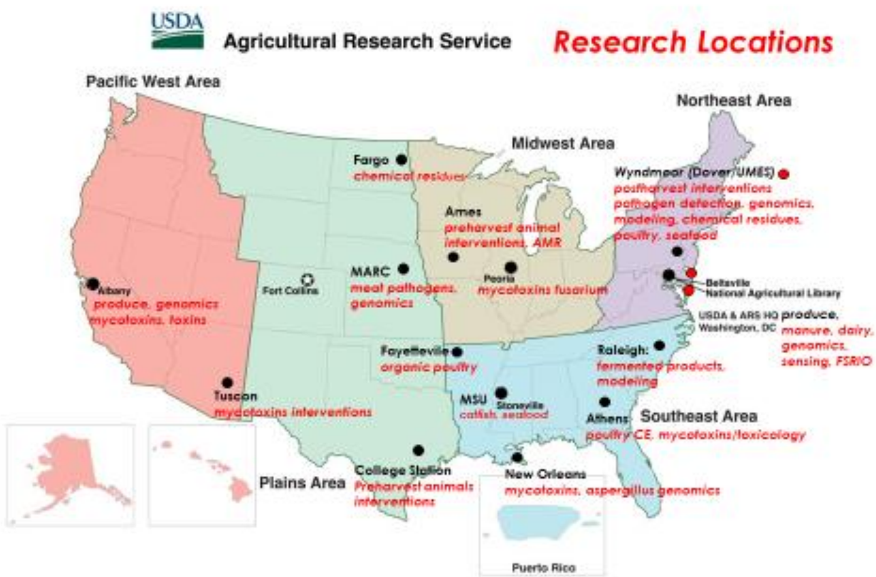
Agricultural Research Service
National Program 108
Food Safety
2024 Project Report Accomplishments by Research Focus



Agricultural Research Service

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Background

The National Food Safety Research Program (NP 108) is one of 16 National Programs (NP) within the USDA-Agricultural Research Service (ARS) Office of National Programs (ONP). The National Programs are organized within four broad Program areas: Nutrition, Food Safety and Quality (NFSQ); Animal Production and Protection (APP); Natural Resources and Sustainable Agricultural Systems (NRSAS); and Crop Production and Protection (CPP). The National Food Safety Research Program (NFSRP) is part of NFSQ. ***http://www.ars.usda.gov/research/programs.htm.***

The National Program structure allows ARS scientists to collaborate with other researchers to address food safety issues, needs or concerns. Within other ARS National Programs, in particular (NP107) Human Nutrition; (NP306) Product Quality and New Uses; (NP103) Animal Health; (NP106) Aquaculture; (NP303) Plant Diseases, and (NP216) Sustainable Agricultural Systems Research; nationally within the U.S. Federal Government, industry and academia; and internationally with public health/food safety regulatory agencies and research institutes in over 60 different countries.

NP108 Vision Statement:

The Programs vision is to enhance and protect public health and agriculture through the development of technologies, strategies, and data that safeguard food from pathogens, toxins, and chemical contaminants during production, processing, and preparation, thus increasing the safety of the U.S. food supply.

Mission Statement:

The Programs ***mission*** is to provide through research, the means to ensure that the U.S. food supply is safe for consumers; and that food and feed meet foreign and domestic regulatory requirements. Food safety research seeks ways to assess, control or eliminate potentially harmful food contaminants, including both introduced and naturally occurring pathogenic bacteria, non-pathogenic bacteria, viruses and parasites; bacterial toxins, fungal toxins (mycotoxins) and plant toxins; non-biological-based chemical contaminants, and foreign materials. Food safety is a global issue; thus, the research program involves both national and international collaborations through formal and informal partnerships. Accomplishments and outcomes are used in national and international strategies delivering research results and advances to regulatory agencies, commodity organizations, industry, academia, other researchers, and consumers.

Current Relationship of this National Program to the USDA and ARS Strategic Plans and Goals:

As of 2022 (through 2026) Food Safety now falls under Goal 4 in the USDA Strategic Plan, and Objective 4.3 Prevent Foodborne illness and Protect Public Health.

https://www.usda.gov/sites/default/files/documents/usda-fy-2022-2026-strategic-plan.pdf

As of 2023 (through 2026) Food Safety also fall under Goal 4 in the ARS Strategic Plan, and Objective 4.3 Prevent Foodborne illness and Protect Public Health.

https://www.ars.usda.gov/ARSUserFiles/00000000/Plans/ARS%20Strategic%20Plan%202023_2026.pdf

