Multistate Research Activity Accomplishments Report

Project/Activity Number: NC-1206
Project/Activity Title: Antimicrobial Resistance
Period Covered: May 14, 2018 – Feb 11, 2019
Date of This Report: April 19, 2019
Annual Meeting Date(s): Feb 11-12, 2019

Participant Attending the Meeting:
Timothy Johnson - Purdue University (chair)
Bing Wang - University of Nebraska (secretary)
Renata Ivanek - Cornell University (out-going chair)
Noelle Noyes - University of Minnesota
Abdelhamed, Hossam - Mississippi State University
Alam, Md Tauqeer - University of Illinois
Xu Li - University of Nebraska
Emmanuel Okello - University of California -- Davis
Bo Norby - Michigan State University
Joy Scaria - South Dakota State University
Kollanoor Johny Anup - University of Minnesota
Michelle Soupir - Iowa State University
Paolo Moroni - Cornell University
Qijing Zhang - Iowa State University
Silva del Rio Noelia - University of California -- Davis
Terry Lehenbauer - University of California -- Davis
Yrjo Grohn - Cornell University
Mervalin Morant - USDA-NIFA
Dawn M. Sievert - Centers for Disease Control and Prevention
Karin Hoelzer - Pew Charitable Trusts
George Smith - Michigan State University (project administrator)

Absent from the 2019 Meeting:

Alan Mathew - Purdue University
Carlos Vilalta - University of Minnesota
Catherine Logue - Iowa State University
Christina Hamilton - University of Wisconsin
Fabio Lima - University of Illinois
Francis Welcome - Cornell University
Gireesh Rajashekara - Ohio State University
Jennifer Granick - University of Minnesota
Jeongmin Song - Cornell University
Johann Coetzee - Kansas State University
Ludek Zurek - Kansas State University
Paul Plummer - Iowa State University
Peter Davies - University of Minnesota
Peter Johnson - USDA-NIFA
Randall Singer - University of Minnesota
Richard Pereira V. - University of California -- Davis
Roderick Mackie - University of Illinois
Sandra Godden - University of Minnesota
Sophia Kathariou - North Carolina State University
Sung Woo Kim - North Carolina State University
Timothy Goldsmith - University of Minnesota
Timothy Johnson - University of Minnesota
Torey Looft - USDA-ARS
Yung-Fu Chang - Cornell University
Raghavendra Amachawadi - Kansas State University
Sun, Gongqin - University of Rhode Island
Sharif S. Aly - University of California – Davis

Minutes of Annual Meeting

On February 11 (Day 1), the meeting was called to start by Renata Ivanek, followed by a brief self-introduction around the room. Multistate research project structure and business items were discussed. Participants then gave Individual research presentations, first focusing on Objective 1-3 followed by those on Objective 4-5. All presentations were given in a "lightning" style, i.e., up to 5 minutes per person. After all presentations, the group discussed the common trends between groups and future research directions. Before wrapping up Day 1 meeting, the group discussed future meeting dates and location to be finalized by a poll after the meeting. Tentatively, the NC-1206 annual meeting in 2020 could be held in Atlanta, GA in May, 2020. Bing Wang was selected as the secretary. On February 12 (Day 2), the meeting was called to start by Tim Johnson, followed by an introduction of three guest speakers. The three guest speakers are Dawn Sievert, the Associate Director for Antimicrobial Resistance Division of Foodborne, Waterborne and Environmental Diseases at CDC, Karin Hoelzar, the Senior Officer of Health Programs (antibiotic resistance) at the Pew Charitable Trusts and Mervalin Morant, the National Program Leader of Food Safety at USDA NIFA. The guest speakers gave presentations on the major activities and visions on antimicrobial resistance in their divisions, followed by a round table discussion among the speakers and meeting participants. After a brief break, participants discussed the potential funding opportunities and research needs around Objective 1-5 of the MSP NC-1206. Before wrapping up Day 2 meeting, some suggestions were provided by participants regarding improving multistate collaboration, such as sharing our expertise and capacity and needs of collaboration, which could be collected and shared among the group after the meeting by Tim Johnson and Bing Wang. George Smith gave an administrative update and offered some suggestions on preparing the meeting reports.

Accomplishments:

Objective 1. Enhance surveillance and monitoring of antibiotic resistance and develop improved diagnostic tests.

New York
- Using classical statistical methods we have assessed the variation of multidrug resistance in the publicly available NARMS data.
- As an alternative to classical methods we have developed graphical network method and current work focuses on machine learning.

North Carolina
- _Surveillance of erm(B) in erythromycin-resistant Campylobacter coli and Campylobacter jejuni._ erm(B) does not appear to have yet infiltrated _Campylobacter_ populations in North Carolina, but other have observed erm(B) in _C. coli_ isolates in China.
- Surveillance of prevalence and AMR profiles of _Listeria, Salmonella_ and _Campylobacter_ from floodwaters associated with hurricane Florence (collaboration with Angela Harris, Natalie Nelson, William Miller, Ryan Emmanuel). We pursued the isolation of _Listeria_, _Salmonella_ and _Campylobacter_ from Hurricane Florence (September 2018) floodwaters in eastern North Carolina.

