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USDA ARS Antimicrobial Resistance (AMR) and Alternatives to Antibiotics (ATA) Accomplishment Summary

2016-2018

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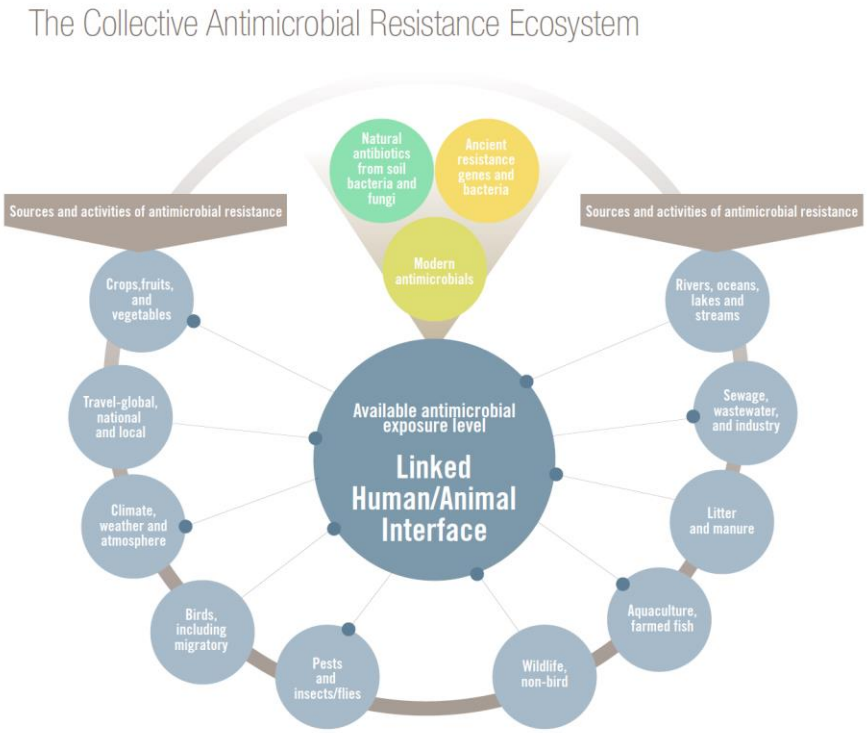
INTRODUCTION

Currently, antimicrobial resistance (AMR) is considered one of the most serious public health threats. In 2012, the USDA One Health Joint Working Group sponsored a stakeholder workshop and developed the USDA Antimicrobial Resistance Action Plan[1] based on input from the workshop to describe how the USDA proposed to obtain and disseminate science-based, actionable information about antibiotic drug use, its potential role in the development of antibiotic resistance in pathogens associated with food-producing animals, and the relationship of drug use and resistance patterns to livestock management practices. The USDA AMR Action Plan forms the foundation for USDA inputs into the Combating Antibiotic Resistant Bacteria National Action Plan.



As the intramural research arm of USDA, the Agricultural Research Service (ARS) has the directive to perform solution-oriented, hypothesis-driven research that benefits agriculture and public health by ensuring a safe, secure food supply that has minimal environmental impacts. ARS uses a systems-based, One Health approach to conduct research on the multifactorial components, termed the Antimicrobial Resistance Ecosystem, that contribute to the development and persistence of AMR. ARS has the expertise and infrastructure to conduct research that helps explain the different factors associated with AMR in agricultural settings and to develop tools that mitigate AMR for the benefit of human, animal, and ecosystem health.

Multiple USDA ARS national programs[2] address antimicrobial resistance through research conducted in the laboratory, on the farm, and at processing facilities. These national programs include NP 101, Animal Production; NP 103, Animal Health; NP 106, Aquaculture; NP 108, Food Safety; NP 212, Soil and Air; NP 301, Plant Health; and NP 306, Product Quality and New Uses.

This report highlights the research accomplishments of ARS scientists in those national programs between 2016 and 2018 as they relate to increasing the understanding of the collective antimicrobial resistance ecosystem, developing alternatives to antibiotics, and new strategies to mitigate the spread of AMR.

[1] https://www.usda.gov/sites/default/files/documents/usda-antimicrobial-resistance-action-plan.pdf
[2] https://www.ars.usda.gov/research/programs/

ACCOMPLISHMENTS BY SUBJECT AREA

Animal Health and Production

Use of the biotherapeutic G-CSF as a prophylactic and alternative to antibiotics to prevent post-weaning *Streptococcus suis* in swine

NP 103: Animal Health
Location: Ames, IA

Due to the increased pressure to reduce antibiotic use in production animal medicine, alternatives to antibiotics ATA for the treatment of bacterial infections need to be explored. Biotherapeutics such as immunomodulators are attractive candidates to stimulate or restore the ability of the immune system to combat infections, especially during times of stress and potential immune dysfunction. Cytokines offer a specific method of stimulating or restoring the ability of the swine immune system to combat infections and should pose no food safety issues because they occur naturally in animals. Granulocyte-colony stimulating factor (G-CSF) stimulates proliferation and release of neutrophils from the bone marrow. These professional phagocytes are critical to the innate control of many bacterial infections, including those caused by *Streptococcus suis*. ARS developed a replication-defective adenovirus vector that expresses G-CSF and showed that it elicits a sustained neutrophilia, lasting nearly 3 weeks, which may be beneficial to prevent bacterial diseases during times of peak incidence.

Streptococcus suis is a bacterium that is an important and common cause of systemic disease such as meningitis, polyserositis, arthritis, and septicemia in pigs; contributes to the porcine respiratory disease complex; and is also a zoonotic threat. Streptococcal disease has been reported to affect more than 50 percent of the finishing herds, 75 percent of the breeding herds, and 95 percent of the nursery herds in the United States (NAHMS 2012 survey). Strain variability and lack of cross-reactivity have made development of efficacious vaccines difficult, and thus there is significant antibiotic use to control this disease. Neutrophils are believed to be important for clearance of *S. suis* in vivo; therefore, ARS also determined whether the presence of increased circulating neutrophils induced by the administration of G-CSF would decrease the incidence or severity of disease with *S. suis*. Pigs given G-CSF had an improved clinical outcome and lower bacterial loads when subsequently infected with *S. suis*. This research indicates that the use of G-CSF in pigs to induce an increase in circulating neutrophil numbers may be a useful antibiotic alternative for the prevention of streptococcal and other bacterial diseases in swine, especially during times of stress and pathogen exposure that occurs after weaning.

