb, HE, Granier, SA, Nightingale, KN, den Bakker, HC, Marault, M, Bugarel, M, and Loneragan, GH. Characterization of colistin resistance mechanisms in Salmonella
- Ison, J.J., A.E. Mather, G.H. Loneragan, M. Bugarel, I. Berta-Vanrullen and S.A. Granier. A Comparison of the Active and Passive Salmonella Surveillance Systems for Antimicrobial Resistance
- Bugarel M., Ison S., Webb H., Nightingale K., den Bakker H., Loneragan G. Characterization of the mechanisms of resistance to β-lactams in various bacterial strains isolated from bovine fecal samples.
- S.A. Ison, G.H. Loneragan, S.J. Trojan, J.J. Ison, M.M. Brashears, H.M. Scott. Variation in antimicrobial susceptibility and prevalence of Escherichia coli and Salmonella isolated from United States feedlot cattle.
- Webb, HE, Bugarel, M, den Bakker, HC, Granier, SA, Nightingale, KN, Scott, HM, Loneragan, GH. Preliminary Exploration of [rare but important beta-lactamase] Resistance in Cattle Populations

ASM meeting

- Gregory H. Tyson, Patrick F. McDermott, Cong Li, Yuansha Chen, Daniel A. Tadesse, Sampa Mukherjee, Sonya Bodeis-Jones, Claudine Kabera, Stuart A. Gaines, Guy H. Loneragan, Tom S. Edrington, Mary Torrence, Dayna M. Harhay and Shaohua Zhao. Whole-genome sequencing effectively predicts antimicrobial resistance phenotypes of Escherichia coli. (Abstract submitted by FDA)

- Marie Bugarel, Sarah Ison, Kendra Nightingale, Guy Loneragan. Characterization of the mechanisms of resistance to β-lactams in various bacterial strains isolated from bovine fecal samples in the United States

U.S. Meat Animal Research Center (MARC)

Dr. John W. Schmidt led the research in collaboration with Drs. Getahun Agga, Terrence M. Arthur, Dayna M. Brichta-Harhay, and Tommy L. Wheeler. Research was conducted from 2013 to 2015 and consisted of two studies.

Study 1:

The goals of this study were to determine the prevalence and concentrations of third-generation cephalosporin-resistant (3GC^r) *E. coli*, trimethoprim-sulfamethoxazole-resistant (COT^r) *E. coli,* 3GC^r *Salmonella enterica* and nalidixic acid-resistant (NAL^r) *S. enterica* in the beef continuum from production through processing. Additionally 3GC^r *E. coli* and COT^r *E. coli* isolates were screened for the presence of virulence-associated markers of extra-intestinal pathogenic *E. coli* (ExPEC), which has been associated with human urinary tract infections.

Results: The prevalence and concentrations of the above described foodborne bacteria were determined in feces and hides both at the feedlot and at the processing plant. Samples were collected from pre-evisceration and final carcasses from three lots of feedlot cattle (N=184). In addition, prevalence of the pathogens was also determined on strip loin steaks from 103 of the carcasses. Three groups of beef cattle were used in the study, two groups from one Nebraska feedlot (n=74 each), and 1 group (n=36) was from a different Nebraska feedlot. The three groups were harvested on different days at the same slaughter/processing plant.

3GC^r *Salmonella* were detected on 7.6% of hides during processing, but was not detected on any carcass or strip loin steak. NAL^r *S. enterica* was detected on one hide. While 3GC^r *E. coli* and COT^r *E. coli* were detected on 100% of hides during processing. 3GC^r *E. coli* and COT^r *E. coli* were detected on 0.5% of final carcasses and none on strip loin steaks. The low prevalences of these organisms on final carcasses and their absence on strip loins demonstrate that current sanitary dressing procedures and processing interventions are effective against antimicrobial-resistant bacteria.

A total of 542 *E. coli* isolates were screened for the presence of ExPEC virulence genes. Only 2 COT^r *E. coli* isolated from hides were positive for ExPEC suggesting cattle and beef may not be an important source of these bacteria.

Study 2:

The goals of this study were to determine the occurrence and variability of of 3GC^r *E. coli*, COT^r *E coli*, 3GC^r *Salmonella,* and NAL^r *Salmonella* and erythromycin-resistant (ERY^r) *Enterococcus* spp. populations within and on Nebraska cattle feedlots.

Seven cattle feeding yards were sampled on 2 occasions. Questionnaires regarding specific antimicrobial use were also provided to the producers.

