



Antimicrobial Resistance (AMR) and Alternatives to Antibiotics (ATA) Accomplishment Summary 2019-2021



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Introduction

Animal Production and Protection



Crop Production and Protection



Natural Resources and Sustainable Agricultural Systems



Nutrition, Food Safety, and Quality



Locations

Pacific West Area

Albany, CA
Riverside, CA
Corvallis, OR
Hageman, ID

Plains Area

Clay Center, NE
Lincoln, NE
Manhattan, KS
College Station, TX

Midwest Area

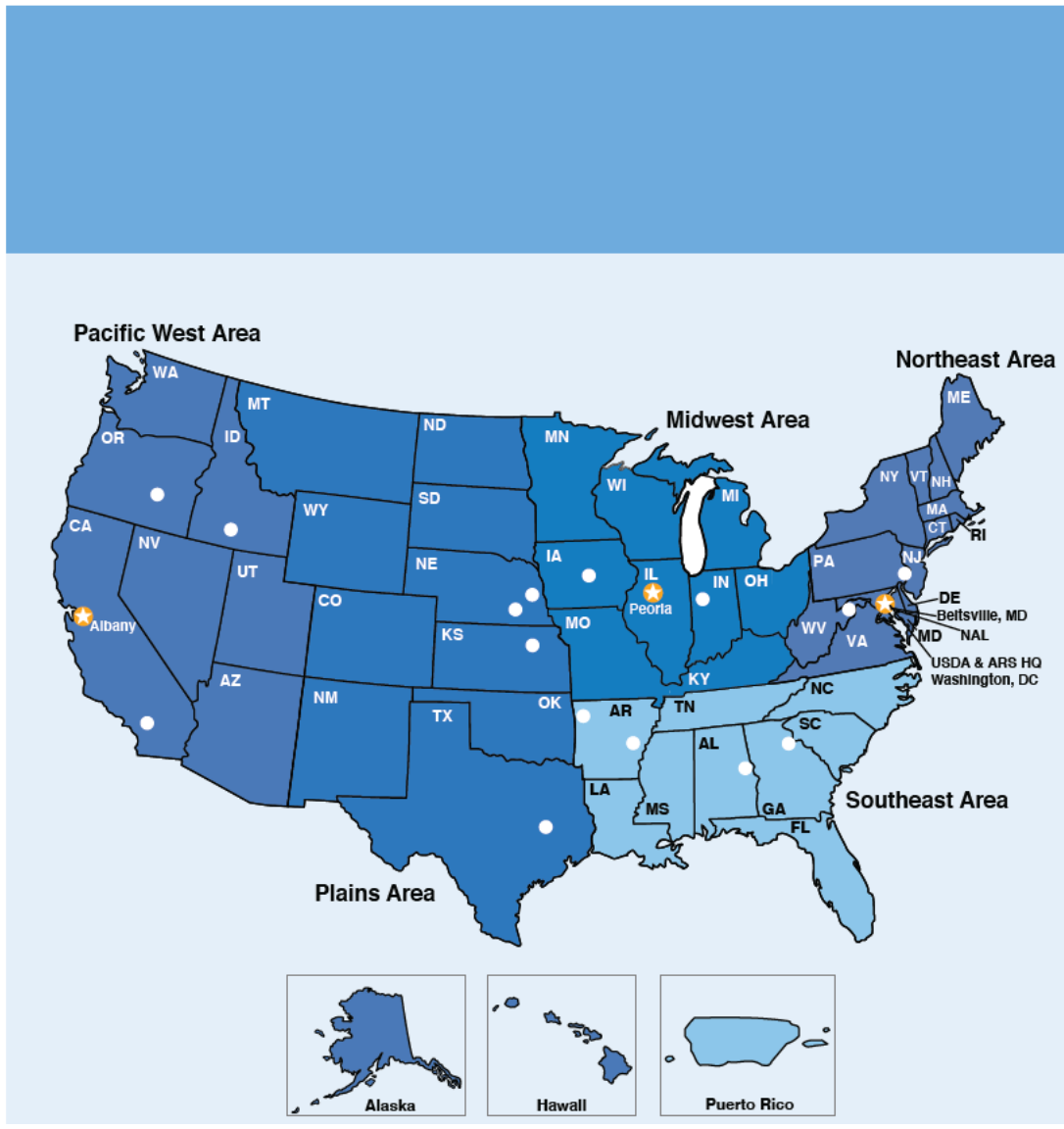
Ames, IA
Peoria, IL
West Lafayette, IN

Southeast Area

Athens, GA
Auburn, AL
Fayetteville, AR
Stuttgart, AR

Northeast Area

Beltsville, MD
Wyndmoor, PA
Leetown, WV





Antimicrobial resistance (AMR) occurs naturally in bacteria and is a serious threat to animal, plant, environmental, and public health. AMR is a complicated issue and there are many factors that contribute to its development in agricultural environments. As the intramural research arm for USDA, the Agricultural Research Service (ARS) has the mission to develop and transfer solutions to agricultural problems of high national priority. ARS scientists conduct research using a One Health approach (animal, plant, environment) that helps explain the different factors associated with AMR in agricultural settings and to develop tools that mitigate AMR and improve animal, plant, environmental, and public health. The research spans the entire spectrum of agricultural production and food safety including developing new antibiotics and antibiotic alternatives and understanding factors leading to AMR in agricultural environments and in foodborne bacteria.

ARS AMR Vision

Provide global leadership for innovative, equitable, and sustainable research solutions for AMR in agriculture

ARS AMR Mission

Promote agricultural resilience to AMR and protect the health and safety of animals, plants, environment, and the public through cutting-edge research solutions and outreach

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By the Numbers

➤ **268 AMR publications**

What to Know More?

- [Alternatives to Antibiotics Resource Center | Animal Health Care \(usda.gov\)](#)
- [AMR: USDA ARS](#)
- Action Plans
 - [Animal Health](#)
 - [Animal Production](#)
 - [Food Safety](#)
 - [Plant Diseases](#)
 - [Soil and Air](#)
- [United States Department of Agriculture Antimicrobial Resistance Action Plan \(usda.gov\)](#)

Highlights

Critical Antimicrobial Resistance

