FY 2016 Annual Report for National Program 108 Food Safety

Executive Summary

Food Safety falls under Goal 4 of the Agency Strategic Plan**: Enhance Protection and Safety of the Nation's Agriculture and Food Supply**. For the Nation to have safe and affordable food, the food system must be protected at each step from production to consumption. The production and distribution system for food in the United States encompasses a diverse, extensive, and easily accessible system that is open to the introduction of pathogens (bacteria, viruses, and parasites), bacterial toxins, fungal toxins (mycotoxins), and chemical contaminants through natural processes, global commerce, and intentional means. In response to these threats, crop and livestock production systems must be protected during production, processing, and preparation from pathogens, toxins, and chemicals that cause disease in humans.

To ensure the security of production systems, Agricultural Research Service (ARS) conducts basic, applied, and developmental research resulting in new technologies, new and improved management practices, pest management strategies, sustainable production systems, and methods of controlling potential contaminants. These ARS activities are key to providing a safe, plentiful, diverse, and affordable supply of food, fiber, and other agricultural products.

Mission Statement

To provide through research, the means to ensure that the food supply is safe for consumers and that food and feed meet foreign and domestic regulatory requirements. Research seeks ways to assess, control or eliminate potentially harmful food contaminants, including both introduced and naturally occurring pathogenic bacteria, viruses and parasites, toxins and non-biological-based chemical contaminants, mycotoxins and plant toxins. Food safety is a global issue; thus, the Program involves both national and international collaborations through formal and informal partnerships. Accomplishments and outcomes are utilized in national and international strategies delivering research results to regulatory agencies, commodity organizations, industry and consumers for implementation.

Vision Statement

To increase public health through the development of technologies which protect food from pathogens, toxins, and chemical contaminants during production, processing, and preparation thus increasing the safety of the food supply.

Component 1. Foodborne Contaminants

The production, processing, and distribution system for food in the United States is a diverse, extensive, and easily accessible system. This open system is vulnerable to introduction of contaminants through natural processes and global commerce, and by intentional means. Thus, the food supply must be protected from pathogens, toxins, and chemical contamination that cause disease or injury to humans. The ARS Food Safety Research Program seeks ways to assess and control potentially harmful food contaminants. ARS will conduct research and provide scientific information and technology to producers, manufacturers, regulatory agencies, and consumers to support their efforts to provide a secure, affordable, and safe supply of food, fiber, and industrial products.

Problem Statement 1. Population Systems

The goal of this research area is to identify and characterize the movement, structure, and dynamics of microbial populations within food-animal and plant systems, across the entire food continuum, from production through processing. At a microbial level, the diversity and complexity within environments and food matrices may alter the makeup of the populations, as well as cause change through spatial and temporal influences, or by the competitive or synergistic relationships among pathogens and commensals. Microbial populations can influence the safety of food, and the various environments in which they survive determine the success and impact of the microorganism. In turn, microorganism(s) may influence the conditions prevailing within the environment which also impacts their survival or ability to thrive. An example of identifiable area of study would include biofilms and the association of quorum sensing.

Components and emphasis for understanding and characterizing microbial populations and their environments must include epidemiology, ecology, and host-pathogen relationships. Epidemiologic studies of microbes within their environment, allows an analysis of the population therein. As such it enables the development of improved detection methods, provides a framework for integration of microbial genomic data with disease, and a mechanism to evaluate risk factors for microbial intervention and/or control. Ecologic studies determine the attributes and changes in various communities, that is, changes to populations in the same space. Such studies allow for a better understanding of the interactions and relationships, and the transmission and dissemination of pathogens and toxins in and among food producing animals and crops. Host-pathogen relationship studies provide an understanding of the acquisition of genetic traits, such as the development and movement of resistance genes; traits connected with colonization and evolution of virulence; and the role of commensals. Where appropriate, a metagenomics approach to selected research areas will be developed to determine the attributes of the ecological communities in which pathogens are found. Knowledge of the attributes, interactions and relationships within the community in which the pathogen lives is critical to the development of control and intervention strategies.

Within this Problem Statement it was critical to differentiate Food Safety from Animal Health. Certainly there will be some overlap; however, this will be addressed at the Office of National Programs level. There will be an emphasis on how pathogens persist in animals and the related environment, and this will drive mitigation and prevention strategies, as well as guidelines, policy and regulation.

Anticipated Products

- Improved epidemiological methods that allow the collection of quantitative data on the pathogen load within the food safety continuum.
- Capability to predict how environmental, nutritional, and/or biological factors influence or control the attributes and changes in ecological communities and within microbial populations.
- A foundation for developing appropriate intervention strategies based on mechanisms for transmission and dissemination of pathogens and toxins in and among food producing animals and crops.
- A risk-based framework that allows the integration of genomic data with disease outcome
- Descriptions of genetic traits associated with colonization and the evolution of virulence, including the development and movement of resistance genes, and the role of commensalism in resistance gene acquisition.

Potential Benefits/Impact

- Improves and enhances knowledge of how microbial populations in agriculture can potentially affect and impact public health.
- Delineates how microbial pathogens are transmitted and disseminated in and among food producing animals and plant crops (includes mycotoxin related research) allowing for future development of improved/alternate (environmentally compatible) intervention and/or control strategies.
- The critical factors which influence fitness characteristics related to microbial persistence colonization, survival, and growth allowing for future development of improved/alternate (environmentally compatible) intervention and/or control strategies.