Maryland

- Evaluating different manure management practices in controlling spread of antimicrobial resistance from 11 NE US dairy farms (Collaboration between Stephanie Lansing and Curt Gooch at Cornell University) across NY, PA, and MD at all points of manure system (digestion, composting, solid-liquid separation, lagoon storage) over 18 months with 8 sampling events. ARG prevalence varied based on location and time, but ARG prevalence did not correlate to antimicrobial usage or tetracycline concentrations across farms and collection dates.Antibiotics residuals in dairy manure digesters were often at or close to zero, but there were spikes with cow treatments, up to 45,000 ng/g dry weight.
- Evaluating transformations of antibiotics and antimicrobial resistance genes during anaerobic digestion of dairy manure. Results showed >95% reduction of Sulfa-based (SDM) drugs during digestion, but inconsistent (0-80%) removal of tetracycline (TC), due to adsorptive nature of TC. The reductions in SDM residuals did not correspond with reductions of Sul1 genes, with greater reductions for TetM genes.There were so significant decreases in methane production with additions of the highest antibiotics residuals seen on-farm (1 mg/L), with the increase to 10 mg/L of tetracycline and sulfamethoxine resulting in less than 10% reduction in biogas production.
- Evaluating transformations of antibiotics residuals during field composting of dairy manure. Variable results were seen in composting studies, as the piles were not managed for thermophilic temperatures, with turning too frequent and interruption of the thermophilic bacteria. In addition, the piles were only maintained for 30 days before field application, as opposed to compost piles of 3 months used in the composting industry.
- Effect of high temperature digestion and composting temperature on AMR in dairy and beef systems. We are assessing effect of high temperature treatment in dairy manure and modeling antibiotic residual transformations in dairy and beef manure with high temperature and grass strip treatments, respectively on antibiotic residuals, antimicrobial genes (ARG), and bacteria (ARB). Researching the most promising thermal-based AMR manure treatment technologies in dairy operations, including anaerobic digestion using thermohydrolysis pre-treatment used in wastewater industry, thermophilic digestion (55 ℃), and high-temp, rotary drum composting.

Indiana
- In collaboration with Purdue engineers, veterinarians and animal diagnostic lab we are working on developing a rapid diagnostic test to diagnose and recommend treatment for bovine respiratory disease. Developed assays for the four most common bovine respiratory disease (BRD) pathogens. Worked to improve the agreement between BRD pathogen resistance genotypes and phenotypes by identifying additional resistance marker genes

Nebraska
- Detection of AMR ICE associated genes in BRD lung samples has high positive predictive value of isolation of AMR *M. haemolytica.* A data collection and analysis pipeline were established for optimizing the multiplex real time PCR assay for detection of antimicrobial resistant genes in cattle samples. A user-friendly tool was developed to transform the data analysis pipeline

Objective 2. Determine the ecology and mechanisms involved in resistance and transmission of resistance.

Iowa
- *Conducting column experiments to identify manure-derived genes that are not present in soils and that can be used to track resistance through environmental pathways*

North Carolina
- *Ecology of multidrug-resistant (MDR) strains of Campylobacter jejuni and C. coli from turkeys* (collaboration with William Miller).
    o MDR strains of *Campylobacter jejuni* and *C. coli* extensively colonized turkeys grown in proximity to other turkey farms.
    o Use of a paired-farm model showed that such strains could not be detected in turkeys grown at a distance from other farms.
    o *Campylobacter* survival in turkey feces and water from the turkey farms depended on the strain's genotype.
    o *Campylobacter* strains with certain genotypes and MDR profiles varied in their capacity to persist in poultry feces and water.
    o MDR *C. coli* resistant to erythromycin and multiple other antimicrobials exhibited impaired fitness in naturally-contaminated turkey feces or farm water.
- *Impact of therapeutic-level treatment on Campylobacter from cattle* (collaboration with Derek Foster).
    o *Campylobacter jejuni* was isolated from cattle treated via (i) two therapeutic types of regimens of ceftiufor and (ii) the fluoroquinolone danofloxacin.
    o Isolates before and after treatment were characterized for their species, genotypes and AMR profiles.   Strains with different MICs to tetracycline and ampicillin were noted.
    o The genetic basis for the MIC differences is being investigated

Kansas
- Prevalence of plasmid-mediated quinolone resistance (PMQR), extended-spectrum beta-lactamases (ESBL) and AmpC beta-lactamases in the feces of American crow.

Indiana
- Environmental fate of antibiotic resistance genes in the bovine and swine agroecosystems (*collaboration with Bo Norby). Sampled dairy and swine manure, stored manure, as well as corn field prior to, and immediately, 3 weeks and 6 weeks after manure application at the Purdue University farm. Established partnerships to sample farms in Finland, Michigan, and New York
- Began use of epicPCR, a molecular technique to link a taxonomic marker (16S) and a resistance gene to allow culture independent identification of bacteria that encode resistance genes.
- Effect of carbadox and Zn Cu additives on antibiotic resistance gene profiles. Used a highly parallel qPCR array (WaferGen) to determine the abundance of hundreds of antibiotic resistance genes (ARGs) in feces from pigs fed Zn and Cu, Carbadox or no additive. Each of these treatments resulted in unique ARG profiles and some enriched ARGs
- Plasmid-mediated transfer of antibiotic resistance genes to *Enterococcus faecalis* JH2-2 in poultry litter (*collaboration with Torey Looft). *Enterococcus faecalis* JH2-2 usually

acquires two resistance gene at a time. The same genetic element with the same resistance genes were found with different flanking elements in different plasmids.

Nebraska
- Fate of antimicrobial resistance in the environment: from beef cattle production to manure storage and land application (collaboration between Xu Li and Bing Wang)
    o In-feed antimicrobial use on cattle has limited impact on the persistence of antimicrobial resistance bacteria in the environmental compartments of beef cattle producing feedlots.
    o Resistome composition of samples were similar between treatments.
    o Sufficient manure storage time effectively limits risks of contamination in the environment before land application, so that the potential contamination in the environment was eliminated.