Albany, CA: Colistin is a “last-resort” antibiotic used to treat bacterial infections in humans that cannot be treated with other antibiotics, but a gene has recently been discovered that increases AMR resistance to colistin. ARS scientists found that the prevalence of this gene in meat and poultry processing facilities is very low. This finding confirms that colistin will remain effective in treating bacterial infections in humans and highlights the safety of the U.S. food supply.

Athens, GA: ARS scientists described the expansion and persistence of large segments of DNA called megaplasmids responsible for antimicrobial and metal resistance in *Salmonella infantis*, one of the most prevalent foodborne pathogens causing human illness.

Clay Center, NE: ARS researchers developed a quantitative risk assessment which determined that people who consume ground beef from conventionally raised cattle compared to non-antibiotic fed cattle are not more likely to be exposed to antibiotic-resistant bacteria.

Alternatives to Antibiotics

Fayetteville, AR: ARS researchers developed a safe and effective strategy for controlling *Salmonella* and *C. jejuni* biofilms by combining the antimicrobial efficacy of Generally Recognized as Safe (GRAS) status phytochemicals from plants with nanotechnology to develop natural disinfectants with significant antibiofilm efficacy against *Salmonella* and *C. jejuni*.

Wyndmoor, PA: ARS scientists showed that an inexpensive powder called ZnO powder can be used in food processing environments to significantly reduce *Salmonella* and *Campylobacter* levels.

Corvallis, OR: ARS researchers found that pyroligneous acid, which is produced by heating plant biomass at high levels in no-oxygen environments, prevents the spread of *Verticillium dahlia*, a pathogen that infects more than 400 crops and causes significant crop losses.

Albany, CA, and Ames, IA: ARS scientists developed an ointment that was effective for digital dermatitis, a ruminant disease that often requires antibiotic therapy.

Auburn, AL: ARS researchers developed a vaccine for hybrid catfish against virulent *Aeromonas hydrophila*, a bacterium that causes serious infections and foodborne illness.

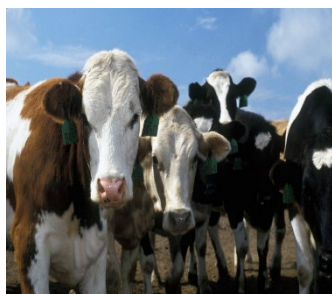
Detection and Mitigation Tools

Athens, GA: ARS scientists and colleagues developed two new genomic pipelines to rapidly process and annotate whole genome sequences from foodborne pathogens. AMRFinderPlus will be used worldwide to identify AMR, stress response, and virulence genes in whole genome sequences of foodborne pathogens. Reads2Resistome performs genome assembly and in-depth genome characterization of pathogen whole genome sequences.