Problem Statement 2. Systems Biology

Systems biology involves an integrated, multidisciplinary approach to study the complexities of biological components, a central problem to food safety. Identifying the components and players within the system allows the genetic components of bacterial, viral, and fungal pathogens and food-borne parasites, and their expression and products to be identified and directly related to the microorganisms. In order to study systems, quantitative technologies such as "omics" [genomics, proteomics, transcriptomics, metabolomics and metagenomics] combined with bioinformatics can be applied. There is an increased need for data gained from systems studies to be directly used for both pre- and postharvest food safety. For example, whole genome sequencing efforts using next generation sequencing (NGS) have increased and allowed regulatory agencies to identify and resolve outbreaks of foodborne illness (often for attribution purposes). It is recognized however, that the use of NGS requires extensive collaborations with other researchers.

The main goal of research developed in response to this Problem Statement is to utilize omic-technologies and apply them to the study of foodborne pathogens in complex food systems. For example, research will elucidate how microbes cause disease and assess their prevalence, pathogenicity (ability to infect and cause disease) and virulence (the severity of disease). Understanding pathogenicity and virulence is critical for intervention and control strategies, modeling, and providing data for the development of risk assessments by regulatory agencies. Pathogens have the capacity to readily and rapidly adapt and evolve, so pathogenicity and release of virulence factors is an issue at all stages of the food safety continuum. The prevalence and patterns of contamination in food sectors may vary considerably and needs to be assessed and evaluated carefully. Differences in microbial prevalence, pathogenicity and their virulence are observed across different food production and processing systems, at different sampling times, and by using various methods. Contamination patterns reveal variation in the pathogenicity and virulence and the presence of persistent or sporadic strains and evidence of bacterial transfer from production environments to processing, and from processing environments to food. Continual outbreaks of industry related bacterial contamination emphasizes the continued need to examine pathogens in order to avoid public health risks.

Ongoing implemented microbial control strategies may lose their effectiveness, forcing the development of new production processes and products to maintain and improve the safety of foods. This in turn may restart the cycle of pathogen adaptation resulting from the changed environment and its stresses. Risk assessment(s) conducted by our regulatory stakeholders are also predicated on understanding the pathogen, the dose response, the behavior in foods, and any positive or negative influences that may affect virulence. Assessing the virulence of foodborne organisms and differences among serotypes/strains is critical in implementing new surveillance and intervention strategies. A critical issue within this Statement is the need to differentiate between microorganisms that are relevant to agriculture versus food safety and public health.

Anticipated Products

- Identities of the critical/required genetic components that make specific microorganisms pathogenic versus non-pathogenic, or highly versus weakly virulent.
- Principles relating regulatory mechanisms that control or impact gene expression with a microorganism's biology, for example, pathogenicity and virulence.
- Information relating how stress factors such as climate change affect pathogen gene expression.

Potential Benefits/Impact

- Provides knowledge of which genes are required for a microorganism to become a pathogen; generates data on genes that contribute to variations in pathogenicity, and how gene expression is involved in virulence and/or persistence viability in animal, plant and food systems.
- Generates data for the specific development of molecular pathogen phylogenetics, allowing for improved and faster molecular tracking, and determination and characterization (attribution) of outbreaks of foodborne illness by regulatory agencies.
- Supports development of improved risk models, and the revision of risk assessments, e.g., pathogens of low virulence may not be considered as necessary for regulatory control.
- Supports improved mitigation strategies and alternative control measures via identification of genes that code for resistance to antimicrobials and disinfectants, for toxin production; for the ability to grow in specific ecological niches; and for the ability to persist in production and/or processing environments.

Problem Statement 3. Microbial Contaminants: Technologies for Detection and Characterization

The challenge is the unequivocal detection and characterization of pathogenic microorganisms entering the food continuum (both pre- and postharvest). Detection and characterization are required at the earliest possible stage of the continuum to provide the necessary data for targeted interventions and reducing the need for recall of food products. Where possible, technologies must be developed that allow the most effective and rapid detection and characterization capabilities.

The focus of the research will be on the most promising technologies (depending on the matrix) or point of use, that is, whether the technology will be used for baseline studies, traceability and/or attribution forensics. This requires that decisions be made relative to what should be detected, and the required level of detection and characterization. It is noted that technologies that have the highest level of detection/characterization capability might not necessarily be the most practical, useful, economically viable, or easily implemented. High-through-put analysis is important, but it may be impractical. Promising technologies will be advanced through technology transfer, and where possible, and appropriate, will undergo validation through national or international bodies from academia, industry, and/or government sectors. Studies that suggest minimal outcome or impact will be terminated, and alternate approaches formulated.

Anticipated Products

- Technologies for multiple agents for trace-back and attribution, and where fiscal and personnel resources are also limited.
- Technologies with improved speed, cost effectiveness, and the capability to provide information for the determination and implementation of subsequent actions.
- Validated technologies that allow uniformity of implementation nationally and internationally.

Potential Benefits/Impact

- Provides validated technologies that have public health, regulatory [monitoring, traceability and attribution], trade, industry, and research use and a commonality of interests between stakeholders and partners.
- Allows improved response times to events, and subsequently allows for the development of mechanisms for treating foods taken out of commerce.
- Provides data to identify areas where interventions are most critically needed, thus assisting the implementation of HACCP programs by Federal agencies, and their regulated industries.
- Enables development and validation of predictive microbial models and helps fill identified data gaps.