During each visit, 42 fecal samples from restrained cattle, 42 hide samples were obtained, and 12 pen soil samples were collected from 3 pens. Overall 1,328 samples were examined, 588 fecal, 588 hide, and 152 pen soil.

Results

Characterization of the isolates is nearly complete.

E. coli results included:

- Generic *E. coli* (*E. coli* regardless of antimicrobial susceptibility or pathogenicity) were detected in all 1,328 samples. A total of 408 generic *E. coli* isolates were examined for the presence of ExPEC virulence markers and 0.5% (2/408) were considered ExPEC. Susceptibilities to 15 antimicrobial agents were determined for 126 generic *E. coli* isolates with 60% of isolates pan-susceptible, 40% of isolates were tetracycline-resistant (TET^r) and 8% of isolates were COT^r. No isolates were consistent with $3GC^r$.
- $3GC^r$ *E. coli* were present in 50% of the samples (35% fecal, 60% hide, 67% pen soil). 359 $3GC^r$ *E. coli* isolates were examined for the presence of ExPEC virulence markers, bla_{CMY}, and bla_{CTX-M} . Detection rates were: 71% bla_{CMY}, 24% bla_{CTX-M}, 2% both bla_{CMY} and bla_{CTX-M}, 3% neither bla_{CMY} nor bla_{CTX-M}. None of the $3GC^r$ *E. coli* isolates were ExPEC. Susceptibilities to 15 antimicrobial agents were determined for 116 $3GC^r$ *E. coli* isolates with 100% were $3GC^r$, 92% were TET^r, 77% were amoxicillin-clavulanic acid-resistant (AUG^r), 77% were cefoxitin-resistant (FOX^r), 3% were COT^r.
- COT^r *E. coli* were present in 62% of the samples (50% fecal, 70% hide, 79% pen soil) and 377 COT^r *E. coli* isolates were examined for the presence of ExPEC virulence markers, *sul* genes, and *dfrA* genes. No COT^r *E. coli* isolates were ExPEC. However, *dfrA* genes were detected in 86% of isolates and *sul* genes were detected in 100% of isolates. Susceptibilities to 15 antimicrobial agents were determined for 119 COT^r *E. coli* isolates with 100% were COT^r, 85% were TET^r, 2% were $3GC^r$.
- Beef cattle are likely not a significant source of ExPEC since only 2 of the 1,114 (0.2%) isolates screened appeared to be ExPEC.

Salmonella results include:

- Generic *Salmonella* were present in 12% of the samples (2% fecal, 21% hide, 17% pen soil). Generic *Salmonella* were detected at 5 of the 7 feedlots examined.
- $3GC^r$ *Salmonella* were present in 3% of the samples (0% fecal, 5% hide, 5% pen soil) were detected at 3 of the 7 feedlots examined.
- NAL^r *Salmonella* were present in only 3 of the 1,328 samples (0.2%) including 2 hide samples from feedlot D and 1 hide sample from feedlot G.
- Serotypes and susceptibilities to 15 antimicrobial agents have been determined for 112 generic *Salmonella* isolates, 38 $3GC^r$ *Salmonella* isolates, and 3 NAL^r *Salmonella* isolates. Serotypes and antimicrobial susceptibilities clustered by feedlot. For example:
    - At feedlot A the majority of isolates were Newport. All Newport isolates from feedlot A and had the same "AUG-AMP-FOX-3GC-CHL-STR-FIS-TET" resistance pattern (AMP = ampicillin, CHL = chloramphenicol, STR = streptomycin, FIS = sulfisoxazole). A minority of feedlot A isolates were pan-susceptible Anatum.
    - The majority of feedlot D isolates were CHL-STR-FIS-TET resistant Meleagridis, but pan-susceptible Anatum and pan-susceptible Schwarzengrund were also present.
    - The majority of feedlot G isolates were TET resistant Montevideo.
- All AMP-FOX-3GC-CHL-STR-FIS-TET resistant *Salmonella* were screened for the presence of bla_{CMY} and bla_{CTX-M}. All had bla_{CMY} and lacked bla_{CTX-M}.

Enterococcus results include:

- Generic *Enterococcus* spp. were detected in 99.6% of samples (99% fecal, 100% hide, 100% pen soil). Susceptibilities to 16 antimicrobial agents have been determined for 117 generic *Enterococcus* spp. isolates.
- ERY^r *Enterococcus* spp. were detected in 92% of samples (83% fecal, 99.5% hide, 100% pen soil). 390 ERY^r *Enterococcus* spp. isolates will be screened for the presence of *ermA, ermB, ermC*, and *mef* genes. Susceptibilities to 16 antimicrobial agents will be determined for 117 generic *Enterococcus* spp. isolates.