Animal Production and Protection Research



Animal Production and Protection (APP) research improves the health, well-being, and production efficiency of livestock, poultry, and aquatic food animals and ensures an ample and safe food supply. APP research programs deliver scientific information and tools U.S. food animal industries need to meet U.S. consumer demands for appealing and nutritional animal products, compete successfully in worldwide trade, and contribute toward global food security. AMR research in APP is focused on developing alternatives to antibiotics (ATA) that can prevent and mitigate diseases which often need to be treated with antibiotics, and developing new therapeutics that do not inadvertently support the survival, spread, and evolution of antibiotic resistant bacteria. This ARS research gives producers valuable support in promoting animal health and optimizing antibiotic use.



Aquaculture

Using organic acids and essential oils in rainbow trout and hybrid striped bass feeds to reduce antibiotic use

Stuttgart, AR, and Hageman, ID

ARS researchers and collaborators at the Bozeman Fish Technology Center in Montana conducted feeding trials with hybrid striped bass and rainbow trout to test the efficacy of these feed additives on fish performance and health. Their results indicate that both organic acids and essential oils improve growth rates and feed efficiency in rainbow trout.

Yersinia ruckeri flagellin as vaccine support in aquaculture

Leetown, WV

Infectious diseases are the chief cause of production loss and antibiotic use in aquaculture. ARS scientists evaluated using *Yersinia ruckeri* (*Yr*) flagellin as an adjuvant to stimulate the immune response generated by vaccines. They found a mutant *Yr* strain simplified the purification of flagellin needed to enhance vaccine immune responses and could become a cost-effective adjuvant in aquacultural vaccine production.

Mechanisms of virulence and novel control measures for virulent *Aeromonas hydrophila*
Auburn, AL

Virulent *Aeromonas hydrophila* (vAh) is a major pathogen of farm-raised catfish. ARS scientists and Auburn University collaborators conducted research to better understand mechanisms of vAh virulence in channel catfish and developed a more robust and reproducible challenge model that in turn advanced developing and evaluating a new and effective immersion vaccine for hybrid catfish.

Ruminants

New Reversible Biocides for Digital Dermatitis Treatment

Ames, IA, and Albany, CA

Digital dermatitis is the leading cause of lameness in dairy cattle; a major economic and welfare problem, it is often treated with topical antibiotics in footbaths, prompting concerns of environmental contamination with antibiotics. ARS scientists developed “reversible” biocides that work as an antibiotic at high concentrations but that become inactive when diluted in water. They also developed an ointment that is effective against digital dermatitis and studies are continuing to support future commercialization.

The role of house flies in harboring and disseminating antimicrobial-resistant bacteria at beef feedlots

Manhattan, KS

Cattle given antibiotics to prevent or control bacterial infections can inadvertently support the evolution of antimicrobial resistant (AMR) bacteria that are shed in manure. ARS scientists found that house flies using feedlot manure as a food source and breeding habitat carried AMR bacteria, indicating that flies may serve as a bridge between AMR bacteria sources and animal feed and water. They also found that female house flies carried a significantly higher load of AMR bacteria than male flies. These results strongly suggest that house fly control should be implemented to reduce AMR bacterial dissemination at confined cattle operations.

Therapeutic Use of Antimicrobial Proteins in Bovine Bacterial Infections

Ames, IA

White blood cells in cattle produce small antimicrobial proteins that kill bacteria. ARS researchers studied the antimicrobial activity *in vitro* of synthetic analogs of small antimicrobial proteins on bacterial pathogens associated with bovine respiratory diseases (BRDC), mastitis, and chronic enteritis (Johne’s disease). One of the small antimicrobial proteins was highly effective in killing respiratory pathogens such as *Mannheimia haemolytica* and Johne’s disease pathogen *Mycobacterium avium subspecies paratuberculosis*. Follow-up animal model studies will determine whether additional modifications to these proteins will preserve their antimicrobial activity against BRDC pathogens.

Supplementing dairy calves with L-glutamine to reduce antibiotic use

West Lafayette, IN

The primary cause of pre-weaning mortality in dairy calves is enteric disease. This is associated in part with separating dams from newborn dairy calves, which creates stress that can harm a calf's intestinal development. ARS scientists found that giving newborn dairy calves supplements of the essential amino acid L-glutamine improved their intestinal health and also improved subsequent growth performance and disease resistance. These findings support the development of alternative solutions to using therapeutic antibiotics in animal agriculture to treat enteric diseases.

Poultry

Interactome Approach to Improve Growth Performance without Antibiotic Growth Promoters (AGP) and Understanding of AGP mechanisms

Beltsville, MD

ARS scientists identified changes in intestinal metabolite levels in chickens fed a diet supplemented with different strains of *B. subtilis*, a bacterium that may have health benefits. Changes in the levels of the intestinal biochemicals provided a distinctive biochemical signature unique to each *B. subtilis*-supplemented group. These results provide the foundation for future studies to identify biochemicals that might be used to improve poultry growth performance without antibiotics.