A subunit vaccine against *Streptococcus suis* in swine

NP 103: Animal Health
Location: Ames, IA

Streptococcus suis is a bacterium that is an important and common cause of meningitis and arthritis in pigs that costs the swine industry millions of dollars in losses annually. Unfortunately, few efficacious vaccines are available for this disease. ARS researchers with collaborators from the University of Cambridge identified five candidate proteins of *Streptococcus suis* that were selected for inclusion in an experimental vaccine by identifying bacterial genes required for survival of the bacterium in the pig. The proteins were delivered to pigs with different adjuvants that help stimulate an immune response. The vaccine was found to be effective at preventing disease caused by *S. suis*. In addition, antiserum from the vaccinated pigs was reactive against *S. suis* bacteria of differing serotypes, thus indicating a potential for cross-protection. These proteins are now being developed into a vaccine

by a biologics company that can be used by swine producers to protect against this devastating and costly swine disease.

Ability of methicillin-resistant *Staphylococcus aureus* to adhere to human and swine skin

NP 103: Animal Health
Location: Ames, IA

Methicillin resistant *Staphylococcus aureus* (MRSA) is a bacterium that causes severe disease in humans and can be acquired from healthcare settings (HA-MRSA), community settings (CA-MRSA), or contact with livestock including swine (LA-MRSA). Although LA-MRSA infrequently causes disease in pigs, there have been concerns in the public health community that livestock may act as a reservoir for MRSA isolates affecting people. To understand the implications of LA-MRSA isolates for swine producers and the risk these isolates may pose outside of the agricultural setting, ARS researchers compared MRSA isolates from healthcare settings (HA-MRSA) and from swine-associated sources for the ability to attach to human and swine skin. The HA-MRSA and LA-MRSA isolates tested were found to adhere equivalently to human skin and a comparison of genes involved in initial attachment revealed a similar genetic potential for adherence. However, the genes that contribute to persistent colonization and disease with *S. aureus* were absent in the LA-MRSA isolates. This suggests that humans in contact with swine have the potential to become transiently colonized with these LA-MRSA isolates but that these isolates are probably less likely to cause disease in humans compared with human isolates acquired from health care settings.

Use of *Lactobacillus acidophilus* fermentation products as an alternative to subtherapeutic antibiotic use in nursery pig diets

NP 101: Animal Production
Location: Clay Center, NE

This project evaluated *Lactobacillus acidophilus* fermentation products (LAFP) as a replacement for antibiotics in nursery swine diets and to identify its effect on both nursery and subsequent production performance. Nursery pig diets containing LAFP were compared with nonmedicated diets and diets containing traditional antibiotics. Pigs were evaluated for growth performance, gut health, pathogen shedding, and expression of antibiotic resistance genes in feces. LAFP did not improve the growth of pigs in this study, presumably due to the high health status of the pigs in the trial. However, expression of antibiotic resistance genes in pigs that were fed LAFP was reduced by approximately 40 percent compared with gene expression in pigs that consumed antibiotics. Results from this experiment show that LAFP does not improve growth performance in nursery pigs with relatively low negative pressures on growth. Even in healthy, fast-growing pigs LAFP reduced the expression of antimicrobial resistance genes, indicating that LAFP may be a good option for reducing the presence of antimicrobial-resistant bacteria in swine production.

Attenuated immune response to Bovine Respiratory Disease by feeding a prebiotic/probiotic blend

NP 101: Animal Production
Location: Lubbock, TX

Bovine Respiratory Disease (BRD) continues to be the most significant disease affecting beef cattle production, and results in over $900 million in losses annually. Additionally, even in the face of improved vaccines and other

management tools, the incidence of BRD has not decreased. ARS scientists collaborated with an industry partner to determine whether feeding a prebiotic/probiotic blend would improve the immune response to a dual viral-bacterial BRD challenge. Results showed that supplementing calves with a prebiotic/probiotic blend may have helped calves recover more quickly from the BRD challenge, as supplemented calves had a reduced fever response to the challenge and had decreased values of inflammatory immune markers. A quicker recovery from a disease challenge can lead to reduced production losses associated with illness and reduced treatment costs, both of which are the main contributors of losses associated with BRD infections. As consumers continue to advocate for the reduced use of pharmaceuticals in livestock production, feeding a prebiotic/probiotic blend may provide an acceptable alternative with health benefits for the over 11.5 million calves being fed each day in the United States.

Discovered an antibiotic alternative for newly weaned and transported piglets

NP 101: Animal Production
Location: West Lafayette, IN

Weaned and transported pigs are inherently stressed by these procedures which are unavoidable. Traditionally, antibiotics have been used to help these compromised animals to prevent disease and morbidity. Currently, antibiotic use is being curtailed and alternatives must be identified to ensure the health and welfare of swine during these procedures. ARS researchers conducted an experiment to determine the threshold for efficacy of L-glutamine (an essential amino acid) as an antibiotic alternative for swine producers following weaning and transport. It was determined that a range between 0.20 and 0.50% was the level at which L-glutamine would provide a positive effect on swine welfare and productivity. These results can be directly utilized by swine nutrition companies in the development and marketing of the antibiotic alternative for use in the swine industry. These results will provide the swine industry with the most effective level to provide L-glutamine to pigs following weaning and transport as an alternative to antibiotics.