Problem Statement 4. Chemical and Biological Contaminants: Detection and Characterization Methodology, Toxicology, and Toxinology

Toxicology examines the relationship between dose and its effects on the exposed organism, whereas ***toxinology*** deals specifically with animal, plant, and microbial toxins produced by or accumulated in living organisms, their properties and their biological significance for the organisms involved. Both kinds of studies are required to reduce risks arising from contamination of food by chemical and biological contaminants.

The regulation and control of veterinary drugs, chemical residues, heavy metals, persistent organic pollutants, and biological toxins derived from bacteria, fungi and plants are an integral component of any food safety program. To protect public health and the environment, regulations have been passed and implemented that set limits on contaminants in edible agricultural products. Compliance and enforcement of these regulations is a critical role of the ARS National Program's stakeholders that requires the availability of practical detection and characterization methods for veterinary drugs (antibiotics, beta-agonists), chemical residues (dioxins, pesticides), heavy metals (As, Pb, Cd), and organic pollutants (polybrominated diphenyl ethers). In addition to regulatory monitoring, there is a need to understand the biological effects of any inadvertent contamination by humans or animals. In addition to toxicological and toxinological studies, this Problem Statement also includes research directed towards methods for detection and identification of mycotoxins, toxicity evaluation, and mechanism of action.

Accomplishments and promising technologies within this research area will be quickly advanced through technology transfer and where appropriate, will undergo validation through national or international bodies such as the Association of Official Agricultural Chemists (AOAC). These studies require multidisciplinary approaches to meet the challenge, and accomplishments may have far reaching effects regarding food biosecurity, regulations and trade issues.

Anticipated Products

- New and validated technologies that when implemented provide tangible benefits through a more effective and efficient means of monitoring the food supply and environment where food is grown.
- Improved methods that assist researchers conducting toxico/toxinological studies.
- Toxico/toxinological data providing basic and applied knowledge on the effect of exposure to biological toxins.

Potential Benefits/Impact

- Provides technologies and data for regulatory use, and for better scientific and regulatory decision-making, reducing the likelihood of tolerance limit-errors, protection of consumers, and prevention of economic losses resulting from inappropriate regulatory actions.

Problem Statement 5. Intervention and Control Strategies

Intervention and control strategies will assist in reducing or eliminating pathogens in food animals and their derived products, seafood, and plant crops during production and processing. Reduced shedding of zoonotic pathogens by food producing animals, and contamination of seafood and plant material will subsequently help reduce the pathogen load during slaughter/ harvesting and subsequent processing and storage. Some food processing/storage technologies have the ability to inactivate microorganisms to varying degrees; however, the intensities required can result in adverse functional and/or sensory properties, combined with a significant reduction in quality. Consequently, there remains a continued need to develop and subsequently combine new and/or innovative processing technologies. Interventions can be additive and/or synergistic, leading to improved control over pathogen growth without potential changes in food quality or reduction in nutrition. Research after an approved period that yields no outcome, or requires the purchase of expensive equipment will be terminated, and alternate approaches formulated. If alternate approaches cannot be found, the project will be redirected to another priority. Unintended or unanticipated consequences of processing intervention strategies such as changes in virulence, production of toxins, pathogen resistance, selection of resistant strains, or changes in microbial ecology should be considered for further investigation.

The challenge is that the pathogen load on a product must be significantly reduced by any processing intervention strategy to avoid the consequences in food production resulting from "dirty in, dirty out" processing. There is also the concern that during processing the initial microbial load can be reduced but recontamination occurs with different strains or serotypes present or resident within the processing environment. Such concerns are valid because there are numerous observations that the pathogens present on product prior to processing are different from those found after processing. This variation in pathogen type has significant public health concerns since those pathogens initially found on the product may not be responsible for any foodborne outbreak and/or clinical outcome.

Research should also address, where possible, the integrated lethality for an intervention process. The purpose of the process lethality determination is to provide processors with science-based validation of the effectiveness of a specific process to destroy any microorganism of concern. For example, a thermal process needs to account for many variables including the initial pathogen load, multiple pathogens, pathogen strain variability, food structure, and the heating and cooling profile of the product. In-plant validation should be conducted to verify the intervention(s). The entire lethality process is incorporated into a systems approach to developing pathogen intervention or control strategies. Problem Statement 5 addresses a wide range of food products including animals, shellfish -seafood, and plant materials. The Problem Statement also includes biocontrol technologies for food crops contaminated by mycotoxins, such a tree nuts, corn and grains.

It is critically important within these studies that for development and validation of any process intervention a common or representative core set of pathogens or surrogates be used. This is critically important in order to make the intervention research results comparable both within and external to the Program. Core sets of strains for different pathogens will be made available through the ARS bacterial culture collection. If a specific strain is not available in the collection, ONP will facilitate researchers obtaining the appropriate isolate.