Conclusions:

Study 1 found a very low prevalence of food-borne pathogens on final carcasses and their absence on strip loins suggests that current sanitary dressing procedures and processing interventions are effective against AMR bacteria.

Enumerating on selective media and plating enrichments on selective media provided detailed data on specific critically important AMR bacteria (eg. 3rd-generation cephalosporin-resistant *E. coli*). It was demonstrated that *E. coli* resistant to antimicrobials critically important to human medicine were present on 100% of cattle hides when processing begins and occasionally these resistant *E. coli* were present on hides at levels that resulted in carcass contamination. It was also demonstrated that *Salmonella* resistant to antimicrobials critically important to human medicine were on 8% of hides when processing begins. Importantly, it was determined that currently employed sanitizing interventions at beef processing plants were effective against antimicrobial-resistant bacteria since *E. coli* resistant to antimicrobials critically important to human medicine were present on 1% of final carcasses and no final products and no *Salmonella* resistant to antimicrobials critically important to human medicine were found on any carcass or final product.

It was also demonstrated that pen surface soil samples were more effective than hide sponge samples or fecal swab samples for the detection of *E. coli*, *Salmonella*, and *Enterococcus* resistant to antimicrobials critically important to human medicine in cattle feedlots. This is important since there will be increased pressure to expand surveillance of antimicrobial resistance to cattle production environments. Samples of pen surfaces are quicker and easier to obtain than fecal and hide samples. Samples of feces and hides must be obtained when cattle are processed though a squeeze chute, a process that can stress cattle and imposes a cost on feedlot operators. Pen surface samples do not require movement of animals so they can be obtained any time and more pens can be sampled during a visit.

The results of both studies strongly suggest that beef cattle are not an important source of ExPEC.

It was recognized that there was a need for studies with greater sampling depth and breadth. A current project at MARC has revealed that levels of resistant-bacteria, especially 3rd-generation cephalosporin-resistant *E. coli* in cattle feces, vary widely by season. These results suggest that additional research may reveal more complexity in the identification of specific antimicrobial resistance genes and antimicrobial-resistant bacteria that have prevalences or concentrations that vary by season and region. A related problem is the lack of "on-farm" antimicrobial use data. It is widely assumed that antimicrobial use is the largest driver of antimicrobial resistance occurrence. However, it is very difficult to determine if factors other than antimicrobial use contribute to occurrence of resistance if use data is not obtained.

There is a concern by cattle feedlot managers that any sampling, but especially collection of antimicrobial use data is a no-win situation as the data will become public and could be used against their operations.

Publications:

Manuscript:

- J. W. Schmidt, G. E. Agga, J. M. Bosilevac, D. M. Brichta-Harhay, S. D. Shackelford, R. Wang, T. L. Wheeler, and T. M. Arthur. 2015. Occurrence of Antimicrobial-Resistant *Escherichia coli* and *Salmonella enterica* in the Beef Cattle Production and Processing Continuum. *Applied and Environmental Microbiology*. 81:713-725. Manuscript available online at: http://aem.asm.org/content/81/2/713

Texas A&M/Kansas State University

This study was conducted by H. Morgan Scott, J. Vinasco-Torres, Neena Kanwar, Naomi Ohta, and Guy Lonergan.

The goal of this study was to test a variety of methods to detect and characterize rare resistance phenotypes (and, genotypes) among *E. coli* and other *Enterobacteriaceae* of feedlot and dairy cattle; specifically, those harboring carbapenemases and ESBLs, and to explore multiple and varied impacts of antibiotic use on levels of resistance and prevalence of enteric pathogens such as *Salmonella enterica*. The overarching objective was to identify potential weaknesses of the current NARMS design as it considers an extension to on-farm sampling and testing, along with capturing and analyzing antimicrobial usage data.

In a trial, cattle were administered ceftiofur (EXE) once and/or chlortetracycline (CTC) over three-5-day periods. Fecal samples were collected and the AMR profile ascertained. *Salmonella* prevalence had decreased significantly on Day 4 in pens, where all steers were treated with ceftiofur (EXE). Addition of CTC to the feed further reduced the prevalence of *Salmonella.* The number of *Salmonella* isolates was very low on Day 4 and 14 due to the treatments. However, *Salmonella* levels returned to near-normal levels without additional CTC treatment. CTC treatment decreased the prevalence of *Salmonella* significantly on Day 14. Salmonella prevalence remained at the same level in the pens without EXE and CTC treatments throughout the trial. On Day 26, Salmonella in the pens with EXE treatment returned to approximately 40% of the cattle tested.