Objective 3. Develop and evaluate interventions (including alternatives to antibiotics) that reduce antimicrobial resistance in food production systems.

Iowa
- *Completed manure mitigation experiments, tested centrifugation, 2-phase storage, anaerobic digestion*
- *Completed qPCR on 16S, tet, and erm genes*

Michigan
- In collaboration with MSU Animals Science faculty, Molecular Genetics faculty, and Large Clinical Sciences faculty and clinicians we were awarded a USDA NIFA Foundational grant to animal veterinarians and clinicians to determine the impact of Ceftiofur use on antibiotic resistant bacterial populations in dairy cattle. Logistics of conducting the studies have been completed.

Kansas

- Worked in both cattle and swine production systems – alternative to antibiotics – heavy metals (copper, zinc), probiotics, essential oils, and also tested different route of administration of antimicrobials.

Indiana
- Determination of resistance gene profiles when alternatives to antibiotics are included in animal diets. Swine experiments were completed with treatment groups that received the normal diet amended with Zn and Cu, beta glucan, wheat bran, glutamine, dextrin, and other complex oligosaccharides. High throughput qPCR arrays (WaferGen) were used to determine the resistance gene profile and 16S rRNA gene sequencing was also completed. Analysis is still underway.

New York
- A tomato protein(s) displays an outstanding bactericidal activity to antibiotic-resistant *Salmonella* spp and other bacteria in the same group.
- This protein is functional in the acidic environment.
- These unique features render this protein extremely useful to develop as an effective antimicrobial intervention in a combination of lactic acid.

Nebraska
- Develop innovative manure management practices to reduce antibiotic resistance genes and antibiotic resistant bacteria in beef cattle manure. Composting was tested for its efficiency in reducing antibiotic resistance genes in beef cattle manure during manure storage.

Objective 4. Quantify animal health, public health, social, economic, and environmental impacts of antimicrobial interventions in food production systems.

New York
- Project 1: Perceptions about antibiotic use and resistance in dairy farming (*collaboration with Paolo Moroni and Francis Welcome)
    - The main goal is to use a mixed-methods (qualitative and quantitate) approach to elucidate the attitudes, beliefs and behaviors of dairy farmers and veterinarians in New York State (NYS) regarding antibiotic use and antimicrobial resistance (AMR) and the knowledge, willingness and abilities amongst them to amend antibiotic use, particularly antibiotics of medical importance. A secondary goal is to evaluate the perceptions of consumers in US and veterinarians internationally regarding antibiotic use and AMR.
    - Studies in progress: (i) Interviews with dairy farmers in NYS (15 conventional, 5 organic); (ii) Interviews with veterinarians in NYS (n= 9); (iii) Pilot International survey of dairy veterinarians (n=57, 16 countries); (iv) Survey of US Public through the Cornell National Social Survey (CNSS) 2017 about perceptions regarding antibiotic use in dairy farming (n=1,000); (v) Survey of US Public through the CNSS 2018 about perceptions regarding the interface between organic dairy farming and the antibiotic use laws (n=1,000).
    - Preliminary findings indicate that: (i) conventional farmers need an economic incentive to reduce antibiotic use on their farms; (ii) there are generational differences and peer influences among veterinarians that appear to affect their antibiotic use practices; (iii) there is a need for a measurable goal for judicious use of antibiotics; (iv) the policy level interventions and fines regarding antibiotic (mis)use are not popular but are effective; (v) consumers are concerned about AMR but not informed about antibiotic use in dairy.
- Project 2 (new): A mathematical modeling study to identify new and improved approaches to control antimicrobial resistance (AMR) on heifer raising operations. (*collaboration with Paolo Moroni, Francis Welcome, Bo Norby, Bing Wang)
    - Goals are to: (i) Evaluate the dynamics of AMR in generic *Escherichia coli* on a heifer raising operation; (ii) Evaluate the dynamics of AMR and spread of *Salmonella* Dublin on a heifer raising operation; and (iii) Develop and disseminate education materials about control of AMR and associated infections in dairy farming.
    - Preliminary results suggest that increased nutrients from deposited manure may support the proliferation of antibiotic resistance irrespective of antibiotic use.

Iowa
- Began a new study of the impact of prairie strips as an edge-of-field mitigation strategy for reducing AMR export through surface runoff, included rainfall simulation experiments and flume studies
- Completed long term monitoring of soil and drainage water impacted by swine manure application.
- Began study of poultry manure derived resistance genes in soils with a long term history of poultry manure application (20 years)

Maryland

- In collaboration with Cornell University and University of Nebraska, as well as partners at USDA-ARS and University of Maryland-Baltimore County, we are assessing perceptions of farmers and veterinarians in order to develop communication materials that are more tailored to the biases, current level of understanding, and perception. Investigating stakeholder perceptions (and misconceptions) of AMR prevalence and conveyance in the environment of farmers, veterinarians, and other stakeholders using interviews, focus groups, surveys, and Q-sort analyses.
- We are organizing a workshop with the leaders in AMR social science research and AMR communication (objective #5) to identify research to date, identify holes in the literature, write a review article, and create a data of social science-based AMR information in agriculture. Workshop participants from the application pool and invited participants have been selected. While planning is taking place now, the workshop and follow-up visits will take place in Mary 2019

Nebraska
- Established a farm-to-fork quantitative microbial risk assessment model with five modules, including feedlot, abattoir, retail and transport, cooking and cross-contamination after cooking. Exposure risks among consumers were compared among different types of beef products (ground beef, intact beef cuts *versus* non-intact beef cuts), and between different primary production systems (conventional *versus* raised without antibiotics).
- Ground beef may pose greater risk of exposure to beta-lactam resistant *E. coli* than beef cuts. Restriction of antibiotic use may have limited impact on reducing the human exposures to antimicrobial resistance through beef consumption pathway.