A new natural antibiotic alternative for swine

NP 108: Food Safety
Location: College Station, TX

Livestock farmers are under increasing pressure to reduce their use of antibiotics to control disease during production; consequently, there is need for new technologies to help farmers maintain optimal health and well-being of their animals. ARS scientists in collaboration with scientists at the Norman E. Borlaug Institute for International Agriculture determined the efficacy of the plant *Nigella sativa* (black cumin) as a potential substitute for conventional antibiotics in swine production. The research established that feeding black cumin dramatically improved growth efficiency of the pigs and helped them resist colonization by *Escherichia coli,* which is particularly pathogenic for young pigs. These results provide important information on a potential new feed additive that when combined with other feed ingredients and good management can help pig farmers improve the health and well-being of their young animals. Ultimately, these results will help pig farmers find new ways to safely and economically produce high-quality and wholesome pork products at less cost to the American consumer.

Evaluation of the effects of antibiotic alternatives on chicken gut microbiome using comparative metagenomic analysis

NP 103: Animal Health

Location: Beltsville, MD

With the movement toward reducing antibiotic use in the poultry industry, the incidences of coccidiosis and necrotic enteritis, which are caused by *Eimeria* species and *Clostridium perfringens* (CP), respectively, are increasing. These diseases are responsible for estimated economic losses of more than $9 billion annually. Very little information is known about how *Eimeria*, and CP, and antibiotics affect the poultry gut microbiome composition. In this study, single and dual infections with the two pathogens were performed. DNA or messenger RNA were extracted from gut microbiome contents and the chicken intestinal mucosa was subjected to sequencing analysis to identify the microbiome bacterial populations and gene expression changes.

Infections with *Eimeria maxima* (EM) and CP and administration of an antibiotic induced significant differences in the microbiota genomic population and cecal mucosal gene expression of infected chickens. For example, EM, CP, EM/CP (dual infections), or antibiotic administration caused changes (i.e., upregulation or downregulation) in bacterial communities identified at the genus level by 16S rRNA sequencing in the cecal microbiota. RNA-Seq analysis of cecal mucosa in EM and naïve control groups revealed 332 upregulated and 363 downregulated genes with significant differences ($P \leq 0.05$), including several significant immune-related gene families. These findings provide new insights into host-pathogen interaction and enhance our understanding of the molecular mechanisms of avian coccidiosis and necrotic enteritis, which will help lead to the rational design of mitigation approaches and the development of alternatives to antibiotics to maintain the balance in gut microbiota and improve the host immune responses.

Novel immunomodulatory host-derived antimicrobial peptides as antibiotic alternatives to mitigate enteric pathogen-mediated gut damage in commercial broiler chickens

NP 103: Animal Health
Location: Beltsville, MD

Increased occurrence and severity of infectious diseases, especially avian necrotic enteritis (NE) and coccidiosis, have been occurring with the removal of antibiotic growth promoters. In the European Union, where antibiotic growth promoters were banned in 2006, limited availability of drug alternatives to manage these re-emerging infectious diseases poses a major challenge. Therefore, alternatives to antibiotics (ATAs) are urgently needed. ARS researchers and their colleagues developed a chicken natural killer (cNK)-lysin antimicrobial peptide to serve as an alternative for treating avian coccidiosis. We know that NK-lysin and a synthetic peptide, cNK-2, corresponding to the core α-helical region of cNK-lysin, are toxic to coccidia parasites. Both cNK-lysin and cNK-2 also exhibit immunomodulatory activities as evidenced by the induction of protective chicken chemokines in relatively low doses. ARS tested the feasibility of incorporating a nanoparticle or liposome to stably deliver cNK-lysin peptides as a feed additive. NK-lysin showed direct killing of coccidia sporozoites before their invasion of gut tissues, and modulated host immunity. This is the first host defense protein that shows direct killing effects on all *Eimeria* species. Effective application of the outcome of this study will reduce cost involved with coccidiosis and necrotic enteritis since coccidiosis is the primary risk factor for necrotic enteritis.

Antibiotic growth promoters alter the chicken intestinal metabolome

NP 103: Animal Health
Location: Beltsville, MD

Although dietary antibiotic growth promoters have long been used to increase growth performance in commercial food animal production, the biochemical details associated with these effects remain poorly defined. A

metabolomics technique was used to characterize and identify the biochemical compounds present in the intestine of broiler chickens fed a standard, unsupplemented diet or a diet supplemented with the antibiotic growth promoters virginiamycin or bacitracin. Compared with control chickens fed an unsupplemented diet, the levels of 218 biochemicals were altered in chickens given the virginiamycin-supplemented diet, whereas 119 biochemicals were altered when chickens were fed the bacitracin-supplemented diet. The changes in the levels of intestinal biochemicals provided a distinctive biochemical signature that was unique to each antibiotic-supplemented group. These biochemical signatures were characterized by increases in levels of metabolites of amino acids, fatty acids, nucleosides, and vitamins. These results provide the framework for future studies to identify natural antibiotic alternatives to improve poultry growth performance without the use of in-feed antibiotics.

Nanoparticles improve vaccines against coccidiosis

NP 103: Animal Health
Location: Beltsville, MD

Coccidiosis, a gut disease of poultry, costs U.S. producers $350 million annually due to poor weight gain in affected animals and the costs of treatment. It is also listed by the World Organization for Animal Health (OIE) as a high priority disease for which improved vaccines would significantly reduce the need for antibiotic administration. Current vaccines against coccidiosis consist of low doses of highly infectious organisms, and better vaccines are needed. ARS scientists discovered that attaching a protective vaccine antigen to nanoparticles significantly improved efficacy. Chickens given the vaccine by oral administration at hatch showed improved weight gain and feed conversion efficiency compared with chickens that were vaccinated with the same antigen but without nanoparticles. This technology may markedly improve the health and welfare of poultry flocks, reduce the costs of poultry production, and reduce the need for antibiotics in poultry by decreasing the occurrence of concomitant bacterial infections.

Novel prebiotics and probiotics reduce stress/pathogen induced mortality and production losses in chickens

NP 108: Food Safety
Location: Fayetteville, AR

Stress, specifically cold stress, can significantly decrease performance and increase susceptibility to infections in poultry flocks. Prebiotics may alter intestinal ecology by modulating inflammation through the production of short-chain fatty acids. Probiotics can directly alter the intestinal microbiome resulting in similar effects. ARS researchers at Fayetteville, Arkansas, investigated the potential of prebiotics and probiotics to protect intestinal health under cold stress and pathogen (*Escherichia coli*) challenge in poultry. Although the prebiotic studies did not prevent effects of cold stress or *E. coli* challenge, the probiotic treatment was consistent in reducing production losses. Continuous inclusion of probiotics resulted in increased body weight and decreased mortality. This research demonstrates the effectiveness of probiotics and their beneficial effects on performance and protecting chicks from infections and various stressors.