Anticipated Products

- Improved intervention strategies to eliminate and/or control microorganisms in animals and their derived products, seafood and plant production, processing and storage systems. Interventions have the ability to inactivate microorganisms to varying degrees; therefore, the goal is to maximize intervention effectiveness while minimizing sensory/quality deterioration, and possible accumulation of toxic chemical by-products.
- Improved intervention strategies for various sized operations, utilizing environmentally compatible technologies.
- Improved intervention strategies focusing on the use of combinations of new or innovative technologies for (minimal) processing, thus mitigating the potential for the development of resistance.

- Improved interventions based on an understanding of their modes of action and effects on the microbial ecology of a food product, since inadequate suppression of spoilage could create an opportunity for human pathogen growth and toxin production.

Potential Benefits/Impact

- Provision of critical intervention strategy data to regulatory/action agencies, industry, and commodity organizations that allows for the development, evaluation, and implementation of Good Agricultural Practices (GAPs); Good Manufacturing Practices (GMPs) or regulations based on sound science.
- Enables methods/strategies for the evaluation of any developed interventions and controls.
- Provides production control interventions that reduce downstream contamination, which subsequently reduces disease risk.

Problem Statement 6. Predictive Microbiology/Modeling; Data Acquisition and Storage; Genomics Database

The tenet of predictive microbiology is that the behavior of any microorganism is deterministic and able to be, within limits, predicted from knowledge of the microorganism itself and the microorganism's immediate environment. However, it has been stressed by stakeholder groups that research should also include a greater emphasis on probabilistic modeling to balance the deterministic approaches. This would benefit predicting the behavior of pathogens under stressed conditions (more relevant to the food industry) where growth/inactivation is stochastic.

Behavioral predictions and models are accepted (globally) as an integral part of microbial risk assessment used to support food safety measures by both food safety regulatory bodies and industry. The Program does not develop or conduct Risk Assessments (RA), where RA is defined as the determination of a quantitative or qualitative value of risk related to a specific situation and a recognized hazard. However; the Program does conduct research and provide data when requested by our regulatory stakeholders (FSIS, FDA) for their use in conducting risk assessments.

The Program develops various modeling programs including; the Pathogen Modeling Program (PMP), a package of models that can be used to predict the growth and inactivation of foodborne bacteria, primarily pathogens, under various environmental conditions. In addition, the Predictive Microbiology Information Portal (PMIP) is geared to assist food companies (large and small) in the use of predictive models, the appropriate application of models, and proper model interpretation. The vision is that the PMIP will be the highway for the most comprehensive websites that bring together large and small food companies in contact with the information needed to aid in the production of the safest foods. The PMIP links users to numerous and diverse resources associated with models (PMP), databases (ComBase), regulatory requirements, and food safety principles.

All predictive models developed must be available for external examination, review and utility. If predictive models are developed for internationally accepted high priority pathogen-food combinations, then they could have a major impact for food companies in the USA and other countries producing and exporting food to the USA. This will require significant interactions with risk assessors and involvement in international initiatives such as National Advisory Committee on Microbial Criteria for Foods (NACMCF), Codex Alimentarius Commission (CODEX), Food and Agriculture Organization (FAO), and the World Health Organization (WHO). Collaborations with stakeholders must be strengthened with regards to what research needs to be conducted so as to effectively utilize the inherent ARS expertise and modeling systems mechanisms.

Data acquisition and storage: ARS and international institutes, including Institute Food Research (IFR-UK) and the University of Tasmania (UTas), as well as associate members University of Querétaro, Mexico; Unilever Research, UK; Agricultural University of Athens, Greece; National Food Research Institute, Japan; Hokkaido University, Japan; and Rutgers University also developed and maintains a publically available global food safety database**,** ComBase **-** *a Combined dataBase for predictive microbiology* – which is the number-one web-based resource for quantitative and predictive food microbiology in the world. Its main components include a database of observed microbial responses to a variety of food-related environments and a collection of relevant predictive models. The purpose and goal of ComBase is to provide an electronic repository for food microbiology observations and to make such data and the generated predictive tools freely available and accessible to the entire food safety community. Data acquisition and use is an interdisciplinary research challenge that translates into safer products and improved public health.

Genomics as a functional and critical part of omic-technologies holds great promise for improving the early detection, prevention and control of current and emerging foodborne pathogens, thus contributing to improved food safety and consequently public health. Genomics have the potential as a partner or replacement of culture-based techniques. Food safety regulatory agencies, USDA and the FDA, have discussed and are planning to implement the increased use of genomics, in particular partial and/or whole genome sequencing (P/WGS) for both regulatory monitoring, attribution and potentially for revising risk assessments.

Implementation of such a redirection requires developing a coordinated system of genomic sequencing technology for routine testing. Critical within this issue is the development of an ARS database from our national and international sequencing/annotation efforts. For this work, a common or representative core set of bacterial pathogens or surrogates will be available. Additional data from isolates studies obtained from national and international collaborations will be incorporated. Allied to the sequencing efforts will be meta-data descriptors. This research will be part of a larger international initiative, the Global Microbial Identifier (GMI), a global, visionary taskforce including more than 30 countries who share an aim of making novel genomic technologies and informatics tools available for improved global infectious disease diagnostics, surveillance and research, by developing needs and end-user based data exchange and analysis tools for characterization of all microbial organisms and microbial communities.

Anticipated Products

- Predictive microbiology [models] that have validity and usefulness while addressing the limitations of the predictive ability. Studies leading to development of these models will include “real food systems” not just broth models or model food systems.
- A shared informational database done in-part through the continued development and

expansion of the international collaborative project Combase. This will include data from industry/academia that pertains to "real food production/processing systems."

