**Regional Multistate Project NC1206: Annual Report 2022**

**Project/Activity Number:** NC 1206

**Project/Activity Title:** Antimicrobial Resistance

**Period Covered:** August 5, 2021-October 27, 2022

**Date of Report:** December 22, 2022

**Annual Meeting Dates:** October 27 and 28, 2022

**Participants**

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**Brief Summary of Minutes of Annual Meeting**

* Prior to the official start of the meeting, Dr. Paul Plummer coordinated a tour of the National Veterinary Services Laboratory for NC1206 members.
* Opening: The meeting started with a welcome by Dr. Erika Ganda, acting president and Dr. Plummer, host. Dr. Ganda passed the president position to Dr. Emmanuel Okello and Drs. Ganda, Plummer, and Okello co-moderated the meeting.
* Project Renewal: Dr. George Smith, scientific advisor for the NC 1206 project also attended the meeting. Dr. Smith was pleased with the successful project renewal for the next 5 years, a success credited to Dr. Ganda’s leadership. Dr. Smith reiterated the purpose of the meeting, which is to share results of our research and to develop possible collaborations. He emphasized the importance of leadership submitting the meeting reports and encouraged the group to keep up the good work.
  + Question (Dr. Ganda) Signs of project success: True collaborations developing along with joint publications from members in the group.
  + Question (Dr. Ganda) How to ensure success/things to avoid: Schedule the next meeting prior to leaving the current one. Emphasize the collaborations made by team members. Carefully elect new leaders.
  + Question (Dr. Plummer) Estimated number of multistate projects: 300-400 projects.
* Research updates: Drs. Ivanek, Looft, Wang, Li, Okkello, Ganda, Cazer, Plummer, Bearson, and Kelli Maddock presented current research and future collaboration interests.
* Guest Speakers:
  + Lonty Bryant, Merch Animal Health: Presentation focused on advances in diagnostic medicine and the relevance of antimicrobial resistance (AMR) based on antimicrobial susceptibility test (AST) results or polymerase chain reaction (PCR). Suggested need to explore AMR and treatment outcomes as related to animal health.
    - Discussed ideas regarding measurement of treatment outcomes in relation to AMR and clinical outcome. Discussed use of antibiograms to make treatment choices and the impact of the microbiome and in cases of dysbiosis.
* New leadership: Kelli Maddock will serve as the project Secretary for the next year cycle, which was supported and confirmed by the group.
* Annual meeting schedule, 2023: Two potential meeting dates were presented to the group. A survey with proposed meeting dates was sent to members of the group for follow-up after the meeting.
  + January 25-26, 2023- Chicago, IL with the CRWAD conference (22-24)
  + May 18-19, 2023- Gainesville, FL with the NIAMRRE conference (16-18)
  + With 11 votes, Gainesville, FL was selected as the meeting place for the next NIAMRRE conference.

**Accomplishments**

Objective 1: Develop knowledge and tools to improve antimicrobial stewardship

1. *iAMResponsible™* is a collaborative education and extension effort about antimicrobial stewardship that was developed by several institutions. [MD, NE, NY, MI]
   1. **Activities:** Communication strategies and tools to best convey AMR knowledge (prevalence, treatment, stewardship, and perceptions). Well Said Media will create video, web, and print materials. Support and dissemination will occur via iAMResponsible social media accounts.
   2. **Outputs:** Over 150 pieces of content were created and distributed over the past year. Social media platforms are rapidly growing and have a global audience. Based on results of a 2020 survey of social media followers, *iAMResponsibleTM* has become a trusted resource for communicating AMR-related information and guidance to food producers and consumers worldwide.
2. One Health Graduate Online Course. [NE, MD].
   1. **Activities:** Graduate-level course on the fundamentals of AMR development, transmission, risk to humans, animals, and the environment delivered by experts in the One Health concept (interconnection between people, animals, plants, and their shared environment).
   2. **Output:** Six institutions participated in the Spring 2022 course (University of Nebraska- Lincoln, North Carolina State University, Washington State University, Oklahoma State University, University of Minnesota, University of Maryland) and 18 students were enrolled in the Spring 2022 course. Students learned from academic, industry, and government leaders about multiple elements of the AMR crisis. Students developed communication skills and contributed to new materials for use in the iAMResponsible team’s outreach database and on the website. Additional institutions were invited to participate in the Spring of 2023 course offerings.