Aquaculture

Host genetic background influence on early molecular signatures of vaccine responsiveness

NP 106: Aquaculture
Location: Leetown, WV

Infectious disease is responsible for 80% of losses in U.S. trout aquaculture and the primary reason for medicated feed application. ARS scientists bred rainbow trout for pathogen resistance or susceptibility and evaluated their survival in response to infection when they were vaccinated or unvaccinated. Scientists were able to demonstrate that breeding and vaccination are additive approaches for reducing disease, while identifying genes that are being targeted for new breeding strategies or vaccine development. Production and distribution of the disease-resistant ARS-Fp-R line of trout has led to reduced on-farm outbreaks of bacterial Coldwater disease and less need for medicated feed application on-farm.

Food Safety

Development of immunoassays to monitor colistin-resistant bacteria in food-producing animals, production environments, meat, and poultry

NP 108: Food Safety
Location: Albany, CA

Colistin is an antibiotic that is considered the drug of last resort for treating infections caused by multidrug resistant bacteria. The recent discovery and rapid spread of mobile colistin-resistance in bacteria is undermining our ability to treat severe infections and threatening human and animal health and safety. To prevent further transfer of colistin resistance, practical and reliable detection methods for the plasmid-borne colistin-resistant gene product, MCR, are needed. ARS developed standards and novel polyclonal and monoclonal antibodies against the MCR proteins. A sandwich enzyme-linked immunosorbent assay was established using these antibodies. The assay can effectively identify meat samples (ground beef, chicken, and pork) contaminated with colistin-resistant bacteria in 0.4 cfu/g of meat. This is the first immunoassay with the ability to detect colistin-resistant bacteria and should be useful for screening samples to determine contamination of colistin-resistant bacteria on animals and in the environment; and in fresh produce, meat, and poultry, thus reducing human risk of foodborne infections with possibly no antibiotic treatment options.

Impact of raising beef cattle without antibiotics on occurrences of antimicrobial resistance

NP 108: Food Safety
Location: Clay Center, NE

There is a significant societal concern that traditional antimicrobial use patterns for food-animal production have contributed to the occurrence of antimicrobial resistance (AMR) in human infections. In response to this concern, ARS researchers compared fecal AMR levels between U.S. beef cattle produced conventionally, with no restrictions on antibiotic use other than regulatory compliance, and U.S. beef cattle raised without antibiotics. Fifty of 67 individual microbial AMR levels were not different between production systems, whereas 17 of 67 levels exhibited significant increases in conventional animals. However, although these increases in AMR were statistically significant, they were so small that they are not likely to be biologically significant. More importantly, cattle raised without antibiotics typically grow slower, so they must be fed 50 days longer and thus they produce about 2,500 pounds more manure. Therefore, the 31 percent increase in the amount of manure from cattle raised

without antibiotics more than offsets the small reduction in a few resistances and may actually increase the total AMR in the environment. Thus, compared with conventional production, beef cattle production without antibiotics would not be expected to reduce the amount of AMR contributed to the environment compared with conventional production.

Colistin resistance gene (*mcr-1*) in U.S. meat animals

NP 108: Food Safety
Location: Athens, GA

In 2015, a gene (*mcr-1*) for colistin resistance was found that increases the ease and rate at which resistance can spread to different bacteria. In 2016, ARS researchers were the first in the U.S. to find this bacterial gene in the stomach contents of livestock. From more than 2,000 cecal samples, two isolates of *Escherichia coli* were found that carried the *mcr-1* gene. Isolates were characterized by whole genome sequencing, resistance profiling, and plasmid mobility studies. The total genomic DNA of the isolates were sequenced, and the U.S. isolates were determined to have descended from isolates found in China, but they were substantially different, so there was not a direct link. At this time, it is unknown how the resistance gene traveled from China to the United States.

Antibiotic resistance profiles of bacteria from all-natural, antibiotic-free broilers

NP 108: Food Safety
Location: Athens, GA

Isolates of *Campylobacter*, *Listeria*, and *Escherichia coli* from 15 flocks raised on pasture from 6 different farms throughout the 2014 growing season were characterized for antimicrobial resistance. Isolates were collected from a variety of environmental samples (feces, soil, carcass rinses, and ceca). The antibiotic resistance patterns were found to be diverse among the different isolates, with resistance profiles being farm specific under certain circumstances. These data highlight the importance of including background antibiotic resistance profiling in future studies because high levels of resistance, including multidrug resistance, can be found on farms that have never used antibiotics during production. This research directly contributes to a better understanding of the larger problem of emerging antibiotic resistance.

Human-associated antimicrobial resistant *Staphylococcus aureus* in table eggs from Pakistan

NP 108: Food Safety
Location: Athens, GA

Staphylococcus aureus is a bacterium that can be commonly found on the skin or in the nasal passages of most humans and animals. It can cause a number of diseases in humans including staphylococcal food poisoning characterized by vomiting and diarrhea. Eggs are usually considered safe and are naturally protected by the egg shell and a semipermeable membrane; however, bacteria such as S*taphylococcus aureus* may enter and contaminate the eggs by crossing both the egg shell and the membrane. In addition, there is increasing interest in the presence of antimicrobial resistance in *Staphyloccus aureus*, specifically methicillin-resistant *S. aureus* (MRSA). ARS researchers isolated staphylococci from table eggs in Pakistan and MRSA from the products were characterized. MRSA were recovered from a portion of the eggs and all were resistant to multiple antimicrobial agents, and molecular analysis revealed characteristics of MRSA that are known to cause human infections. Results from this study showed that MRSA are present in table eggs from Pakistan, which may be transmitted to humans. The genetic similarities of MRSA present in the eggs to that of humans may suggest human-to-poultry

transmission of MRSA via contamination. This information is of importance to consumers and personnel who handle eggs in Pakistan because safe handling and cooking methods can prohibit colonization and infection with MRSA.