- A computer-based system and database to aggregate, share, mine and translate genomic data for microorganisms in real-time through a direct link using user-friendly platforms.

Potential Benefits/Impact

- Generates data on the responses of microorganisms to both defined and changing environmental conditions, and translates these data into mathematical models and user friendly software tools available on the internet at no cost. These must be readily usable by national and international regulatory and public health agencies, and industry, to assist in ensuring the safety of the food supply.
- An internet-based database ensures that data-mining and acquisition will continue to be coordinated. Genomic database and bioinformatics efforts become increasingly important so that biologists have the ability to gain information that will foster technological innovation, and an understanding of the genetic basis of foodborne microorganisms.

Problem Statement 7. Antimicrobial Resistance

The discovery of antibiotics transformed human and veterinary medicine and saved millions of lives in the United States and around the world. The rise/increase of antibiotic-resistant bacteria represents a serious threat to both animal and human health and the economy. The concern for the development of antimicrobial resistant (AMR) bacteria has resulted in the development of both national and international strategies to address the issue. In 2014, the President signed an Executive Order, and a strategy was developed by multiple agencies to begin addressing AMR at the National level. Even though the USDA is not the lead regulatory agency for antibiotic use and AMR, USDA-ARS is an important part of the solution.

Areas of concern include detecting, measuring, and assessing the amount of AMR bacteria within the production animal populations with an emphasis on foodborne pathogens. In addition, developing alternative strategies to minimize the use of antibiotics in production animals while maintaining and improving animal health and ensuring safe food for consumers is a critical need. Currently, the role of feeding antibiotics to production animals on the development of AMR bacteria and the impact on public health is not well defined. In addition, there is a critical need for the development of alternative strategies to reduce the level of antibiotic use as well as developing mitigation strategies for foodborne AMR bacteria in food producing animals. These areas are cross-linked with Problem Statements 1 and 2 within the Action Plan

Anticipated Products

- Improved detection techniques facilitating the speed, ability, and accuracy of detecting foodborne AMR bacteria in food producing animals and their products.
- Improved strategies to reduce antibiotic use and the number of AMR bacteria in the food supply.

Potential Benefits/Impact

- Provides support for both stakeholders and regulatory agencies in developing strategies to address foodborne AMR bacteria.
- Improves strategies to reduce the use of antibiotics in production animals while maintaining their health and growth efficiency. This is critical for feeding an ever growing population while also addressing a serious public and animal health concern.

Selected Accomplishments for FY 2016

Antimicrobial carryover. *Salmonella* bacteria are a major cause of foodborne illness. The pathogen can be transmitted to humans through the consumption of contaminated foods, especially poultry. The USDA Food Safety Inspection Service (FSIS) monitors contamination of poultry through a testing program to protect consumers. However, recently there has been critical concern that the level of *Salmonella* contamination is higher than recognized due to carry-over of intervention chemicals (sanitizers) such as chlorine on whole poultry carcasses. These sanitizers are used to reduce pathogens in poultry processing, and may inadvertently be transferred into the collection broth used for testing by FSIS inspectors. To alleviate any concerns that the FSIS monitoring tests were underestimating pathogen levels, ARS researchers from Athens Georgia, developed a modified collection broth capable of neutralizing a wide range of sanitizers. FSIS validated, approved, and implemented this new protocol in their collection of regulatory samples for *Salmonella* testing in July 2016. This new protocol now provides a statistically more accurate test and reporting of *Salmonella* levels in poultry, making for a safer food for consumers.

Egg candling lights for official grading. For poultry production farmers and regulatory agencies use a specific light source to show details within shell eggs (termed candling), specifically to learn which eggs are fertile and will hatch into chicks, and to maintain egg quality assurance. The official graders with the USDA-Agricultural Marketing Service (AMS) were concerned that the current light source was not accurate enough and thus requested ARS to develop a new light source for candling. ARS scientists from Athens, Georgia, designed both a portable battery-powered and a stationary high intensity white light emitting diode (LED). This new light source provided greater accuracy for the AMS who are now replacing all of their existing obsolete candling lights with this new technology. ARS transferred the technology to a U.S. commercial partner for industrial production.

Detection of pesticides and environmental contaminants. Improved analytical methods (more accurate, cheaper, and faster) are needed to better monitor pesticides and persistent organic pollutants in meat and poultry by food safety regulatory agencies such as the USDA-FSIS. ARS researchers in Wyndmoor, Pennsylvania, developed and validated a new, easy, reliable, cost-effective, high-throughput analysis method for 192 diverse pesticides and 51 environmental contaminants found in cattle, swine, and poultry muscle. The new technology is based upon the previously ARS-developed QuEChERS approach now used worldwide. The new method was successfully applied to the analysis of known contaminated meat samples that validated the utility of the method, which was transferred to the FSIS for implementation in routine regulatory monitoring of contaminants in meats.