Plant-based supplements that promote growth and enhance innate immunity

Beltsville, MD

ARS scientists and colleagues at South Korea's Rural Development Administration studied the benefits of supplementing broiler chicken diets with *Allium hookeri* (AH). They found young broiler chickens that received optimum levels of AH supplements exhibited improved growth and antioxidant responses.

Identification of vaccine candidates against necrotic enteritis

Beltsville, MD

Necrotic enteritis (NE) caused by the bacterium *C. perfringens* (CP) costs the global poultry industry more than \$6 billion in losses annually. ARS scientists identified four recombinant CP proteins as vaccine candidates and used them to develop a single experimental vaccine that was highly effective in protecting broiler chickens from NE infection.

Swine

How stress affects antibiotics in pigs

West Lafayette, IN

Pigs that are being weaned or transported are sometimes given antibiotics to reduce the effects of stress on their intestinal health. ARS researchers found intestinal health improvements in pigs that were given stress hormone blockers were similar to the intestinal health improvements in

pigs that were given antibiotics. These findings suggest that stress hormone blockers could be an ATA in reducing stress-related issues in some pigs.

Effect of antibiotics on the development of *Streptococcus suis* biofilms

Ames, IA

Streptococcus suis (*S. suis*) is the leading bacterial swine pathogen worldwide and sometimes infections are lethal. ARS researchers found that low levels of some antibiotics commonly used in swine production support the development of *S. suis* biofilms, which then can potentially increase the pathogen's survival and persistence within the swine respiratory tract and between animals. These findings highlight the need for appropriate antibiotic use in swine production for disease prevention and transmission.

Characterizing fungi in the porcine mycobiome

Beltsville, MD

The gastrointestinal tract (GI) microbiome in animals is a critical component of host health. ARS scientists performed the first in-depth analysis of fungal populations, or mycobiome, in the piglet GI tract and found a dynamic shift in fungal populations at the time of weaning, including the dominance of the fungus *Kazachstania slooffiae* in weaned piglets. These findings could support developing interventions and dietary modifications to enhance piglet performance and health, developing alternatives to antibiotics, and increasing swine herd productivity.

Crop Production and Protection Research

Crop Production and Protection research delivers science-based information, genetic resources, and technologies for increasing crop productivity, improving economically and environmentally sustainable methods of crop production, and protecting crops from diseases and pests.



Developing biological controls to mitigate fungicide use in row crops

Lincoln, NE

There are concerns that using antifungal compounds in crop production may contribute to the development of pathogenic *Aspergillus fumigatus* that is resistant to current fungal treatments. Biological control agents might be an alternative to the use of antifungal compounds. ARS scientists used next-generation, genome-wide sequencing of 17 *Pseudomonas* isolates to identify genes in bacterial strains isolated from drought-tolerant sorghum roots. They identified several genes that enhance biological control abilities, including genes associated with plant root colonization, biofilm formation, iron sequestration, plant hormone production, and chitin degradation. These results could help advance the development of biocontrol agents to mitigate fungal pathogens that are particularly insidious under drought conditions.

Tests for an antimicrobial protein combination to control of Fusarium head blight in wheat

Peoria, IL

Fusarium head blight is a serious fungal disease that can reduce wheat yields and produce mycotoxins that harm the health of humans and animals. ARS scientists found that two antimicrobial and generally regarded as safe (GRAS) compounds significantly reduced mycotoxin levels in wheat head compared to mycotoxin levels in the wheat head treated with only *Fusarium* spores. These results suggest that the antimicrobial GRAS compounds might be able to replace or augment synthetic chemicals used to combat *Fusarium* plant pathogens.

Natural Resources and Sustainable Agricultural Systems Research

Natural Resources and Sustainable Agricultural Systems research develops technologies and strategies that help farmers, ranchers, and other managers effectively steward the diverse agricultural mosaic spread across the Nation. Their studies run the spectrum of agroecosystem research, from livestock grazing expansive natural western rangelands to crops grown in the fertile Midwestern Heartland and Southern States to high-value produce cultivated in the coastal valleys and plains.





How Conservation Pasture Management Affects Antimicrobial Resistance Bacteria in Water and Soil Systems

Fayetteville, AR

AMR bacteria in soil and water is a significant public health concern. ARS scientists tracked the movement and fate of AMR bacteria in soil after broiler litter and cattle manure were applied as fertilizer. They found that long-term conservation pasture management practices—such as riparian buffer strips and rotational grazing and select animal manure inputs such as poultry litter—minimize the presence and abundance of AMR genes in grassland soils.