Genomic sequencing of antimicrobial resistant *Escherichia coli* from Nigeria

NP 108: Food Safety
Location: Athens, GA

Widespread use of antimicrobials in economically challenged countries is a driver of worldwide antimicrobial resistance. In order to effectively combat antimicrobial resistance, monitoring of resistance in these regions is necessary. *Escherichia coli* is a common commensal of the intestinal tract of humans and animals but can also be an opportunist pathogen associated with illnesses. In order to effectively treat these infections, it is important to understand the mechanisms of resistance in *E. coli*. To determine resistance in *E. coli*, ARS researchers isolated and sequenced the genomes of multidrug-resistant *E. coli* from humans and chicken carcasses from samples taken in Nigeria. Multiple genes conferring resistance to the critical human use beta-lactam drug class were identified in those isolates. The isolates also harbored genes conferring resistance to other drugs used to treat human infections. This information is essential to food safety and human health for development of control strategies for combating antimicrobial resistance in economically challenged countries.

ColE1 plasmids contribute to the fitness of *Salmonella* Heidelberg in poultry litter

NP 108: Food Safety
Location: Athens, GA

Salmonella enterica subsp. *enterica* serovar Heidelberg (S. Heidelberg) is a clinically important serovar linked to foodborne illness, and commonly isolated from poultry, and ColE1 plasmids contribute to the fitness of *Salmonella* Heidelberg in poultry litter. Investigations of a large, multistate outbreak in the United States in 2011 indicated that poultry litter (PL) as an important extra-intestinal environment that may have selected for specific S. Heidelberg strains. In this study, ARS researchers performed a series of controlled laboratory experiments to assess the evolution of two *S.* Heidelberg strains (SH-2813 and SH-116) in PL that was previously used to raise three flocks of broiler chickens. Whole genome sequencing was performed on 86 isolates recovered after 0, 1, 7, and 14 days of evolution in PL. Only strains carrying an IncX1 (37kb), 2 ColE1 (4 and 6kb), and 1 ColpVC (2kb) plasmids survived more than 7 days in PL. Competition experiments showed that acquisition of these plasmids from the commensal poultry litter flora resulted in healthier chickens and was associated with an increased copy number of IncX1 and ColE1 plasmids.

Resistome analysis of dairy calves and lactating dairy cows

NP 108: Food Safety
Location: Beltsville, MD

The role of dairy cattle in the transmission of antibiotic resistant bacteria (ARB) to humans is unclear. However, they are reservoirs of ARB and further dairy calves harbor more ARB than adult cows. ARS scientists sequenced the genomes of bacteria (metagenome) found in the feces of preweaned dairy calves and lactating dairy cows from 12 farms. Results indicated that feces of preweaned dairy calves have a significantly different bacterial community population than lactating cows. Similarly, the resistomes (the totality of antibiotic resistance genes in the bacteria) were significantly different between the adults and calves. The relative abundance of antibiotic resistance genes was highest in feces from the younger animals. Results of this analysis support the observation

that dairy animals are colonized with antimicrobial-resistant bacteria at a very young age and indicates that more information is needed to determine the factors that affect this early colonization in order to determine mitigation approaches that dairy operations could use to potentially decrease the abundance of antibiotic resistance in these animals.

Defined collateral effects of the in-feed antibiotic, carbadox, on the total gut bacterial community

NP 108: Food Safety
Location: Ames, IA

Carbadox is an antibiotic not used in humans but widely used in U.S. pig farms. It is important to study possible side-effects of carbadox use because it has been shown to promote bacterial evolution, which could indirectly impact antibiotic resistance in bacteria of clinical importance. Carbadox can induce phages in swine gut bacteria and *Salmonella enterica* serovar Typhimurium, a foodborne pathogen. Phages are viruses that infect bacteria and are important because they kill bacteria and they transfer genetic material between cells. ARS researchers fed carbadox to nursery-aged swine to characterize its impact on the swine gut microbiota, particularly phages. Two days after feeding, gut bacteria in the carbadox-fed pigs expressed different genes than the gut bacteria in the nonmedicated pigs. The differences indicated that the gut bacteria in the carbadox-fed pigs were not multiplying or metabolizing carbohydrates as they normally would, and that phages were being induced in the gut microbiota. In addition, phage genetic material encoded antibiotic resistance genes that could provide resistance to antibiotics that are important in human medicine, indicating that human-relevant antibiotic resistance genes are mobile between bacteria via phages. This research highlights the collateral effects of antibiotics and demonstrates the need for scientists, policymakers, regulators, and farmers to consider diverse antibiotic effects whenever antibiotics are being used or new regulations are considered.

Plant-based compounds reduce foodborne *Campylobacter* or *Salmonella* on poultry meat and eggs.

NP108: Food Safety
Location(s): Fayetteville, AR

Investigations into alternative to antibiotic strategies to improve postharvest food safety and shelf life of poultry products show that plant-based, compounds (eg. carvacrol, caprylic acid, eugenol, Beta-resorcylic acid, trans-cinnamaldehyde) and probiotic cultures are very effective in reducing *Campylobacter* or *Salmonella* on poultry meat and eggs. They also found that edible coatings such as chitosan, gum Arabic or pectin fortified with phytochemicals consistently reduced *C. jejuni*. These plant phytochemicals can potentially provide the poultry industry (both conventional and organic) with economical, effective control strategies for effective control of Campylobacter at various stages of poultry production and processing. Additional research is needed to determine mechanisms of action and to identify compound delivery options for industry applications.

Reduction of *Salmonella* and *E. coli* in beef cattle using sodium chlorate as an alternative to antibiotics.