Detecting food adulterants. Food adulteration is a critical and serious international issue. It is usually considered an act whereby food is contaminated with the intention to maximize economic profits. However, it consequently often results in reduced safety and quality which may lead to illnesses and deaths. For example, recent intentional contamination of milk-based infant formulae with melamine to mask the true protein level resulted in many illnesses and deaths, and an international recall of product. To provide regulatory agencies with the ability to detect adulterated products, for example milk powder, ARS researchers in Beltsville, Maryland, developed a line-scan Raman chemical imaging system sensitive to 50 parts per million. The new imaging system, now under Patent review, can be used for rapid, nondestructive, continuous, and quantitative measurement of chemical adulterants present in dry powdered food ingredients.

Reevaluation of produce metrics. The consumption of fresh produce is associated with many outbreaks of foodborne illness. The current metrics used by the California Leafy Greens Marketing Agreements (LGMA) for leafy greens to determine the microbial safety of fresh produce were reevaluated by ARS scientists at Beltsville, Maryland. Studies examined several LGMA rules currently employed to prevent fecal contamination being introduced to crops: the 60-day interval between flooding of production fields and replanting of crops, and the 9 meter (30 foot) "no harvest" zone from the edge of the flood zone. In an intentionally flooded spinach field with a -5% slope, Escherichia coli populations declined more slowly in Fall trials than in Spring trials. E. coli in soils and on spinach plants were detected (9 meters) away from the edge of the flood. These results suggest that the LGMA metrics for fresh produce production should be revised to include considerations of field and weather conditions that may promote bacterial movement and survival.

Meat safety inspection. Current meat inspection in slaughter plants for food safety and quality attributes, including potential fecal contamination, is usually conducted through visual examination by human inspectors. Improvement in inspections can be done using fluorescence detection methods, however, these conventional methods require ambient darkness. ARS researchers in Beltsville, Maryland, developed a handheld fluorescence-based imaging device (HFID) and display monitor for use under ambient lighting in food processing plants, to highlight contamination on food and equipment surfaces. This study validated the effectiveness of the HFID in enhancing visual detection of fecal contamination on red meat, fat, and bone surfaces of beef under varying ambient luminous intensities. Overall, diluted feces on fat, red meat and bone surfaces of beef under ambient light were detectable in the 670-nm single-band fluorescence images. This technology also validated by the U.S. Military (for their use) will support and improve meat safety inspection programs as implemented by U.S. processors and regulatory inspectors.

Decontamination technology. The U.S. is the second leading producer and exporter of pistachios, providing 24 percent of the world total production. In 2014, the U.S. produced 257,000 tons of pistachios valued at $1.6 billion. Pistachios have unfortunately been recalled due to contamination with Salmonella bacteria and this contamination has caused several outbreaks of salmonellosis, impacting public health and the pistachio industry. ARS researchers in Albany, California, developed a novel intervention technology based on sequential infrared heating and hot air. The technology dries pistachios and results in a one million times reduction in bacteria on pistachio kernels and shells, respectively, and has a 34% energy saving compared with traditional hot air drying alone. This technology now provides the tree nut industry with an efficient and effective approach to produce high quality safe pistachios and reduce the incidence of foodborne illness caused by this food product.

Extending the shelf life of fresh milk. Milk is a major consumer food. Pasteurization of milk using heat has long been the standard method to extend the shelf-life of dairy products, as well as a means to reduce microbial load and the risk of food-borne illness. ARS-funded scientists at the Center for Food Safety Engineering (CFSE), Purdue University, West Lafayette, Indiana, tested a novel new pasteurization method using low temperature, short time (LTST) where milk is dispersed in the form of droplets and treated with low heat/pressure variation over a short treatment time. LTST was found to be very effective, reducing the level of microorganisms by over 10 million fold. The CFSE-developed BARDOT/BEAM detection technology was used to demonstrate that the only survivors of LTST were a few organisms that do not grow at refrigeration temperatures. The impact of the LTST process was an extension of the milk shelf-life from 14-35 days to 63 days with no loss in quality. The new process and subsequent extended shelf-life will positively impact the dairy industry in terms of shipping and overall product sustainability, and consumer product safety and quality.

<u>Price tag of processing controls.</u> Processing interventions (controls) are implemented to improve product safety while maintaining quality. The cost of implementing interventions is often an unknown, especially for foods such as fresh produce. Interventions for tomatoes are a major concern for industry and regulators. ARS researchers in Wyndmoor, Pennsylvania, developed a formal cost model analysis for one known intervention: competitive exclusion microbes (CEM) for biocontrol of Salmonella enterica on tomatoes. The study found that the unit costs of CEM biocontrol range from $0.05-$0.95 per kg of tomato for the small-scale and $0.0041-$0.0075 per kg for the large CEM production models. Since total variable costs for CEM were 95% of total production costs, while fixed costs were only 5% of total costs, CEM is, therefore, best suited for large-scale application. Overall, the estimated total annual cost of CEM for control of Salmonella enterica on tomatoes ($0.0058 - 0.073 per kg) is greater than sodium hypochlorite (~$0.00046 per kg) or gaseous chlorine dioxide ($0.02 - $0.21 per kg). For high value produce, CEM may complement existing intervention technologies if efficacy and delivery systems can be optimized and associated factors such as product quality are evaluated.