Objective 5. Create and deliver programs on antibiotic stewardship in food production systems through education and outreach.

Maryland
- In collaboration with Cornell University and University of Nebraska, as well as partners at USDA-ARS, University of Maryland-Baltimore County, and Ithaca College, we are creating video, web, and print information on antimicrobial resistance prevalence, treatment, stewardship, and perceptions. Ithaca College's Park Production will be creating video content, with additional web and print communication materials.
- Facilitate optimal distribution and utilization of AMR-related food safety information and resources at the state, regional and national levels via on-demand access to science-based information, educational resources, and decision-support tools and evaluate the impact of these efforts among the intended audience Ithaca College's Park Production will be creating video content, with additional web and print communication materials.
- We are organizing a workshop with the leaders in AMR social science research (objective #4) and AMR communication to identify research to date, identify holes in the literature, write a review article, and create a data of social science-based AMR information in agriculture. Workshop participants from the application pool and invited participants have been selected. While planning is taking place now, the workshop and follow-up visits will take place in Mary 2019

Impacts:

- The major advantages of a graphical network methods is recognize the pattern of multidrug resistance in BIG DATA ultimately without human decision (machine learning).
- Macrolides are a leading drug for treatment of human campylobacteriosis. Identification of novel genes conferring macrolide resistance has important public health implications, especially if these are found to lack the negative fitness impacts previously reported for macrolide resistance in *Campylobacter jejuni*.
- Extreme weather events such as hurricanes can have massive impacts in the movement and distribution of pathogens via floodwaters. Such issues are especially acute for eastern North Carolina, a region highly dense in swine and poultry farms.
- Ecological determinants underlying the prevalence of *Campylobacter*, including MDR strains, in conventional turkey farms remain poorly understood. Further knowledge can be valuable for science-based design o improved interventions.
- Post-excretion survival of *Campylobacter* is critical for dissemination within and between flocks, as well as contamination of the natural environment (e.g. surface water)
- Therapeutic treatment of cattle with antibiotics can result in shifts in prevalence, species and strain distribution, and AMR profiles. Further understanding of such outcomes can be critical in designing adequate therapeutic interventions while minimizing the risk for adverse outcomes for *Campylobacter* prevalence or resistance traits.
- Gathering information useful to developing approaches for promoting antibiotic use change among dairy farmers and veterinarians. General public has a strong leverage in influencing antibiotic use through their purchasing behaviors and needs to be better informed about the use of antibiotics in dairy farming.
- Improved understanding of the effect of manure management and environmental factors on the dynamics of antibiotic resistance in heifer raising operations may lead to improved management of AMR with positive economic and health impacts.
- Will provide veterinarians with guidelines for the judicious use of antibiotics as well as novel control methods that can be used to limit the flow and pool of resistance genes and resistant bacterial hosts in dairy cattle and the farm environment.
- Evaluated alternative to antibiotics and their impact on development/propagation of antimicrobial resistance in both cattle and swine production systems.
- Improved understanding and or the knowledge on antibiotic alternatives and their use in food animals.
- Working toward providing veterinarians a rapid test to aid in the antibiotic prescription decision-making process and will increase antibiotic stewardship and hopefully decrease resistance
- Improve understanding of the impact of soil manuring and the environmental fate of antibiotic resistance genes
- Zn and Cu are already available and used as antibiotic alternatives, but may co-select for all the resistance genes that prompted the Veterinary Feed Directive rules. We are investigating their impact on the animal microbiome and resistome.
- Quantifying and understanding the horizontal transfer of resistance plasmids will allow us to judge the impact of animal management practices. The exogenous gene capture is also a fairly novel method to study the ecology of antibiotic resistance genes.

- We are testing marketed alternatives to antibiotics for their selective pressure for antibiotic resistance genes. This will aid companies and producers to increase their antimicrobial stewardship.
- We have published a review article where we discussed potential impacts in public health of research outcomes from the ongoing studies. Offering insights into effective strategies to control and mitigate antibiotic-resistant Salmonella and other Enterobacteriaceae is an intended outcome. Furthermore, if allowed, we could further develop a treatment method for efficient control of contaminated foods using information learned from the current study.
- Provide science-based evidence informing decision makings on the prioritization of management strategies for controlling food animal originated antibiotic resistance
- improve understanding of transfer of antimicrobial resistant bacteria and genes along beef cattle manure management pathway; indicate the significance of appropriate manure management practices as effective antimicrobial resistance control measures; provide data for establishing a quantitative microbial risk assessment model to evaluate the significance of an environmental pathway in posing human risk of exposure to livestock related antimicrobial resistance
- provide diagnosticians a new AMR diagnostic method; enable a "self-service" model of method development process that would allow for a "real-time" advancement of the PCR method for AMR diagnosis
- This manure composting technology offers producers an alternative manure management practice to reduce the environmental loads of antibiotic resistance genes through manure application.
- Quantified on-farm antibiotic residuals and ARGs throughout different on-farm manure management systems, began to understand how different manure treatment processes (solid liquid separation, composting, digestion, lagoon storage) affect AMR. Was able to correlate (or see lack of correlation) between antibiotic administration and antibiotic residuals in the manure management systems.
- While many studies of the effective of composting to reduce antibiotic concentrations are lab-based or under ideal conditions, the piles maintained by the farmers of the sick cow bedding, did not show decreases in tetracycline. In fact, the relative concentration increased, as the quantity of solids and moisture decreased with compost time. The effect of pile management needs to be further considered with assessing the effect of composting on AMR.
- Many studies of the effective of digesters to reduce antibiotic concentrations use concentrations that are not related to field concentration (0 - 1mg/L). We found that at these low concentrations, the effect on biogas production was minimal, and the effect on antibiotic degradation was inconsistent for tetracycline, but high for sulfamethoxine, with inconsistent reduction is Sul1 genes and greater reductions in TetM genes during digestion.