Identifying Novel Solutions that Inhibit Dispersal and Infectivity of Soilborne Pathogens (INSIDIOuS-Pathogens)

Corvallis, OR

New strategies and compounds are urgently needed to control soilborne fungal pathogens, including *Verticillium dahliae*, a destructive fungi that infects more than 400 crops. ARS scientists used laboratory assays to determine that pyroligneous acid, which is derived from plant biomass, prevents the proliferation of *V. dahliae* at several stages of its fungal lifecycle. They also found that its anti-fungal activity is limited to *V. dahliae* and *Fusarium oxysporum*, another fungus that can infect plants. This research suggests that pyroligneous acid has the potential to disrupt disease cycles without impacting beneficial fungi.

Dissemination of anthropogenically induced AMR in the agricultural environment

Riverside, CA

ARS scientists performed a container experiment to study spinach and radish uptake of antibiotics, antimicrobial resistance genes, and antibiotic resistant bacteria from soil irrigated with treated municipal wastewater. The wastewater contained the antibiotics sulfamethoxazole, sulfapyridine, and trimethoprim. The scientists observed that trimethoprim was taken up more readily than the other antibiotics and that in general, uptake levels in spinach were higher than uptake levels in radish.

Nutrition, Food Safety, and Quality Research



The ARS national programs in Nutrition, Food Safety, and Quality advance the maintenance of a healthy and safe food supply and improve the economic viability and competitiveness of U.S. agriculture by enhancing the quality and utilization of agricultural products for producers and consumers. AMR research is conducted to protect human health, identify novel technologies to prevent and/or treat resistance, understand factors that promote the development and spread of AMR, and identify solutions that reduce the impact of foodborne AMR, particularly in human pathogens carrying resistance to antimicrobials of critical concern to human health.



Beef and Dairy

Potential of non-antibiotic compound to inhibit *Escherichia coli* and *Salmonella* growth
College Station, TX

The emergence of antibiotic resistant bacteria in animal agriculture has raised concerns that infections caused by pathogenic bacteria will be more difficult to treat. ARS researchers established the potential of a non-antibiotic compound called methylsulfonylmethane (MSM), a commonly used anti-inflammatory agent, to inhibit the growth of multidrug resistant *Escherichia coli* and *Salmonella* isolated from cattle. MSM antibacterial activity may prove useful during pre- or post-harvest food processing as a disinfectant. These results provide livestock producers and food processors with a valuable antibiotic alternative.

Probiotic feeding reduces *Salmonella* in beef cattle
Clay Center, NE

Feedlot cattle purchased from auction barns are often at higher risk for disease and foodborne pathogen colonization due to stresses from weaning, transportation, and handling, all of which can affect the immune function of cattle. As a preventative measure, antimicrobials have been used to reduce disease, especially bovine respiratory disease. ARS researchers and West Texas

A&M University collaborators found that chromium propionate treatments improved white blood cell and immune function parameters and found that cattle treated with a *Bacillus* probiotic shed reduced levels of *Salmonella*. This report identifies a potential dietary approach for reducing the risk of human foodborne illness associated with beef consumption.

Exposure to antimicrobial-resistant bacteria among U.S. ground beef consumers

Clay Center, NE

Some U.S. consumers believe that raising cattle without the use of antibiotics reduces human exposure to antibiotic resistant bacteria in ground beef. ARS researchers and University of Nebraska-Lincoln collaborators performed a retail-to-fork quantitative exposure assessment for eight different antibiotic resistant bacteria and determined that typical consumer ground beef handling and cooking practices reduced the probability of consuming antibiotic resistant bacteria to less than 1.7 percent. This quantitative risk assessment determined that consuming ground beef from conventionally raised cattle does not increase the probability of exposure to antibiotic resistant bacteria.

Genomic features associated with antimicrobial-resistant *Escherichia coli* in veal calves

Beltsville, MD

Veal calves remain an understudied reservoir of antimicrobial resistance (AMR), and strategies to reduce AMR in the food chain are needed to provide a safer food product to the consumer. ARS scientists sequenced the genomes of more than 80 *Escherichia coli* isolates from veal calf feces and analyzed these data to identify all AMR genes and virulence factors (VFs). Since some VFs are involved in survival within the host, statistical analyses were conducted to determine if VFs are significantly associated with the multidrug-resistant (MDR) trait. The researchers found a strong significant association between the MDR trait and iron-scavenging genes and outer membrane proteins. These findings may be used to identify specific drug targets and management strategies that can be leveraged to reduce foodborne pathogens such as *S. enterica* in dairy animals.