Objective 2: Develop and evaluate antimicrobial use, resistance transmission, mitigation strategies, and stewardship programs in food systems from a One-Health perspective.

1. Connecting AntiMicrobial Resistance Agricultural Decisions and Environmental Systems, (CAMRADES) is a project aiming to assess the effectiveness of various mitigation strategies for reducing risk to human health in agro-ecosystems by developing an adaptable framework. [NE, IA]
   1. **Activities:** The project was initiated in Spring 2022. All research groups successfully recruited students for the proposed studies. Both research teams started water sampling and analysis of microbiome and resistome in samples from natural watershed.
   2. **Output:** While research is still on-going, the project will integrate predictive models of AMR transport and associated risk to human health, and improve stakeholder understanding of AMR, potential risks and mitigation strategies, along with motivating adoption of research-based practices to protect human health.
2. Evaluation of the effect of high temperature treatment of diary manure and modeling antibiotic residual transformation in dairy and beef manure with high temperature and grass strip treatments, respectively, on antibiotic residuals, antimicrobial genes, and bacteria. [MD, NY, NE]
   1. **Activities:** New extraction techniques will be developed to improve extraction efficiencies of manure to determine antibiotic quantitation in manure and biosolid matrices using LC tandem MS-MS. Thermal-based manure treatment technologies will be employed, including anaerobic digestion, thermophilic digestion, and high-temp, rotary drum processing.
   2. **Output:** While research is on-going, the ultimate impact is to be determined; however, the antibiotic detection method is under review with additional publications to follow.
3. Environmental fate of antibiotic resistance genes in the bovine and swine agroecosystems. [MI, IN]
   1. **Output:** Improve understanding of the impact of soil manuring and the environmental fate of antibiotic resistance genes
4. Plasmid-mediated transfer of antibiotic resistance genes to *Enterococcus faecalis* JH2-2 in poultry litter. [IN, USDA]
   1. **Activities:** Quantification and understanding the horizontal transfer of resistance plasmids judge the impact of animal management practices.
   2. **Output:** Development of a novel method to study the ecology of antibiotic resistance genes.
5. Whole genome sequencing and phenotypic antimicrobial resistance of *Salmonella enterica* serovar Dublin. [PA, ND]
   1. **Activities:** Through connections established at the NC1206 meeting, these laboratories will share *Salmonella* isolates for evaluation of phenotypic and genotypic markers of antimicrobial resistance.
   2. **Output:** Contribution to the body of knowledge surrounding *Salmonella* Dublin resistance mechanisms and potential mitigation measures.
6. Integration of a multi-pronged standardized methodology to identify key diseases and prioritized antimicrobial alternatives in production animals. [IA, CA, OH, GA]
   1. **Activities:** Scoping reviews, expert elicitation, and multi-criteria decision analysis to develop recommendation for alternative practices to manage prioritized diseases that drive the use of medically important antimicrobial agents in production animals.
   2. **Output:** This is an ongoing research project. Expected output include a prioritized list of the diseases that drive the most use of antimicrobials in the four livestock commodities (dairy, beef, poultry, and swine) and a prioritized list of alternative practices to antimicrobial treatments for each of the four livestock commodities

Objective 3: Create and deliver programs on antibiotic stewardship in food production systems through education and outreach.

1. Evaluation of stakeholder perceptions of antimicrobial resistance prevalence and conveyance in the environment. [NY, NE]
   1. **Activities:** Through interviews, focus groups, surveys, and Q-sort analyses, assessment of farmer and veterinarian perceptions of AMR to develop communication materials that are more tailored to biases, current levels of understanding, and perception.
   2. **Output:** Research is on-going, so impact of the results is still to be determined. The Q-sort analyses paper was submitted and is under review.
2. Human Dimensions of AMR in Agriculture Workshop. [MD, NE]
   1. **Activities:** Organized with leaders in AMR social science research and AMR communication to identify current research, identify gaps in the literature, write articles to address limitations of social science-based AMR information in agriculture.
   2. **Output:** Workshop brought together 37 participants, including researchers, students, and agricultural experts focused on surveying and communicating the human and economic dimensions of AMR in agriculture. Working groups allowed for mentoring and collaboration between participants. Three publications with another under review were produced from this workshop.
3. Improving Antimicrobial Resistance Science Communication Education for Veterinary Students. [IA, CA, OH]
   1. **Activities:** Focus groups and surveys to identify gaps in knowledge about AMR, antimicrobial stewardship, and communication skills training among current veterinary medicine students with the aim to develop modules that address these knowledge gaps.
   2. **Output:** The research is ongoing. Expected output is online AMR/antimicrobial stewardship communication modules available to all interested individuals and organizations, targeting veterinary students, veterinary technicians, recent graduates, and professional organizations.