Resistant phenotypes of *Salmonella* were tested against 15 antibiotics. The population dynamics of MDR-*Salmonella* was observed from Day 0 to Day 26. Most of the isolates were pansusceptible on Day 0. EXE treatment of all steers in a pen expanded the MDR- *Salmonella* levels on Day 4. CTC treatment increased the percentage of MDR *Salmonella* by day 14; however, there were a few isolates remaining especially when previously treated with ceftiofur. MDR phenotypes increased dramatically on Day 26 in all the antibiotic-treated groups. These results suggest that *Salmonella* populations in Canyon Texas feedlot are likely to be mostly pan-susceptible in the absence of antibiotic treatment.

Conclusions

The amount of fecal samples used for each sample was small due to sample limitation and one might need to use more for better detection. Results suggest that carbapenemases remain or, extremely rare in U.S. cattle populations, which is good news for industry and public health. However, the lack of a

consistently valid approach to their detection frustrates attempts to quantify the confidence in their absence.

Both EXE and CTC are effective in reducing the overall number of *Salmonella* positive steers during the period of antibiotic activity but the reduced bacterial levels were not sustained after antibiotic treatment was withdrawn. This further supports treatment to reduce foodborne pathogens with antibiotics not an effective control strategy.

Publications

Manuscript:

- N Ohta, KN Norman, B Norby, SD Lawhon, J Vinasco, H denBakker, GH Loneragan, HM Scott. Population dynamics of enteric Salmonella in response to antimicrobial use in beef feedlot cattle. Sci Rep. 2017 Oct 30; 7(1): 14310.

Submitted Abstracts

- N. Ohta, H.M. Scott, S. Lawhon, K. Norman, J. Vinasco, Bo Norby, G.H. Loneragan. Population dynamics of multi-drug resistant *Salmonella* in feedlot cattle treated with ceftiofur or chlortetracycline. 2014 Conference of Research Workers in Animal Disease. Poster presentation December 2014.
- N. Ohta, K.N. Norman, S. Lawhon, J. Vinasco-Torres, B. Norby, G.H. Loneragan, H.M. Scott. Population dynamics of multidrug-resistant *Salmonella* in feedlot cattle treated with ceftiofur or chlortetracycline. 4th ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens. Oral presentation. May 2015
- N. Ohta, K.N. Norman, S. Lawhon, J. Vinasco-Torres, B. Norby, G.H. Loneragan, H.M. Scott. The effect of ceftiofur or chlortetracycline treatments in transition of multidrug-resistant *Salmonella* population in feedlot cattle. 6th Symposium on Antimicrobial Resistance in Animals and the Environment. June 2015

Poultry

This research was conducted by Drs. Randall Singer at the University of Minnesota and Charles Hofacre, University of Georgia. This project began in 2011 and includes three studies that were supported by NARMS through 2015. The project currently remains active under the FDA Cooperative Agreement Program for Antimicrobial Use and Resistance Data Collection.

Study 1:

The *goal* of the initial study was conducted October 2011 to February 2012 to determine the feasibility of collecting samples from poultry farms to isolate *Salmonella* and *Campylobacter.*
Results: Approximately 400 boot sock samples were collected from broiler houses and 100 samples from turkey houses. The overall prevalence of *Salmonella* was 22% for broilers and 40% for turkeys. Serotype Kentucky was most commonly associated with broilers while Reading was the prominent serotype from turkeys. Approximately 53% of the *Salmonella* isolates were pan-susceptible, microbial resistance included; 2% were resistant to 1, 19% were resistant to 2, 6% were resistant to 3, 10% were resistant to 4 and 10% were resistant to 5 or more antimicrobials.

Of the 287 unique *Campylobacter* isolates, 267 were obtained from broiler chickens and 20 were obtained from turkeys. Among the chicken isolates 171 were *C. jejuni*, and 96 were *C. coli.* Among the

turkey isolates, 17 were *C. jejuni* and 2 were *C. coli*. Approximately 50% of the *Camplylobacter* isolates were pan-susceptible, 29% resistant to 1 antimicrobial, 12% were resistant to 2 antimicrobials and 8% resistant to 3.