<u>Reducing contamination of poultry meat.</u> Campylobacter bacterial contamination of poultry is responsible for millions of cases of foodborne illness worldwide. Developing viable, cost-effective, generally recognized as safe (GRAS) processing interventions against this bacteria are a critical need. ARS scientists in Athens, Georgia, optimized a novel in-package ozonation technology to reduce Campylobacter and other spoilage species contamination on chicken breast filets. Significant reductions of natural bacterial flora and surface-applied bacterial pathogens were achieved using this novel technology. Currently, this technology is being expanded to include major food spoilage bacteria (Pseudomonas) and additional food safety pathogens (Salmonella). This novel technology, when implemented, will provide commercial poultry processors a method to significantly reduce bacterial pathogens and other bacterial spoilage flora on packaged breast filets, therefore, increasing the quality and safety of the final product as it leaves the processing plant.

<u>Breeding chickens resistant to pathogens</u>. Pathogenic bacteria live commensally in poultry and may cause foodborne illness if consumed by humans. New approaches are needed to produce poultry that are not colonized by these harmful bacteria given that absence of the pathogens in living birds will largely translate into pathogen-free meat products for human consumption. Breeding chickens resistant to Salmonella and Campylobacter infection is considered, along with vaccination, to be a potential long-term intervention in controlling these pathogenic bacteria

poultry production. ARS researchers at College Station, Texas, have identified a population of roosters from the Athens Canadian Random Bred (ACRB) lineage, a 1950s meat-type chicken, with differential expression of key immune markers to serve as sires for the generation of a F1 population of chickens selected for a more efficient innate immune responsiveness. Development of microbial pathogen-resistant birds would be a dramatic success in enhancing the microbial safety of poultry meat products reaching the consumer.

Antimicrobial food containers. Various types of containers are used for the storage and transportation of fruits and vegetables. Unfortunately, some containers are easily contaminated and can transfer microorganisms from the container to the food, resulting in cross contamination. Having a pathogen-free container would be a major technology advancement. ARS researchers in Wyndmoor, Pennsylvania, developed a coating formulae and application methods to produce food containers with antimicrobial surfaces. Specifically, Titanium dioxide (TiO2) nano-powders, which are ingredients in food coloring, were incorporated into zein or other polymers and used to form antimicrobial coating on the container surface. The surface coatings are then activated by placing them in visible light. This surface coating after activation was shown to kill > 99% of Escherichia coli O157:H7 on the container surface. The impact of the work is that the formula can be applied to various different types of containers made of metal, wood, plastics, paperboard, etc., used to store or transport a variety of foods, especially fruits and vegetables to inhibit bacterial cross-contamination of products.

Detection using bacteriophage. Rapid detection of foodborne pathogens, especially the adulterant Escherichia coli O157:H7 is of vital importance for public health worldwide. Studies funded by ARS at the Center for Food Safety Engineering, Purdue University, West Lafayette, Indiana, developed a technology using a modified bacteriophage (bacterial virus) specific for E. coli O157:H7. The scientists added a bioluminescence gene from shrimp to the bacteriophage genome, and after bacteriophage infection of E. coli caused the pathogen to glow. The developed detection/diagnostic tool is very specific and very sensitive, detecting as little as 5 cells of the pathogen in less than 7 hours during enrichment. The coupling of low phage concentrations and the ease of integration into current regulatory protocols provide a low-cost method for the detection of E. coli O157:H7 potentially leading to improved pathogen surveillance and a safer food supply. Additionally, the technology itself can be broadened to manipulate other bacteriophage specific for a wide range of other pathogens, including Salmonella and Campylobacter.

Alternative to animal bioassays. Staphylococcal food poisoning caused by [entero]-toxins the bacterium produces is among the leading causes of food-borne illness outbreaks. The current method (bioassays) for detection of enterotoxin activity is to use animals such as monkeys or kittens. Such methods are inappropriate and impractical, besides being expensive, having low sensitivity and poor reproducibility, especially for a large number of samples. To address non-animal use, ARS researchers in Albany, California, developed a robust cell based assay that combines the use of a genetically engineered T cell-line (immune response cell line) expressing the luciferase reporter gene with a B-cell line (antibody producing) that presents the toxin to the engineered T cell line. Exposure of the above mixed culture to, for example, active staphylococcal enterotoxin type E, results in a concentration dependent bioluminescence that can be measured quantitatively. The technology is extremely sensitive to as little as 1 fg/mL

(0.000,000,000,000,001 g/ml) of active SEE. This is > 100 times more sensitive than a typical ELISA assay and animal bioassay making this new method an economical and effective alternative to current detection technologies. Additionally, this innovative technology can be expanded to develop similar detection systems for other biological toxins that require the use of animal bioassays.

Inorganic arsenic in rice. Arsenic (As) is a naturally occurring heavy metal found in both soil and water. Arsenic poisoning is a medical condition resulting from increased levels in the body, which may cause a variety acute and of long-term sequelae. Natural As is the primary source of the inorganic As often found in rice. Research has suggested that consumption of rice based products may have severe toxic effects, including limiting cognitive and immune system development, potential premature births, and cancer due to long-term chronic exposure. The US Food and Drug Administration (FDA) who regulate heavy metals in foods developed a method to measure inorganic arsenic in rice which is expensive, costing about $200 per sample. Because the Food Code (CODEX) established by the United Nations Food and Agriculture Organization (FAO) and the FDA have set limits for inorganic arsenic in rice, they required simpler and much less costly methods for analysis. ARS scientists at Beltsville, Maryland, developed a new method and cross validated it with the FDA method, showing comparable results from a series of diverse rice types and samples. The new, simpler method is rapid and cost-effective, costing less than $40 per sample, an 80% reduction from the FDA method. This new method transferred to various regulatory agencies will benefit rice growers, the rice industry, infant cereal manufacturers, and consumer organization who monitor rice-based products for their safety.