**Impacts**

Antimicrobial resistance is a major concern from the One Health perspective, which must be addressed using a multidisciplinary and collaborative approach across the animal, human and environmental health spheres. The NC 1206 Multi-State Research Project has collaborated to accomplish key studies and extension education activities that advance the knowledge, outreach, interdisciplinary collaboration, and mitigation efforts related to antimicrobial resistance. Social sciences have been integrated into education programs to target potential antimicrobial user biases, increase AMR knowledge, and to reach stakeholders across the food chain; the addition of social sciences strengthens the approach and allows for more targeted education of stakeholders. Several mitigation and resistance mechanism collaborations have developed as a result of this collaboration.

**Funding**

1. CAMRADES connecting antimicrobial resistance, agricultural decisions, and environmental systems: A tool for mitigating AMR and assessing risk to human health in agro-ecosystems. Type: Research/extension. Duration: 01/01/2022-12/31/2026. Amount: $1,000,000. Funding Source: USDA NIFA. **Bing Wang**, **Michelle Soupir**, Daniel Anderson, Adina Howe, Diana Aga, Shannon Bartelt-Hunt**, Amy Schmidt.** [NE, IA]
2. Application of Fourier Transformed Infrared Spectroscopy (FTIR) and Machine Learning Classifiers for Typing of Veterinary Associated Strains of Salmonella spp. Rasmussen, R., Foundation for Food and Animal Research (FFAR) Veterinary Student Research Fellowship. Funded June 2022. **Moxley R.** and **Loy JD.** [NE]
3. Nebraska Beef Council and National Cattlemen’s Beef Association. $55,822, 10/1/2020 – 9/30/2021. **Moxley RA**, **Loy JD**. [NE]
4. UMDNIFA Award # 2018-68003-27467; iAMResponsible NIFA Awards # 2018-68003-27545; UMD Workshop Award: #2019-67017-29114. **Lansing S, Schmidt A.** [MD, NE]
5. USDA NIFA Award #2022-68015-37140. **Plummer P, Okello E,** King A, Sander W.[CA, IA]
6. FDA Cooperative Agreement. **Plummer P** (PI). Collaborators: Karriker L, Lehenbauer TW, **Okello E, Pereira RVV**, Aly SS, Wittum T, Logue C. [IA, CA, OH]

**Appendix I: Publications**

1. **Johnson TA**, Sylte MJ, **Looft T**. In-feed bacitracin methylene disalicylate modulates the turkey microbiota and metabolome in a dose-dependent manner [submitted to Scientific Reports]
2. Carter HF, Wills RW, Scott MA, Thompson AC, **Singer RS, Loy JD**, Karisch BB, Epperson WB, and Woolums AR. Diversity of antimicrobial resistance phenotypes and genotypes of *Mannheimia haemolytica* isolates from bovine nasopharyngeal swabs. Front Vet Sci. 2022 May 11;9:883389. doi: 10.3389/fvets.2022.883389
3. Bo Li, **Xu Li, Bing Wang**, and Tao Yan. A metagenomic approach for characterizing antibiotic resistance genes in specific bacterial populations: demonstration with *Escherichia coli* in cattle manure. Appl Environ Microbiol. 2022 Apr 12;88(7):e0255421*.* [NE]
4. Donner L, Staley ZR, Petali J, Sangster J, Li X, Mathews W, Snow D, Howe A, Soupir M, Bartelt-Hunt S. The Human Health Implications of Antibiotic Resistance in Environmental Isolates from Two Nebraska Watersheds. Microbiol Spectr. 2022 Apr 27;10(2):e0208221. doi: 10.1128/spectrum.02082-21. Epub 2022 Mar 21. PMID: 35311538; PMCID: PMC9045274. [NE, IA]
5. Mware NA, Hall MC, Rajendran S, Gilley JE, **Schmidt AM,** Bartelt-Hunt SL, Zhang Y, **Li X**. Resistome and mobilome in surface runoff from manured soil as affected by setback distance. J Hazard Mater. 2022 May 5;429:128278. doi: 10.1016/j.jhazmat.2022.128278. Epub 2022 Jan 14. PMID: 35065306. [NE]
6. Kaniyamattam K, Hertl J, **Tauer LW**, and **Grohn YT**. (2022). Economics of reducing antibiotic usage for pathogen-specific clinical mastitis through genomic selection and disease management. *Prev Vet Med*. 2022 Jul;204:105642. doi: 10.1016/j.prevetmed.2022.105642. Epub 2022 Apr 6. PMID: 35430445. [NY]
7. Verteramo Chiu, L. J., **Tauer, L. W.,** & **Gröhn, Y. T.** (2022). Pricing efficiency in livestock auction markets: A two-tier frontier approach. *Agricultural Economics*,1–13.<https://doi.org/10.1111/agec.12735> [NY]
8. Verteramo Chiu, L.J., **Tauer, L.W**., Lhermie. G., Kaniyamattam, K., **Gröhn Y.T**. (2022)Benefits of Preconditioning Cattle under Stochastic Feedlot Performance. *Journal of Agricultural and Applied Economics*. 1–18 doi:10.1017/aae.2022.32 [NY]
9. Barrett, J. R., Innes, G. K., Johnson, K. A., Lhermie, G., **Ivanek, R.,** Greiner Safi, A., & **Lansing, D.** (2021). Consumer perceptions of antimicrobial use in animal husbandry: A scoping review. *PLOS ONE*, *16*(12), e0261010