Publications:

- J. Dustin Loy, Laura Leger, Aspen Workman, Michael L. Clawson, Ece Bulut, Bing Wang. Development of a multiplex real-time PCR assay using two thermocycling platforms for detection of major bacterial pathogens associated with bovine respiratory disease complex from clinical samples. Journal of Veterinary Diagnostic Investigation. 2018; 30(6): 837-847. https://doi.org/10.1177/1040638718800170

- Yi Li, Qiaoling Sun, Yingbo Shen, Yangjunna Zhang, Junwen Yang, Lingbin Shu, Hongwei Zhou, Yang Wang, Bing Wang, Rong Wang, Shaolin Wang, Zhangqi Shen. Rapid increase in the prevalence of carbapenem-resistant Enterobacteriaceae (CRE) and emergence of colistin resistance gene mcr-1 in CRE in a hospital in Henan, China. Journal of Clinical Microbiology. doi:10.1128/JCM.01932-17

- Yangjunna Zhang, Bing Wang. Comparison of the efficacy of commercial antimicrobial interventions for reducing antibiotic resistant and susceptible beef-associated *Salmonella* and *Escherichia coli* strains. Journal of Consumer Protection and Food Safety. 2018; 13(1):3-23. https://doi.org/10.1007/s00003-017-1141-x

- Joy, S. R.; Bartelt-Hunt, S. L.; Snow, D. D.; Gilley, J. E.; Woodbury, B. L.; Parker, D. B.; Marx, D. B.; Li, X.,Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry. Environ. Sci. Technol. 2013, 47, (21), 12081-12088.

- 2. Joy, S. R.; Li, X.; Snow, D. D.; Gilley, J. E.; Woodbury, B.; Bartelt-Hunt, S. L.,Fate of antimicrobials and antimicrobial resistance genes in simulated swine manure storage. Sci. Total Environ. 2014, 481C, 69-74.

- 3. Soni, B.; Bartelt-Hunt, S. L.; Snow, D. D.; Gilley, J. E.; Woodbury, B. L.; Marx, D. B.; Li, X.,Narrow grass hedges reduce tylosin and associated antimicrobial resistance genes in agricultural runoff. Journal of Environmental Quality 2015, 44, 895-902.

- Rieke, E.L., M.L. Soupir, T.B. Moorman, F. Yang, A. Howe. 2018. Temporal dynamics of bacterial communities in soil and leachate water after swine manure application. *Frontiers in Microbiology.* 9:3197

- Rieke, E.L., T.B. Moorman, M.L. Soupir, F. Yang, A. Howe. 2018. Novel approach to assessing pathogen presence in an intensively tile drained agricultural watershed. *Journal of Environmental Quality.*

- Choi, J., E.L. Rieke, T.B. Moorman, M.L. Soupir, H. Allen, S.D. Smith, A.C. Howe. 2018. Practical implications of erythromycin resistance gene diversity on surveillance and monitoring of resistance. *FEMS Microbiology Ecology.*

- Zawack KA, et al.: Inferring the interaction structure of resistance to antimicrobials. Prev Vet Med. 2018 Volume 152, Issue 3, Pages 81-88.

- Current work: Zawack KA, et al.: Estimation of Variability in Multi-Drug Antibiotic Resistance. Under review.

- Søgaard Jørgensen P, et al. (Living with resistance project): Promoting antibiotic and pesticide susceptibility to preserve an Anthropocene operating space. Nature sustainability 2018 https://doi.org/10.1038/s41893-018-0164-3

- Lhermie G, et al.: Hot topic: do antimicrobials matter? An assessment of the costs of antimicrobial use restrictions on the U.S. dairy market. Prev. Vet. Med. 2018. Volume 160, Pages 63-67. https://doi.org/10.1016/j.prevetmed.2018.09.028