NP108: Food Safety
Location: College Station, TX

Researchers have developed a novel treatment strategy employing sodium chlorate to rid pathogenic and antimicrobial resistant *E. coli* and *Salmonella* in cattle, swine and poultry. Trials to date support the potential for this feed additive to reduce unwanted bacterial populations. Full realization of these technologies will deliver to livestock producers a viable antibiotic alternative technology that provides an opportunity for producers to reduce pathogens and potentially recoup the costs by improvements in the animals' gross energy utilization. Research is underway to optimize delivery mechanisms and to evaluate the efficacy for reduction of *Salmonella* in lymph nodes, a critical need for the beef and swine industries.

Analysis of virulence and antibiotic resistance mechanisms of *Salmonella* and development of intervention strategies

NP 108: Food Safety
Location: Ames, IA

Antibiotic exposure can induce the expression of disease-associated genes in multidrug-resistant (MDR) *Salmonella*. Chlortetracycline and florfenicol are antibiotics commonly used in veterinary medicine for respiratory and gastrointestinal infections, but the dose used to combat infections does not eliminate many MDR *Salmonella* isolates. ARS scientists found that exposure of MDR *Salmonella* isolates to either chlortetracycline or florfenicol changed the expression levels of more than 50 percent of *Salmonella* genes, including genes involved in the ability of *Salmonella* to move, invade host cells, and survive and replicate inside host cells. Some *Salmonella* do not cause clinical symptoms or disease in poultry, swine, or cattle thus food animals can be unknowingly colonized with MDR *Salmonella*. Treatment of an unrelated disease can expose the colonizing MDR *Salmonella* to sub-inhibitory concentrations of the antibiotic, thereby potentially enhancing expression of *Salmonella* virulence genes and prolonging host colonization and fecal shedding of this human foodborne pathogen. Understanding the potential collateral effects of antibiotics on *Salmonella* will provide information to practitioners to help limit the negative consequences of antibiotic therapy.

Clusters of antibiotic resistance genes enriched together, stay together in swine agriculture

NP 108: Food Safety
Location: Ames, IA

Tracking the source of antibiotic resistance is a complicated issue because antibiotic usage and resistance is widespread. This widespread distribution is due to the location of resistance genes in the deoxyribonucleic acid (DNA) of bacteria on mobile genetic elements, which are portions of DNA that are transferred between bacteria as a method to share their antibiotic resistance genes with their bacterial neighbors. ARS researchers in collaboration with Michigan State University examined the associations between antibiotic resistance genes and mobile genetic elements in fecal and manure samples from Chinese swine farms and United States research swine. Many resistance genes and mobile genetic elements were found enriched together, meaning that when one gene increased or decreased in abundance, partner genes increased or decreased in nearly identical fashion. These findings will help veterinarians and producers understand the risks associated with antibiotic selection and develop best practices for prudent agricultural antibiotic use.

Phytochemical nanoemulsions reduce *Campylobacter jejuni* colonization in broiler chickens

NP 108: Food Safety
Location: Fayetteville, AR

Together, *C. jejuni* and *Salmonella* cause over 2 million annual illnesses, largely due to the consumption of contaminated poultry products. A reduction in gut colonization directly translates into reduced carcass contamination with *Campylobacter jejuni* and *Salmonella* spp. and reduced human infections. Studies by ARS showed that phytochemical nanoemulsions reduce *C. jejuni* colonization in broiler chickens. Potential mechanisms of action of phytochemicals are being assessed by studying their effect on gut microbiome, pathogen proteome, and pathophysiology. The result of this project will be safe, effective, and easy to use antibiotic alternatives to reduce the gut colonization of these two poultry-associated foodborne pathogens. This fundamental mechanistic analysis has shed light on the effect of phytochemicals on *Campylobacter* proteome response, poultry gut health, and microbiome profiles.

Genetic selection for resistance to foodborne pathogens in poultry

NP 108: Food Safety
Location: College Station, TX

Breeding chickens that are resistant to *Salmonella* and *Campylobacter* infection is considered to be a potential long-term intervention in controlling these bacteria in broiler chicken production. ARS scientists developed an innovative selection strategy based on a phenotype of inherently higher pro-inflammatory mediators that showed the profile of the sire was passed onto progeny. This approach is very different from other selection strategies that are seeking to improve resistance against single pathogens. A population of sires and dams from two lines of broiler chickens have been screened, and individuals with naturally high and low levels of key immune markers (IL6, CXCLi2, and CCLi2) have been identified. Selection based on pro-inflammatory mediators could be valuable in light of stricter regulations with respect to antibiotic use and may provide the poultry industry with a viable option for selecting animals with improved robustness, livability, and resistance against a broad range of poultry and foodborne pathogens.

Transmission of antibiotic resistance within biofilm

NP 108: Food Safety
Location: Wyndmoor, PA

Antibiotic resistant pathogenic bacteria pose serious public health concerns and increase the burden of disease treatment and the use of antibiotics as veterinary drugs and growth promoters in food animals has the potential to increase the incidence of antibiotic resistant pathogens in foods. Furthermore, the complex microbial communities (biofilms) found in foods and food processing environments provide a physical barrier that can protect bacteria from a variety of stresses, including antibiotics, and may provide conditions that promote for the spread of genes encoding antibiotic resistance. To better understand the contribution of gene transfer in biofilms to antibiotic resistance in foodborne pathogens, ARS researchers examined the mobility of plasmid-associated antibiotic resistance genes between pathogens in biofilms. They found that many of the plasmids tested were capable of transferring antibiotic resistance to other bacteria within biofilms at equal or greater frequency compared with experimental controls on agar plates. This research enhances our understanding of resistance plasmid transfer within biofilms and may ultimately lead to better control against the spread of antibiotic resistant bacteria and genes.

Irradiation inactivation of antibiotic-resistant bacteria

NP 108: Food Safety
Location: Wyndmoor, PA

Irradiation effectively reduces foodborne pathogens and spoilage microorganisms on fresh produce. However, limited research is available regarding its effect on antibiotic-resistant bacteria, or on the genes that convey that resistance. In collaboration with Virginia Tech University, ARS scientists inoculated lettuce leaves with a compost slurry containing multidrug-resistant *Escherichia coli* O157:H7 and *Pseudomonas aeruginosa*. The lettuce was washed with a mild sanitizer, irradiated, and stored for 14 days at 4°C. A 1.0-kGy dose of irradiation reduced both pathogens by 99.9 percent, and no significant regrowth was observed during storage. No differences in abundance or distribution of the antibiotic resistance genes were observed between irradiated and control lettuce over time. These results show that irradiation effectively reduces antibiotic-resistant bacteria on romaine lettuce. This information can be used to help design protocols to irradiate fresh and fresh-cut produce, and thereby improve food safety.