Detection of violative residues. Antibiotic residues in food products is considered of serious consumer concern. Currently meat, poultry, and egg products are tested through the U.S. National Residue Program for the presence of more than 100 antibiotic/antimicrobial compounds. Sampling of meat products, for example, conducted by the Food Safety Programs major stakeholder, the USDA-FSIS is done at the processing plant and “swab tests” are used to quickly determine initial negative or positive results. Samples are typically taken from consumable organ meats such as liver or kidney where most compounds accumulate in the highest quantities. Penicillin G is a valuable antibiotic for the treatment of infection in female pigs (sows), however, penicillin G residues deplete very slowly from some sows but rapidly in others. As a result, the marketing of groups of sows treated with penicillin G has to be managed based on animals that clear penicillin G at the slowest rate. ARS researchers in Fargo, North Dakota, demonstrated that an inexpensive test strip could rapidly detect penicillin residues in urine of commercial sows, and that its presence in urine accurately predicted violative (over allowed level) penicillin residues in edible meat and organ tissues. The use of an inexpensive and easy to use test strip will allow the differential marketing of penicillin-free sows and sows retaining drug residues. This ability was welcomed by both industry and regulatory agencies.

Antimicrobial resistance. The impact of potential antimicrobial resistant bacteria in livestock waste runoff has been a growing topic of public concern. A main concern stems from the potential for these bacteria to be transported into the public water supply. ARS scientists at Clay Center, Nebraska, compared the populations of antimicrobial-resistant bacteria and the presence of antimicrobial resistance genes from samples within livestock and municipal waste streams discharged from municipal wastewater treatment facilities, cattle feedlot runoff catchment ponds, swine waste lagoons and environments considered low impact (a municipal lake and a prairie). The study results showed that the prevalence and concentrations of antimicrobial-resistant bacteria were similar among the livestock and municipal sample sources, but there were differences among the antimicrobial resistance genes found in agricultural, environmental, and municipal samples, with municipal samples harboring the highest number of antimicrobial resistance genes. It was concluded that antimicrobial resistance is a very widespread phenomenon where antimicrobial resistance can be found in cattle, swine, and human waste streams, but the higher diversity of antimicrobial resistance can be found in human waste streams.

Escherichia coli in culled dairy cows. Culled dairy cattle are a source of meat for human consumption. Many of these cattle commensally carry bacterial pathogens, the most important of which are the enterohemorrhagic Escherichia coli (EHEC) also known as STEC (Shiga-toxin producing E. coli) which are the cause of serious foodborne illness, chronic sequelae, and deaths in the United States. The most well-known of these STEC types of bacteria is E. coli O157:H7, but there are six additional pathogenic E. coli of concern (referred to as the Big Six) to regulatory agencies such as the USDA-FSIS that cause severe disease in humans. EHEC can be found in most groups of cattle, but it is not known whether any are specific to culled dairy cows harvested for beef. ARS scientists and colleagues belonging to the STEC-CAP research group (a consortium project funded through USDA-National Research Initiative NRI) examined matched fecal, hide, and pre-intervention carcass samples collected from culled dairy cows at harvest. Culture isolation found EHEC in 6.5% of feces samples, 15.6% of hide samples, and 1.0% of carcass samples. It was concluded that EHEC are common on the hides of culled dairy cattle, and that feces are an important source of EHEC contamination of hides. This information is critically important for industry, and regulatory monitoring of animals, and more critically the development of both pre- and post-harvest interventions against these pathogen types.

Poultry litter management. The poultry industry continues to combat the spread of foodborne pathogens and have spent millions of dollars attempting to control Salmonella and Campylobacter in animal production facilities often with minimal results. For example, until recently, the focus has largely been on implementing management strategies for reducing the movement of bacteria from the poultry house environment. Because of increasing cost of new bedding materials, modern broiler producers utilize the same litter for growing out multiple flocks. ARS researchers at College Station, Texas, assessed the changes in bacteria and dispersion of several poultry litter parameters in a commercial broiler house. The results demonstrated that clean-out practices have the potential to reduce contaminant buildup of many chemical pollutants in the litter, such as nitrates and the heavy metals iron, manganese, and zinc, within the broiler production facility. The work also established that proper disposal of litter is necessary for environmental health. This study provides an understanding of the role of management approaches in food safety as well as animal health, and the impact on the environment.

Antibiotic determinants. Cattle are frequently administered macrolide antibiotics such as Azithromycin and Erythromycin for the prevention or treatment of various diseases. In order to examine the effect of macrolide exposure on fecal shedding of resistant bacteria, rapid methods for characterizing resistance gene content were needed. To address this need, ARS researchers in Clay Center, Nebraska, developed a new method to detect seven major antibiotic resistance mechanisms simultaneously. This method was successfully used to screen over 2000 bacterial isolates and revealed subpopulations of bacteria containing antibiotic resistance genes not previously recognized in these organisms. This information is important for scientists, industry, and regulatory agencies concerned with the lateral transfer of antibiotic resistance genes between pathogenic and commensal bacteria and the subsequent development of resistance.