- Lhermie G, et al.: The farm cost of decreasing antimicrobials use in dairy production. *PLoS ONE*, 2018, 13(3): e0194832. DOI: 10.1371/journal.pone.0194832
- Lhermie G, et al.: Weighing the trade-offs between resistance to antimicrobials in public health and their use in agriculture: a review of the options for sustainability assessment. Under review
- Niedermeyer JA, Ring L, Miller WG, Genger S, Parr Lindsey C, Osborne J, Kathariou S. 2018. Proximity to other commercial turkey farms affects colonization onset, genotypes and antimicrobial resistance profiles of *Campylobacter* in turkeys: suggestive evidence from a paired-farm model. Appl Environ Microbiol AEM.01212-18.
- Bolinger HK, Zhang Q, Miller WG, Kathariou S. Lack of Evidence for *erm*(B) Infiltration Into Erythromycin-Resistant *Campylobacter coli* and *Campylobacter jejuni* from Commercial Turkey Production in Eastern North Carolina: A Major Turkey-Growing Region in the United States. Foodborne Pathog Dis. 2018 Nov;15(11):698-700. doi: 10.1089/fpd.2018.2477.
- Parsons C, Lee S, Kathariou S. Heavy Metal Resistance Determinants of the Foodborne Pathogen *Listeria monocytogenes*. Genes (Basel). 2018 Dec 24;10(1). pii: E11. doi: 10.3390/genes10010011.
- Arikan, O[*]., Mulbry, W., Rice, C., Lansing, S., 2018. The fate and effect of monensin during anaerobic digestion of dairy manure under mesophilic conditions. PLoS One 13(2): e0192080. (Citations: 3).
- Arikan, O[*]., Mulbry, W., Rice, C., Lansing, S., 2018. Anaerobic digestion reduces veterinary ionophore lasalocid in dairy manure. Desalination and Water Treatment. Desalination and Water Treatment 108: 183-188.
- Oliver, J.P., Schueler, J.E., Gooch, C.A., Lansing, S., Aga, D[*]., 2018. Performance quantification of manure management systems at 11 Northeastern US dairy farms. Applied Engineering in Agriculture 34(6): 973-1000.
- Hurst, J.J., Oliver, J., Schueler, J., Gooch, C.A., Lansing, S., Crossette, E., Wiggington, K.R., Raskin, L., Aga, D.S[*]., Sassoubre, L.M., 2019. Trends in antimicrobial resistance genes in manure blend pits and long-term storage across dairy farms with comparisons to antimicrobial usage and residual concentrations. Environmental Science & Technology (in-press).
- Wu, F., M. D. Tokach, J. M. DeRouchey, S. S. Dritz, J. C. Woodworth, R. D. Goodband, K. Chitakasempornkul, N. M. Bello, K. Capps, S. Remfry, H. M. Scott, T. G. Nagaraja, M. D. Apley, and R. G. Amachawadi*. 2018. Effects of tylosin administration routes on the prevalence of antimicrobial resistance among fecal enterococci of finishing swine. *Foodborne Pathogens and Disease* (*In Press*).
- Van Bibber-Krueger, C. L., C. I. Vahl, S. K. Narayanan, R. G. Amachawadi, E. A. Taylor, H. M. Scott, and J. S. Drouillard. 2018. Effects of supplemental zinc sulfate on growth performance, carcass characteristics, and antimicrobial resistance in feedlot heifers. Journal of Animal Science (*In Press*).
- Chalmers, G., K. M. Rozas, R. G. Amachawadi, H. M. Scott, K. N. Norman, T. G. Nagaraja, M. D. Tokach, and P. Boerlin. 2018. Distribution of the *pco* gene cluster and associated genetic determinants among swine *Escherichia coli* from a controlled feeding trial. *MDPI Genes*. 18:9(10). pii:E504.

- Williams, H. E., M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, T. G. Nagaraja, R. D. Goodband, J. R. Pluske, K. Chitakasempornkul, N. M. Bello, and R. G. Amachawadi*. 2018. Effects of chlortetracycline alone or in combination with direct fed microbials on nursery pig growth performance and antimicrobial resistance of fecal *Escherichia coli*. Journal of Animal Science (*In Press*).
- Williams, H. E., R. A. Cochrane, J. C. Woodworth, J. M. DeRouchey, S. S. Dritz, M. D. Tokach, C. K. Jones, S. C. Fernando, T. E. Burkey, Y. S. Li, R. D. Goodband, and R. G. Amachawadi*. 2018. Effects of dietary supplementation of formaldehyde and crystalline amino acids on gut microbial composition of nursery pigs. Nature Scientific Reports 8:8164. doi: 10.1038/s41598-018-26540-z.
- Muller, H. C., C. L. Van Bibber-Krueger, O. J. Ogunrinu, R. G. Amachawadi, H. M. Scott, and J. S. Drouillard. 2018. Effects of intermittent feeding of tylosin phosphate during the finishing period on feedlot performance, carcass characteristics, antimicrobial resistance, and incidence and severity of liver abscesses in steers. Journal of Animal Science. 96:2877-2885.
- Amachawadi, R. G., F. Giok, X. Shi, J. Soto, S. K. Narayanan, M. D. Tokach, M. D. Apley, and T. G. Nagaraja. 2018. Antimicrobial resistance of *Enterococcus faecium* strains isolated from commercial probiotic products used in cattle and swine. Journal of Animal Science. 96(3): 912-920.
- Feldpausch, J. A., R. G. Amachawadi, M. D. Tokach, H. M. Scott, S. S. Dritz, R. D. Goodband, J. C. Woodworth, and J. M. DeRouchey. 2018. Effects of dietary chlortetracycline, *Origanum* essential oil, and pharmacological Cu and Zn on growth performance of nursery pigs. Translational Animal Science. 2:62-73.
- Jamborova I, M. Dolejska, L. Zurek, AK Townsend, AB Clark, JC Ellis, I Papousek, A. Cizek, and I Literak (2017). Plasmid-mediated resistance to cephalosporins and quinolones in Escherichia coli from American crows in the USA. *Environmental Microbiology* 19: 2025 – 2036.
- Johnson TA, Sylte MJ, Looft T. In-feed bacitracin methylene disalicylate modulates the turkey microbiota and metabolome in a dose-dependent manner [submitted]
- Yang Y-A, Chong A, & Song J. (2018). Why is eradicating typhoid fever so challenging: Implications for vaccine and therapeutic design. Vaccines. 6(3): E45.