Environmental Antimicrobial Resistance

Antibiotic resistance is more common in natural prairies than in organic farming operations

NP 212: Soil and Air
Location: Lincoln, NE

Bacteria that are resistant to antibiotics are found throughout the natural world, even in areas where no antibiotics have been used before. The use of antibiotics in modern agricultural production is a concern for the public and research community alike because it is thought that irresponsible antibiotic use may lead to the development of antibiotic-resistant microorganisms that could affect human health. ARS researchers found that antibiotic resistance to two classes of antibiotics was detected at high levels in 13 Nebraska organic farming operations. Most of the resistance genes were detected more frequently in nearby natural prairie soils than in soils on the organic farms. This information suggests that farming practices that use manure do not lead to an increase in long-term antibiotic resistance in the soil and will inform and support U.S. policy positions for international trade negotiations around antibiotic use in U.S. agricultural products.

Antibiotic resistance genes and antibiotics can be transported off agricultural fields in subsurface drainage water

NP 212: Soil and Air
Location: Ames, IA

Antibiotic resistance is an increasing medical problem, and the effect of agricultural use of antibiotics on resistance in human pathogens is not clear. Previous reports have indicated elevated levels of antibiotic resistance genes (ARGs) in surface water and groundwater around confined animal feeding operations that administer antimicrobials, but little is known about how their transport from tile-drained fields receiving swine manure affects downstream environments. ARS scientists and collaborators from Iowa State Universityfound higher levels of two genes that confer resistance to antibiotics. Approximately 840,000 swine are raised within the watershed. A companion study also documented the presence of two veterinary antibiotics. The study provides new information establishing that antibiotic resistance genes and antibiotics are being transported off farmed fields in drainage water. These findings provide new science-based information to improve our understanding of this problem.

Development of a cost-effective treatment process to mitigate excessive loading of soils with wastewater from a concentrated animal feeding operation containing antibiotics

NP 212: Soil and Air
Location: Clay Center, NE

Wastewater was collected from feedlot runoff facilities. Portions of the wastewater were spiked with various antibiotics and adjusted to different pH levels and temperatures to determine the effect of environmental conditions on the process. Next, diatomaceous earth (DE), a naturally occurring sedimentary mineral, was added to the spike wastewater samples, mixed, and allowed to bind to the dissolved antibiotics. After binding occurred, the bound material was allowed to settle, and the wastewater without antimicrobials was decanted off. Raw DE containing clay and organic matter effectively removed several antimicrobials from the wastewater. Chemically pretreating the wastewater using the flocculent alum removed many suspended particles and adjusted the pH to optimum levels for the antibiotic binding process. Also, treating the DE with hydrogen peroxide to remove the organic matter coating of the mineral particles improved binding and subsequent settling for removal. This treatment process could help reduce or eliminate the release of excreted antimicrobials used during livestock production. It is anticipated that reducing loading of antimicrobials to the environmental will limit the impact on resistance development. Additionally, these processes have application to treat municipal wastewaters.

Impact of biotic vs abiotic fertilizer on antibiotic resistance in soil: role of manure

NP 212: Soil and Air
Location: Bowling Green, KY

A field experiment consisting of poultry litter, commercial fertilizer and control plots was designed in corn plantation and the impact was monitored during the entire growing season. A total of 576 soil samples collected at six time points were cultured for enumeration and detection of *Escherichia coli* and *Enterococci* resistance against third-generation cephalosporins, tetracyclines, and macrolides. Shotgun sequencing was performed on 72 pooled metagenomic DNA samples from baseline, 7- and 28- days after treatment to determine the total microbiome and resistome. Concentrations of total and tetracycline-resistant *E. coli* and tetracycline-resistant enterococci were significantly increased to levels higher than commercial fertilizer or untreated plots following poultry litter application. Two months after fertilizer application, levels returned to baseline with no significant differences from the commercial fertilizer or untreated plot. The prevalence of third-generation cephalosporin-resistant *E. coli* and erythromycin-resistant enterococci also increased on Days 7 and 28 following fertilization, and subsequently returned to baseline. Total microbiome and resistome populations were significantly enriched in the poultry litter–amended soils on 7 and 28 days following application compared with those in commercial fertilizer or untreated control plots. Data generated from this project are useful in quantitative microbial risk analyses and to guide policy decisions for the management of animal manure as soil amendment.

Pathogenic and antimicrobial-resistant *Escherichia coli* in surface water

NP 108: Food Safety
Location: Athens, GA

Surface waters are important sources of water for drinking, industrial, agricultural, and recreational uses; hence, contamination of water by fecal bacteria, such as *Escherichia coli*, is a environmental and public health concern. Very little data is available on pathogenic or antimicrobial resistant *E. coli* in surface water. To address this, ARS researchers collected water samples quarterly from 2015 to 2017 from the Upper Oconee River Watershed, in

Athens, Georgia. *Escherichia coli* counts to assess the water quality were occasionally above the threshold established by the Environmental Protection Agency for recreational water. Pathogenic *E. coli* were detected in the water including those that cause diarrhea in humans. Antimicrobial-resistant and multidrug-resistant *E. coli* were also detected. Results from this study demonstrated that *E. coli* is prevalent in high levels in the Upper Oconee Watershed. The study emphasizes the role of environmental water as a potential reservoir of resistant and pathogenic *E. coli* that may be pose a risk.