Evaluating antimicrobial-resistant *Escherichia coli* populations as calves age

Beltsville, MD

Young calves on dairy farms carry the highest prevalence of antimicrobial-resistant (AMR) *Escherichia coli*. The early postnatal period is a critical time for colonization of the calf gut by resistant bacteria and weaning plays a key role in reducing resistance in the calf gut. ARS scientists and Pennsylvania State University collaborators evaluated how AMR *E. coli* populations change as calves age and found that about 90 percent of the samples from the pre-weaned calves contained AMR *E. coli*. The study informs scientists and industry of potential targets for mitigating the prevalence of AMR bacteria harbored in young dairy calves.

Witch hazel extract inhibits bacterial pathogens

Albany, CA

WHISOBAX (WH) is a witch hazel extract containing high levels of phenolic compounds that have antimicrobial properties. ARS researchers collaborated with Framingham State University and Marike Polytechnic University researchers and showed that both gram-positive and gram-

negative bacteria are sensitive to WH, including those that are notoriously resistant to antibiotic treatment, such as Methicillin-resistant *Staphylococcus aureus* (MRSA). Additionally, WH enhances the effect of commonly used antibiotics while preventing bacterial disease, which supports eradicating infections while reducing excessive antibiotic use. This discovery provides new options for improved treatment of bacterial infections.

Poultry

New alternatives to antibiotics in poultry production

College Station, TX

Commercial poultry can be colonized by a number of microorganisms that cause foodborne illness, including *Clostridium*, *Salmonella*, *Campylobacter*, and others, and this remains a serious challenge for producers and food processors. ARS scientists and university and industry partners found that chicks infected with *Clostridium* developed significantly less pathological tissue damage when they were fed a diet supplemented with a blend of organic acids and botanicals. This diet supplement can be a viable alternative to traditional antibiotics for ensuring bird health and can help protect them from harmful bacteria that can also cause foodborne illness in humans.

Development plant-based therapeutics with nanotechnology for controlling *Salmonella* and *Campylobacter jejuni*

Fayetteville, AR

Salmonella and *Campylobacter jejuni* (*C. jejuni*) are the leading foodborne bacterial pathogens in the United States and are responsible for more than 2 million illnesses annually. ARS researchers developed a safe and effective strategy for controlling *Salmonella* and *C. jejuni* biofilms by combining the antimicrobial efficacy of Generally Recognized as Safe (GRAS) status phytochemicals from plants with nanotechnology to develop natural disinfectants with significant antibiofilm efficacy against *Salmonella* and *C. jejuni*. Since *C. jejuni* can form biofilms in the processing environment and contaminate poultry products, phytochemicals could potentially be used to control biofilms and reduce the risk of human infections. This research can be used to develop safe and effective phytochemical-based disinfectants and application strategies for controlling pathogen persistence in biofilms commonly encountered in food processing facilities.

Dynamics between horizontal gene transfer and acquired antibiotic resistance in *S. Heidelberg* following in vitro incubation

Athens, GA

Salmonella enterica serovar Heidelberg is linked to foodborne illness and is one of the top five serovars isolated from poultry in the United States and Canada. ARS researchers used commercial broiler gastrointestinal (GI) tracts as a model to investigate antibiotic resistance genes that can be transferred *in vitro* from GI flora to *Salmonella enterica* serovar Heidelberg (*S. Heidelberg*). DNA segments (plasmids) producing AMR enzymes were transferrable between *Escherichia coli* and *S. Heidelberg* strains, but transfer was unsuccessful between *S. Heidelberg*

strains. This study contributes to our understanding of the dynamics of DNA transfer between an important foodborne pathogen and the chicken gut microbiome.

Reused litter reduces transfer of multidrug resistant plasmids from the broiler gut microbiota to *Salmonella* Heidelberg

Athens, GA

Horizontal gene transfer plays an important role in spreading antimicrobial resistance and virulence genes to foodborne bacterial pathogens. ARS researchers completed a 2-week *in vivo* broiler study evaluating the interactions between the broiler gut microbiome, litter age, and antimicrobial resistance acquisition in *Salmonella* Heidelberg. Broilers raised with re-used litter demonstrated a lower transfer of multidrug resistant plasmids from the broiler gut microbiome to inoculated *S. Heidelberg* strain than broilers raised on fresh litter. This study supports the notion that consecutive reuse of litter results in an “unfavorable” environment for *Escherichia coli* and *Salmonella* carrying multidrug resistant plasmids and limits their potential for transfer.