Presentations:
- Guillaume Lhermie, Donald Kenkel, Charles Nicholson, Loren William Tauer, Gröhn, Y.T.: A Dynamic Systems Assessment of Benefits and Costs of Policies to Regulate Antimicrobial Use in U.S. Animal Agriculture Annual Conference of the Benefit –costs analysis society, Washington D.C. USA. March 2018
- Cazer CL, Al-Mamun MA, Kaniyamattam K, Love WJ, Booth JG, Lanzas C, and Gröhn, Y.T.: Applying Machine Learning Techniques to Antimicrobial Susceptibility Data. Antimicrobial Resistance: Research Synergies in Human and Animal Medicine. New York, NY, March 2018. (Poster Presentation).
- Guillaume Lhermie, Loren William Tauer, Gröhn, Y.T.: From dairy farm to consumer: Analysis of the impacts of policies mitigating antimicrobial use in dairy production International Society for economics and social science of animal health, Montpellier, France. May 2018
- Cazer CL, Al-Mamun MA, Kaniyamattam K, Love WJ, Booth JG, Lanzas C, and Gröhn, Y.T.: Association rule mining identifies multidrug resistance patterns in antimicrobial susceptibility data. Bridging the Divide: Machine Learning in Medicine. Ithaca, NY, September 2018. (Poster Presentation). (Second place student poster competition).
- Guillaume Lhermie, Gröhn, Y.T.: Sustainable antimicrobial use in cattle medicine and public health impacts World Buiatrics Congress, Sapporo, Japan, October 2018
- Cazer, C. L., M. A. Al-Mamun, K. Kaniyamattam, W. J. Love, J. G. Booth, C. L. Lanzas, and Gröhn, Y.T.: Applying machine learning techniques to antimicrobial susceptibility data. Proceedings of 2018 International Society for Veterinary Epidemiology and Economics meeting, Chiang Mai, Thailand.
- Guillaume Lhermie, Loren William Tauer, Gröhn, Y.T.: Assessing the aggregate market costs of restrictions on antimicrobial use in U.S. dairy production. International Society for Veterinary Epidemiology and Economics Conference, Chiang Mai, Thailand, November 2018
- Casey Cazer, Guillaume Lhermie, Erin Eldermire, Sarah Murray, H. Morgan Scott, Gröhn, Y.T.: The effect of tylosin on antimicrobial resistance in beef cattle enteric bacteria International Society for Veterinary Epidemiology and Economics Conference, Chiang Mai, Thailand, November 2018
- Ceres, K., Al-Mamuna, M.A., Gröhn, Y.T.: Environmental transmission of Mycobacterium Avium Subsp. Paratuberculosis: an individual based model. International Society for Veterinary Epidemiology and Economics Conference, Chiang Mai, Thailand, November 2018
- Stapleton, S. Cazer CL, and Gröhn, Y.T.: Tylosin phosphate physiologically-based pharmacokinetic model assesses antimicrobial pressure on enteric bacteria. Conference of Research Workers in Animal Diseases. Chicago, IL, December 2018. (Poster Presentation).
- Fan S, Foster D, Niedermeyer J, Kathariou S. 2018. Impact of antimicrobial treatment of cattle on *Campylobacter* spp. North Carolina State University Graduate Student Poster Symposium. June 26, 2018.
- Wemette, M., Beauvais, W., Ceres, K., Wolverton, A.K., Grainer Safi, A., M., Shapiro, M., Welcome, F.L., Moroni, P., Ivanek, R. Attitudes of dairy farmers and the general public towards antibiotic use and resistance in dairy cattle. Applied Animal and Public Health Research and Extension Symposium, Kansas City, MO, October 20, 2018. Oral presentation.