Novel Antibiotics/Antifungals

Overcoming antibiotic resistance using a novel antibiotic modified to have reduced toxicity

NP 306: Product Quality and New Uses
Location: Peoria, IL

Beta-lactam antibiotics are a class of broad-spectrum (i.e., effective against a large variety of organisms) antimicrobials, which include penicillin derivatives and cephalosporins. The use of these important drugs has been limited over the years with the development of antibiotic resistant bacterial strains. Tunicamycin is a powerful antibiotic that can be combined with beta-lactam antibiotics to overcome antibiotic resistance. Scientists have known about this antibiotic for decades, but toxicity in human and animal cells prevented it from being used for therapeutic application. ARS researchers chemically modified tunicamycin into less harmful derivatives. The modified tunicamycins did not show any toxicity to human and hamster cells but were still capable of increasing the efficacy of clinical penicillin-based drugs by 32 to 64 times. This significant discovery now allows older-type antibiotics to once again be effective and is an important step toward combating drug resistance.

Optimization of novel antibacterial oil production from biomass

NP 306: Product Quality and New Uses
Location: Peoria, IL

Liamocins are novel antibacterial oils produced by the fungus *Aureobasidium pullulans*, with specificity for *Streptococcus* spp., that cause important infections of swine and dairy cows. Recently, it was shown that *A. pullulans* produces liamocins when grown on a variety of sugars and polyols; however, production from agricultural biomass substrates had not previously been reported. Among five biomass substrates tested, pretreated wheat straw appeared to be promising as an abundant, low-value agricultural byproduct. Twenty-seven strains from phylogenetic clades 8, 9, and 11 were surveyed for production of liamocins from wheat straw and sucrose. Liamocin yields were highest from strains in clade 11, and higher from cultures grown on sucrose than wheat straw. However, when supplementary enzymes were added to the fermentation, liamocin production on wheat straw was equivalent to that on sucrose. Furthermore, scientists determined that liamocins produced from wheat straw were under-acetylated, resulting in higher levels of liamocin species with the highest activity against *Streptococcus*. Production from such low-cost substrates might be particularly appropriate for bulk agricultural applications, such as in dairy cattle dips for prevention of mastitis caused by streptococcal infections.

Alternative to antibiotics for control of fuel ethanol contaminants

NP 306: Product Quality and New Uses
Location: Peoria, IL

Most fuel ethanol facilities use baker's yeast to ferment sugars from agricultural products to alcohol. Contamination in large-scale production plants is unavoidable, so efforts usually focus on controlling levels of the naturally occurring bacteria that compete for the same sugars that are used to produce ethanol and they often synthesize byproducts that inhibit the ability of the yeast to grow. Chronic and acute contamination problems significantly reduce the economic viability of the U.S. fuel ethanol industry. Whereas antibiotics can be used to control the contamination, the overuse of antibiotics to combat these infections has led to antimicrobial resistance and the presence of antibiotic residues in fuel ethanol co-products. ARS scientists developed an alternative method to control contamination using beneficial bacteria similar to probiotics. These bacteria are able coexist with yeast and inhibit the detrimental effects of contaminating strains. These findings will allow ethanol producers to improve the efficiency of their fermentation and reduce the need for use of antibiotics in their plants.

Making size-controlled silver nanoparticles for antibacterial cotton

NP 306: Product Quality and New Uses
Location: New Orleans, LA

The biocidal performance of silver nanoparticles is strongly dependent on particle size (i.e., the smaller the size, the greater the antibacterial activity); however, they easily aggregate into large particles, which negates the antibacterial efficacy associated with the nanoscopic dimension. Because a critical need exists to develop methods that control the synthesis of nanoparticles, ARS scientists developed an easy, low-cost, and controllable water-based method using low concentrations of polyethylene glycol and ethylene glycol. The nanoparticles that resulted were smaller than those produced via a polyol process in a similar condition. Their minimum inhibitory concentrations (MIC) against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus enterica*, and *Escherichia coli* (4.7, 2.3, 2.3, and 1.2 µg/mL, respectively) were smaller than those described in other studies, confirming the particle size effect. The reduced MICs obtained by controlling the particle size demonstrated that silver nanoparticles are suitable as a targeted disinfecting tool for healthcare and food packaging nonwoven cotton products.

Dry-tolerant antifungal biological control strains as biorefinery products to protect postharvest potatoes

NP 306: Product Quality and New Uses
Location: Peoria, IL

More than 80 percent of fungal strains that cause potato dry rot are now resistant to thiabendazole and there is growing pressure to develop non-azole alternatives. However, chemical substitutes are limited, especially for use on postharvest potatoes destined for food use, so ARS scientists developed bacterial strains that are antifungal biocontrol agents against dry rot, late blight, and pink rot. The strains also reduce sprouting. The new bacteria strains can be grown using switchgrass hydrolyzate and show excellent survival during dry-storage conditions. Production of a broad-spectrum antifungal agent on low-cost renewable substrates and improved dry storage formulations of the biocontrol product are expected to lower costs and expedite application by growers. This new

technology benefits agriculture by providing an antifungal microbial alternative to azole chemicals and by serving as a potential co-product of a renewable lignocellulose biorefinery with the effect of boosting economic feasibility.

Antimicrobial Risk Assessment

Microbial risk assessment related to agricultural operations

NP 212: Soil and Air
Location: Marshfield, WI

Quantitative microbial risk assessment (QMRA) is a method that can predict human exposure to gastrointestinal pathogens via environmental routes, but QMRA predictions have not been validated for many pathogens. ARS researchers compared QMRA predictions to epidemiological measurements collected during outbreaks of waterborne gastrointestinal disease and confirmed that QMRA can reliably estimate human disease rates due to waterborne gastrointestinal pathogens. Policymakers and agricultural engineers can use QMRA to accurately predict the health burden of pathogen exposure, including exposure to pathogens that originate on livestock farms and pollute ground and surface water during manure disposal. Such predictions of health burdens are crucial to evaluating public health and environmental policies related to agriculture.

ADDITIONAL RESOURCES

- Search Engine for all ARS publications related to AMR and ATA- https://www.nal.usda.gov/biblio/antibiotics
- https://www.ars.usda.gov/nutrition-food-safetyquality/food-safety-animal-and-plant-products/docs/antimicrobial-resistance-amr/
- https://www.ars.usda.gov/anrds/agar/agar-home/
- https://www.ars.usda.gov/alternativestoantibiotics/

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