**Appendix II: Scientific and Outreach Presentations**

1. **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. [MN, USDA]
2. **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. [MN, USDA]
3. Rasmussen M, **Moxley R**, and **Loy JD.** Evaluation of artificial neural network-based classifier models to discriminate phenotypes of *Salmonella enterica* using Fourier-Transform Infrared Spectroscopy (FT-IR). National Veterinary Scholars Symposium. Minneapolis, MN. 2022. [NE]
4. **Moxley RA,** **Loy JD**. Genetic analysis and rapid detection of multidrug-resistant *Salmonella enterica* isolates from cattle. Beef Industry Safety Summit, Beef Industry Food Safety Council (BIFSCo), Oral Presentation, Denver, CO, Feb. 28-Mar. 2, 2022. [NE]
5. Lewis GL, Fenton R, **Loy JD** and **Moxley RA**. Genetic analysis of multidrug-resistant *Salmonella enterica* isolates from cattle. National Institute of Antimicrobial Resistance Research and Education (NIAMRRE) 2022 Annual Conference, Lincoln, NE, May 16-18, 2022. [NE]
6. Hertl J, Schukken Y, **Tauer L, Grohn Y** (2022). Effects of pathogen-specific clinical mastitis occurrence in the first 100 days of lactation 1 on future mastitis occurrence in Holstein dairy cows. 16th International Symposium of Veterinary Epidemiology and Economics, Halifax, Nova Scotia, August 7-12, 2022.
7. **Johnson TJ,** Armstrong JR, Johnston B, Merino-Velasco I, Jamborova I, **Singer RS**, Johnson JR, Bender JB. 2022. Occurrence and potential transmission of extended-spectrum beta-lactamase-producing extraintestinal pathogenic and enteropathogenic *Escherichia coli* in domestic dog feces from Minnesota. Zoonoses Public Health, In Press.
8. Manjankattil S, Nair DVT, Peichel C, Noll S, **Johnson TJ,** Cox RB, Donoghue AM, **Johny** **AK.** Effect of caprylic acid alone or in combination with peracetic acid against multidrug-resistant Salmonella Heidelberg on chicken drumsticks in a soft scalding temperature-time setup. Poult Sci. 2021; 100(11):101421.
9. Dewi G, Nair DVT, Peichel C, **Johnson TJ,** Noll S, **Johny AK.** Effect of lemongrass essential oil against multidrug-resistant Salmonella Heidelberg and its attachment to chicken skin and meat. Poult Sci. 2021;100(7):101116.
10. Dewi G, Manjankattil S, Peichel C, **Johnson TJ**, Cardona C, Noll S, **Johny AK.** Effect of Turkey-Derived *Lactobacillus* Probiotics and Trans-Cinnamaldehyde Against Multidrug-Resistant Salmonella Heidelberg in Turkey Poults. International Association of Food Protection, Phoenix, AZ, 2021  
    <https://iafp.confex.com/iafp/2021/onlineprogram.cgi/Paper/26620>