Study 2:

This research study used isolates collected previously from 2 broiler production companies. Profiles were determined by pulse-field electrophoresis (PFGE) to examine relatedness of *Salmonella* isolates. Out of 217 flocks enrolled in the study 156 flocks were sampled at both farm and the plant. A total of 2,276 farm samples and 4,680 plant samples were collected for *Salmonella* testing. Ninety-nine (63.5%) flocks had positive *Salmonella* samples taken at the farm, while 104 (66.7%) flocks had positive *Salmonella* samples taken from the plant. Eighty eight flocks (56.4%) had positive samples taken from both the farm and plant. *Salmonella* isolates from the farm (n=243) and the plant (n=1,048) were serotyped, and of these, 223 isolates from the farm and 450 isolates from the plant from 110 flocks were tested for antibiotic sensitivity. All serotypes found on the farm were also found at the plant, while 11 serotypes found at the plant were not found on the farm. Serotypes Kentucky and Enteritidis accounted for the majority of farm isolates. Overall, 100% of *Salmonella* isolates were susceptible to ciprofloxacin and trimethoprim-sulfamethoxazole. Isolates on the farm and the plant were most frequently resistant to streptomycin, sulfisoxazole, tetracycline, and gentamicin. These results show that the relationship between serotypes and resistance on the farm and at the plant is not necessarily straightforward, but that the serotypes and antimicrobial resistance patterns found in the plant can be identified with on-farm samples.

Study 3:

The goal of the Poultry On-Farm NARMS study was to have a national representation of the United States poultry industry by enrolling companies that collectively account for between 60 and 80% of annual broiler chicken and turkey production. Complexes within these companies were e randomly selected, but because another goal of this project was to collect antibiotic usage information that could be matched with the samples, researchers strove to sample the same complexes year after year. To accomplish these goals, Dr. Hofacre at the University of Georgia and Dr. Singer at the University of Minnesota worked together to coordinate enrollment, sampling and laboratory analyses.

The sampling plan was based on the number of slaughter plants for each broiler and turkey company. For broilers, the sampling methodology and culture were conducted similarly to the pilot project. Four boot sock samples were collected from one house on each farm, with 8 farms sampled per week (32 weekly boot sock samples). Each sample was cultured for both *Salmonella* and *Campylobacter*. Samples were collected the week of slaughter. For turkey sampling, the turkey industry slaughters many fewer birds than broilers. Because turkeys live longer and do not have as many birds per house, a different sampling plan was developed for turkeys but was done similarly as for broilers.

By the end of 2015, more than 60% of the annual U.S. broiler and turkey production had been enrolled in the study. Approximately 184 broiler farms and 31 turkey farms were sampled. The prevalence of *Salmonella* and *Campylobacter* was found to be higher in broiler samples than turkey samples. At the time this summary was compiled, for the broiler samples entered into the database, 50.2% and 20.9% of samples were positive for *Salmonella* and *Campylobacter*, respectively. For turkeys, 21.8% of samples were positive for *Salmonella*, but only one sample was positive for *Campylobacter*.

By the end of 2015, resistance data had been completed for 339 *Salmonella* isolates from broilers and 36 isolates from turkeys. Additional work has since been completed under the auspices of the FDA Cooperative Agreement Program for Antimicrobial Use and Resistance Data Collection

Publications:

Submitted Abstracts

- Hofacre, C. VFD, FDA and NARMS (plus kitchen sink). Association of Veterinarians in Broiler Production, Denver, Colorado. July 25, 2014.
- Antibiotic Use in Poultry Production. Antibiotic Webcast, Watt Publishing. November 4, 2014.
- Hofacre, C.L. and R.S. Singer. Antibiotic Use in the Livestock and Poultry Industry: Principles of Judicious Use. Antibiotic Use Symposium International Production and Processing Expo, Atlanta, Georgia. January 28, 2015.
- Hofacre, C.L., R.S. Singer, R. Berghaus, and P. McDermott. On Farm Poultry National Antimicrobial Resistance Monitoring (NARMS) A Progress Report. Western Poultry Disease WPDC, Sacramento, California. March 22, 2015.
- Singer, R.S., C. Nichols, R.D. Berghaus, and C.L. Hofacre. Tracking *Salmonella* Serotypes and Antibiotic Resistance from the Broiler Farm to the Processing Plant. Submitted to ARAE 2015.

Overall Project Conclusions

The multiple projects described here demonstrates the complexity of on-farm surveillance for AMR. Currently, much of our knowledge is based on the profiles of bacteria obtained at slaughter. However, in the majority of the studies, the AMR profile and bacteria isolated from cohorts of animals tested on-farm and at slaughter were not 100% in agreement. This would not be unexpected as the slaughter plant would have animals from multiple sources and thus have different bacteria and AMR profiles. However, the data collected by these researchers provide an important source of information as we move forward to develop a better understanding of AMR bacteria in production animals, linkage with drug usage and perhaps better identify the relationship between AMR in food producing animals and humans. This information will be important in future policy decisions as the animal industries respond to this problem of global concern.