- Wemette, M., Beauvais, W., Wolverton, A.K., Greiner Safi, A., Shapiro, M., Welcome, F.L., Moroni, P., Ivanek, R. A survey of the United States public's perceptions of antibiotic use in dairy farming. 2018 Conference for Research Workers in Animal Agriculture (CRWAD), Chicago, IL, December 1-4, 2018. Oral presentation.
- Schueler, J., Lansing, S., Felton, G., Hurst, J., Naas, K., Aga, D., Crossette, E., Raskin, L., Wiggington, K., Oliver, J., Gooch, C., 2018. Fate and transport of antibiotics, genes, and nutrients in anaerobic digestion systems (poster). Bioresource Technology for Bioenergy, Bioproducts & Environmental Sustainability. Sitges, Spain. September 16-19, 2018.
- Schueler, J., Lansing, S., Felton, G., Hurst, J., Naas, K., Aga, D., Crossette, E., Raskin, L., Wiggington, K., Oliver, J., Gooch, C., 2018. Fate and transport of antibiotics, genes, and nutrients in anaerobic digestion systems (poster). Bioresource Technology for Bioenergy, Bioproducts & Environmental Sustainability. Sitges, Spain. September 16-19, 2018.
- Morales, J. Y., **R. G. Amachawadi**, J. A. Sorg, H. M. Scott, and K. N. Norman. 2018. Effects of chlortetracycline and dietary zinc and copper on nursery swine *Clostridium difficile* carriage and intestinal microbiota. The 14th Biennial Congress of the Anaerobe Society of the Americas, July 9-12, Las Vegas, Nevada, USA.
- Cochrane, R. A., **R. G. Amachawadi**, S. Remfry, A. B. Lerner, J. T. Gebhardt, T. G. Nagaraja, J. R. Pluske, M. C. Niederwerder, J. C. Woodworth, S. S. Dritz, and C. K. Jones. 2018. Young Scholar Presentation: A Review of Medium Chain Fatty Acids and Their Recent Role in Feed Safety. Midwest Animal Science Meeting, March 12-14, Omaha, NE.
- Sylvester. E. W., R. A. Cochrane, **R. G. Amachawadi**, S. Remfry, A. B. Lerner, T. G. Nagaraja, J. Pluske, M. C. Niederwerder, C. B. Paulk, C. R. Stark, J. C. Woodworth, S. S. Dritz, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, and C. K. Jones.2018. Defining the Minimum Inhibitory Concentration of Synthetic and Commercial Medium Chain Fatty Acid Based Products Against *Salmonella Typhimurium*. Midwest Animal Science Meeting, March 12-14, Omaha, NE.
- Swanson. A. J., R. A. Cochrane, **R. G. Amachawadi**, S. Remfry, A. B. Lerner, T. G. Nagaraja1, J. R. Pluske, M. C. Niederwerder, C. R. Stark, C. B. Paulk, J. C. Woodworth, S. S. Dritz, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, and C. K. Jones. 2018. Determination of the Minimum Inhibitory Concentration of Various Medium Chain Fatty Acid Based Products in *E. coli*, Enterotoxigenic *E. coli*, and *Campylobacter coli*. Midwest Animal Science Meeting, March 12-14, Omaha, NE.
- Wu. F., M. D. Tokach, J. M. DeRouchey, S. S. Dritz, J. C. Woodworth, R. D. Goodband, K. Capps, S. Remfry, K. Chitakasempornkul, N. M. Bello, T. G. Nagaraja, and **R. G. Amachawadi.** 2018. Effects of tylosin administration route on the development of antimicrobial resistance in fecal enterococci of finishing swine. Midwest Animal Science Meeting, March 12-14, Omaha, NE.
- Menegat. M. B., J. C. Woodworth, S. S. Dritz, **R. G. Amachawadi**, T. G. Nagaraja, K. Capps, M. D. Tokach, J. M. DeRouchey, and R. D. Goodband. 2018. Impact of added copper and chlortetracycline on growth performance of nursery pigs. Midwest Animal Science Meeting, March 12-14, Omaha, NE.
- **Amachawadi. R. G.**, S. A. Murray, C. L. Van Bibber-Krueger, K. N. Norman, J. Vinasco, S. D. Lawhon, R. A. Pugh, J. S. Drouillard, T. G. Nagaraja, and H. M. Scott. 2018. Effects of zinc and menthol supplementation on antimicrobial resistance among fecal enterococci in feedlot cattle. 5th International Conference on Enterococci, April 15-20, Chamonix, France.

- Chastain C, Richert BT, Schinckel AP, Johnson TA, Wickware CL, Thayer M, Mills KM, Feldpausch J, Palencia JP, and Radcliffe JS. 2019. Effects of feeding soluble fiber (dextrin) to pigs pre- and post-weaning on growth performance and volatile fatty acid (VFA) production. Midwest Swine Nutrition Conference. Omaha, NE.
- Muurinen J, Virta M, Johnson TA. Soils, livestock production and antimicrobial resistance.
- Johnson TA, Sylte MJ, Looft T. 2018. Bacitracin modulates metabolomic profiles in cecal microbiomes in a dose dependent manner. International Symposium on Microbial Ecology. Leipzig, Germany.
- Looft T, Sylte MJ, Johnson TA. 2018. Bacitracin modulates metabolomic profiles in cecal microbiomes in a dose dependent manner. Keystone symposium: Microbiome, Host Resistance and Disease. Banff, Canada.
- Ece Bulut, Xu Li, Galen Erickson, Amy Schmidt, John Schmidt, Bing Wang. Transfer of antimicrobial resistance from beef cattle with different antimicrobial uses through manure management. Oral presentation. Research Workers in Animal Diseases (CRWAD) 2018 annual meeting. Chicago, IL. December 01-04, 2018.
- J. Dustin Loy, Joshwa Payne, Caitlyn Deal, Enakshy Dutta, Ece Bulut, Michael Clawson, Bing Wang. Moving beyond the MIC: Evaluation of a novel multiplex real time PCR assay for detection of antimicrobial resistance genes in clinical bovine respiratory disease samples. Oral presentation. American Association of Veterinary Laboratory Diagnosticians (AAVLD) 2018 annual meeting. Kansas City, KS. October 18-22, 2018.
- Bing Wang. Human health impact of antibiotic resistance associated with the use of antibiotics in animal agriculture. Oral presentation. Nebraska Farm Bureau – Agricultural Economic & Technology Summit. Kearney, NE. July 17-18.
- Yangjunna Zhang, Sapna Chitlapilly Dass, Tommy Wheeler, Norasak Kalchayanand, Bing Wang. Comparison of the effectiveness of antimicrobial interventions on reducing antibiotic resistant and susceptible beef-associated *Salmonella*. Poster presentation. IAFP 2018 annual meeting, P3-164. Salt lake City, UT. July 08-11, 2018.

Educational and outreach programs:
- Videos on the impact of antimicrobial resistance on food safety and public health, developed for the Food Microbiology, FS 405/505.
- Lansing, S., 2018. Manure Management & Contaminants: Effects of Antibiotics and Pathogens. 2018 Mid-Atlantic Crop Management School. Ocean City, MD. November 13 – 15, 2018.
- Lansing, S., and Lansing, D., 2018. Antibiotic resistance, manure technologies, and value-added treatment. Opportunities and Challenges in Anaerobic Digestion: Maryland and the Northeast Experience. Annapolis, MD. October 2, 2018.

Report:
- Bing Wang. Ecology and Transfer of Antimicrobial Resistant Bacteria and Genes in the Feed-yard and Land-application Environment When Cattle are Fed Different Antibiotics. Research Project Summary Outline - Final Report to National Cattlemen's Beef Association and Nebraska Beef Council. April 30, 2018.