**Appendix III: Other Related Publications**

1. Waltenburg MA, Shugart MA, **Loy JD.** Tewari D, Zhang S, Cole S, Ceric O, Walters MD, Nichols M. A survey of current activities and technologies used to detect carbapenem resistance in bacteria isolated from companion animals at veterinary diagnostic laboratories—United States, 2020. J Clin Microbiol. 2022;60 (3) e02154-21. doi: 10.1128/jcm.02154-21
2. Kalchayanand N, Dass SC, Zhang Y, Oliver EL, **Wang B**, Wheeler TL. Efficacy of antimicrobial interventions used in meat processing plants against antimicrobial tolerant of non-antimicrobial resistant (non-AMR) and antimicrobial resistant (AMR) *Salmonella* on fresh beef. J Food Prot. 2022; 85(8): 1114-1121. <https://doi.org/10.4315/JFP-21-364>
3. Sun Y, Snow D, Walia H, **Li X**. Transmission routes of microbiome and resistome from manure to soil and lettuce. Environ Sci Technol. 2021;55(16): 11102-11112.
4. Shafieifini M, Sun Y, Staley Z, Riethoven J, **Li X.** Effects of nutrient level and growth rate on the conjugation process that transfers mobile antibiotic resistance genes in continuous cultures. Appl Environ Microbiol. 2022. In press, <https://doi.org/10.1128/aem.01121-22>.
5. Sun T, Duarte ME, Kim SW. Dietary inclusion of multispecies probiotics in preventing post-weaning diarrhea caused by F18+ E. coli in pigs. Anim Nutr. 2021;7:326-333. doi:10.1016/j.aninu.2020.08.012. [NC]
6. Casseri E, Bulut E, Llanos Soto S, Wemette M, Stout A, Greiner Safi A, Lynch R, Moroni P, **Ivanek R**. Understanding antibiotic resistance as a perceived threat towards dairy cattle through beliefs and practices: a survey-based study of dairy farmers. Antibiotics. 2022 Jul 25;11(8):997. doi: 10.3390/antibiotics11080997 [NY]
7. Schell R, Bulut E, Padda H, Greiner Safi A, Moroni P, **Ivanek R.** Responsible antibiotic use labeling and consumers’ willingness to buy and pay for fluid milk. J Dairy Sci. (In press) [NY]
8. Preine F, Herrera D, Scherpenzeel C, Kalmus P, McCoy F, Smulski S, Rajala-Schultz P, Schmenger A, **Moroni P**, Krömker V. Different European Perspectives on the Treatment of Clinical Mastitis in Lactation. Antibiotics (Basel). 2022 Aug 16;11(8):1107. doi: 10.3390/antibiotics11081107. PMID: 36009976; PMCID: PMC9404852. [NY]
9. Cremonesi P, Biscarini F, Castiglioni B, Sgoifo CA, Compiani R, **Moroni P**. Gut microbiome modifications over time when removing in-feed antibiotics from the prophylaxis of post-weaning diarrhea in piglets. PLoS One. 2022 Mar 7;17(3):e0262199. doi: 10.1371/journal.pone.0262199. PMID: 35255081; PMCID: PMC8901073. [NY]
10. Manuscript in preparation: **Maddock K,** Webb T, Carson P, Stokka G. Carriage of antimicrobial resistant flora and enteric pathogens among veterinary professionals in the upper Midwest.
11. **Singer, RS**. 2022. Continued abuse of causal inference in studies of antimicrobial resistance: revisiting the confusion between ecological correlation and causation. J Glob Antimicrob Resist 30:485-486
12. Siddique A, Ullah N, Ali A, Patel A, Moore T, Kenney SM, **Ganda E**, Rahman A. Draft genome sequences of 25 Salmonella enterica serovar Agona strains isolated from poultry and associated food products harboring multiple antibiotic resistance genes. J Glob Antimicrobial R. 2022. 29, 131-135. DOI: 10.1016/j.jgar.2022.02.013, ISBN/ISSN: 2213-7165 http://dx.doi.org/10.1016/j.jgar.2022.02.013
13. Siddique A, Azim S, Ali A, Adnan F, Arif M, Imran M, **Ganda E,** Rahman A. Lactobacillus reuteri and Enterococcus faecium from Poultry Gut Reduce Mucin Adhesion and Biofilm Formation of Cephalosporin and Fluoroquinolone-Resistant Salmonella enterica. Animals. 2021. 11(12), 3435. DOI: 10.3390/ani11123435, ISBN/ISSN: 2076-2615 http://dx.doi.org/10.3390/ani11123435