Beta-lactam resistant *Escherichia coli* in both humans and broiler chickens

Athens, GA

Beta-lactam resistant *Escherichia coli*, which infect both animals and humans, are resistant to many types of antibiotics. ARS researchers collected clinical samples from infected patients and healthy broiler chickens from Egypt and examined the samples for the presence of *E. coli*. They found that, although no single clone appeared to be circulating among the beta-lactam resistant *E. coli*, there were some shared characteristics among isolates from the two sources. Additional studies are needed to help researchers and regulatory personnel determine the role of humans and food animals in disseminating beta-lactam resistant *E. coli* and to develop effective antimicrobials intervention strategies.

Plasmid genes support *Salmonella* Infantis persistence

Athens, GA

Salmonella is a common cause of foodborne illness in the United States and is often transmitted to humans from contaminated poultry. The serotype *Salmonella* Infantis increased from 5 to 29 percent of the serotypes isolated from U.S. chicken samples and continues to be a problem. Most of these Infantis are resistant to antibiotics due to a specific gene carried by a large plasmid known as pESI (plasmid for Emerging *Salmonella* Infantis). ARS investigated the role of pESI in the expansion and persistence of Infantis in U.S. poultry by analyzing the DNA sequences of Infantis isolated from poultry and human infections. The specific gene was present in 61 percent of the plasmids, while more than 90 percent of the plasmids contained genes that could help Infantis colonize chickens and persist in poultry production. This information is useful to poultry producers for modifying practices to minimize the impact of the advantages that pESI gives Infantis, thus preventing the selection of antibiotic resistant Infantis and reducing its prevalence and persistence in chickens.

Salmonella sentinel for antibiotic resistant genes acquired by horizontal transfer under antimicrobial selective pressure

Ames, Iowa

Understanding how *Salmonella* in the poultry gastrointestinal (GI) tract acquires antimicrobial resistance (AMR) is key for understanding the role of horizontal gene transfer (HGT) from microbes in the GI tract to foodborne pathogens. ARS researchers evaluated the transfer of tetracycline and ampicillin resistance from the GI microbes to a susceptible *Salmonella enterica* serovar Heidelberg (*S. Heidelberg*) strain in young chicks inoculated shortly after hatch. Birds in three groups (Group 1, no antimicrobial treatment; Group 2, Bacitracin methylene disalicylate (BDM) treated; and Group 3, Zn treated) were inoculated with *S. Heidelberg*. AMR transfer was observed in the absence of antimicrobials, and neither BMD nor Zn appeared to impact AMR acquisition. These data suggest that both the eggshell and environment provide chicks with critical microbes. Similar AMR transfer studies provide a basis to slow pathogens from acquiring new AMR genes and identify the role of antibiotics in HGT *in vivo*.

Swine

Impact of in-feed versus injected antibiotics

Ames, Iowa

Antibiotics are an important tool for limiting disease in swine and are commonly delivered by oral administration via in-feed supplements. ARS researchers administered the antibiotic oxytetracycline orally and by intramuscular injection and then compared antibiotic levels in blood and feces; they also measured intestinal bacteria levels and antimicrobial resistance gene abundance. Compared to injection, oral dosing caused a greater shift in gut bacterial communities and increased the abundance of some antibiotic resistance genes. These findings suggest that injecting antibiotics may help reduce the development of antibiotic resistance in swine production.

Effect of chlortetracycline on *Salmonella*

Ames, IA

The antibiotic chlortetracycline is commonly used in veterinary medicine for treating respiratory and gastrointestinal infections. Many multidrug-resistant (MDR) *Salmonella* isolates are resistant to chlortetracycline, and chlortetracycline treatment for an infection may have unintended consequences in an animal unknowingly colonized with MDR *Salmonella*. ARS scientists found that pigs exposed to MDR *Salmonella* and given a therapeutic dose of chlortetracycline had higher levels of *Salmonella* in the oral cavity (tonsils) and feces when compared to pigs that did not receive chlortetracycline. These findings indicate that producers may want to consider an animal's *Salmonella* status when administering therapeutic antibiotics.

Diagnostics and Alternatives to Antimicrobials

Investigation of mobile colistin resistance in U.S. animal-origin food

Albany, CA

Colistin is a “last-resort” antibiotic used to treat bacterial infections that have resisted treatment with other antibiotics. Recently, a mobile colistin resistance gene, *mcr-1*, was discovered in clinical and animal samples, and could directly threaten human health. ARS researchers evaluated the prevalence of the mobile *mcr-1* gene via screening of more than 5,000 chicken, beef, pork, and catfish samples that were randomly collected by the USDA Food Safety Inspection Service. They found a very low prevalence of the *mcr-1* gene (0.24 percent in raw pork and less than 0.02 percent in all samples). This is the first systemic and large-scale investigation of mobile colistin resistance in U.S. food animal products, and the information obtained is useful for trade and food safety risk assessments.

New tool to identify antimicrobial resistance genes in DNA sequences

Athens, GA

In the United States, antimicrobial resistant bacteria are responsible for 2.8 million human infections and more than 35,000 deaths each year. ARS researchers were part of a multi-agency collaboration that developed the Bacterial Antimicrobial Resistance Reference Gene Database and the AMRFinderPlus gene detection tool. The database includes 7,737 antibiotic, acid, biocide, metal, and stress resistance genes, which can be detected by AMRFinderPlus in whole genome sequences of any bacteria. The new tool allows users to use only AMR genes or to include stress response and virulence genes. This tool increases the number and type of genes that can be targeted to better understand AMR in important human pathogens, thereby improving food safety and public health.

Development of Reads2Resistome

Athens, GA

ARS researchers and Colorado State University collaborators developed Reads2Resistome, a bioinformatic tool that allows users to employ Linux basic commands to analyze bacterial genomes sequenced using either short and/or long read sequencing technologies. Reads2Resistome takes sequence reads as input and performs assembly, annotation, and genome characterization with the goal of producing an accurate and comprehensive description of the bacterial genome and collection of all the AMR genes, virulence genes, and other resistance elements within the chromosome, plasmids, or bacteriophage. Reads2Resistome is the first known pipeline that performs both genome assembly and in-depth genome characterization. It has been made publicly available on GitHub and is accessible to USDA researchers via SCINet.

Olive leaf extract as a natural antimicrobial compound for the food industry

Wyndmoor, PA

New methods are needed to control pathogenic bacteria in the food industry. Olive leaf extract (OLE) is often used as an herbal supplement and is considered beneficial to human health. ARS researchers studied the application of OLE as an antimicrobial agent for controlling major foodborne pathogenic bacteria such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Staphylococcus aureus*. They found that an antimicrobial film prepared with OLE inhibited the growth of foodborne pathogens. These findings suggest OLE

can potentially be used as a natural antimicrobial food packing material to control foodborne pathogens in food and the food processing environment.

Molecular Characterization of Foodborne Pathogen Responses to Stress

Wyndmoor, PA

Listeria monocytogenes is a foodborne bacterium that causes a disease known as listeriosis. Some consumers prefer the use of natural antimicrobials from plant extracts to inhibit the growth of foodborne pathogens because of safety concerns. ARS researchers evaluated 800 different plant extracts from around world for their effectiveness in inhibiting the growth of *L. monocytogenes*. Twelve of the plant extracts showed notable activity against the pathogen, and the concentrations needed to stop bacteria growth were determined. These plant extracts can be used as new preservatives by the food industry to reduce the risk of contamination from *L. monocytogenes*.

Alternatives to Antibiotics in Food Production Environments

Wyndmoor, PA

Concerns about AMR in bacteria have generated needs for new treatment strategies for bacterial infections. Rather than focus on the development of new antibiotics, which can ultimately lead to resistance, this multidisciplinary project concentrated on an alternative strategy using inorganic materials generally recognized by the U.S. Food and Drug Administration as safe (GRAS). Project scientists found that multiple grades of low cost ZnO powders significantly reduced microbial loads of *Salmonella* and *Campylobacter*, two leading common causes of foodborne illnesses, on surfaces in food processing environments. The research identified GRAS materials that do not need to be nano-sized in order to be effective which is important because particle size can affect how they are regulated; therefore this should lower the barrier for entry for adoption.

NK-lysin-derived peptides have strong antimicrobial activity against MDR *Salmonella*

Ames, IA

Small antimicrobial proteins called NK-lysins are produced by cattle white blood cells and have antimicrobial activity against various microbial pathogens. ARS researchers studied the antimicrobial activity of NK-lysins on multidrug resistant (MDR) and non-MDR *Salmonella* isolates and determined the mechanisms these proteins use against *Salmonella*. More improvements to biodegradable NK-lysin encapsulated nanoparticles are currently underway that will improve intracellular *Salmonella* killing activity.

Discovery of new antimicrobial biosurfactants

Wyndmoor, PA

Some very-long-chain (vlc) biosurfactants have improved antimicrobial activity than the better-known medium-chain variants. ARS scientists first developed a quick and simple process to separate the structural variants of the vlc biosurfactants, which led to the discovery of new, more effective environmentally-friendly antimicrobial biosurfactants that could potentially replace current antimicrobial agents already or in the process of being phased out.