Introduction

The National Food Safety Research Program (NFSRP) in collaboration with its regulatory agency and industry customers/stakeholders; and academic partners, conducts research to provide the means to ensure that the U.S.

food supply is safe for consumers, and that food and feed meet foreign and domestic regulatory requirements. The Program research and subsequent accomplishments seek ways to detect, assess, control or eliminate potentially harmful food contaminants, including both introduced and naturally occurring pathogenic bacteria, viruses and parasites, bacterial and plant toxins, fungal-toxins (mycotoxins); and non-biological-based chemical contaminants, including foreign materials such a microplastics. Since food safety is a global issue, the Program involves both national and international collaborations through formal and informal partnerships. Accomplishments and outcomes are used in national and international strategies delivering research results and advances to regulatory agencies, commodity organizations, industry, academia, research and extension agencies and consumers.

Despite many decades of research and changes in food safety regulations and guidance, the safety of the global food supply continues to be a visible public health issue, and a national and international priority. Many critical issues remain. Outbreaks of foodborne illness are still seen as a significant cause of morbidity, mortality, and chronic sequelae. The cost and burden resulting from these events often results in "economic" devastation in its broadest sense (trade issues, health care costs, litigation etc.), both nationally and internationally. Granted there has been a significant recent effort to determine if not resolve the cause of outbreaks through improved attribution determination (use of genomic technologies). However, unfortunately, and despite these concerted efforts the cause of many outbreaks often remains unresolved.

There are still a wide range of continuing food safety concerns: for example, intensive food production and processing, antimicrobial resistance, international trade, consumption habits, travel, immigration of peoples. These are now combined with newer and evolving concerns such as climate change, environmental ecology, pathogen evolution which may increase virulence/pathogenicity; and food adulteration/food fraud. Persistent outbreaks of major commodity-specific foods also still occur that potentially directly affect public health, regulations, industry, and trade. Specific examples include fresh produce, various meat types, and ready-to-eat-foods.

A predominant continuing focus is the implementation of the Food and Drug Administration's (FDA) Food Safety Modernization Act (FSMA), and its various components, for example, the "Produce Rule". The effect of FSMA to government, industry and trade should not and cannot be over-estimated.

Due to FSMA there have been major changes within several food safety/public health agencies, especially within the USDA-Food Safety Inspection Service (FSIS); and the Department of Human Health Services (DHHS) FDA, and the Center for Disease Control and Prevention (CDC). Many of these changes have and will continue to significant impact the NFSRP.

In the ***2016-2020 Action Plan***, research efforts were redirected to problem-solving through technology development, in-line with the Office of Management and Budget (OMB). ARS's job as the chief scientific in-house research agency "is finding solutions to agricultural problems that affect Americans every day from field to table"; ***https://www.epa.gov/agriculture/agriculture-information-Federal-state-and-local-sources***

The mission "ARS delivers scientific solutions to national and global agricultural challenges;" and vision "Global leadership in agricultural discoveries through scientific excellence". ***https://www.ars.usda.gov/ARSUserFiles/00000000/Plans/2018-2020%20ARS%20Strategic%20Plan.pdf***

In the ***2016-2020 Action Plan*** we suggested/argued that scientists nationally and internationally had very different opinions on what aspects of food safety research were critically important. At that time there was no apparent consensus. European and Austral-Asian food agencies were for example, stressing a greater focus on food integrity/fraud. Consequently, they refocused their research and regulatory surveillance programs, and undertook development of Food Crime Units under the auspices of their National Food Regulatory Agencies, for example, ***https://www.food.gov.uk/safety-hygiene/food-crime***.

In addition, there was increased concern regarding antimicrobial resistance; ***https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance;*** and a greater emphasis on assisting emerging nations to develop secure food supplies ***http://www.ifpri.org/topic/food-security.*** The latter, however, is not a food safety issue per se.

Ensuring adequate supplies of food does encompass some challenges to food safety when global climate change, water availability, and plant or animal diseases are considered. More apparent/important was food loss due to food waste, often a consequence of inadequate food storage; foods considered inedible due to for example, (mycotoxin) contamination; or foods not considered perfect in size or shape, known as the "ugly produce' or "wonky veg". The latter is considered responsible for ~40% of food waste. ***https://olioex.com/food-waste/the-problem-of-food-waste/***

In the past few years these differing opinions on food safety have somewhat coalesced, and a consensus is more apparent. Thus, in the development of the ***2021-2025 Action Plan***, the National Program Leaders (NPLs) for Food Safety focused on the most critical issues and needs. These are still described under the previously defined single-Component, Foodborne Contaminants; and the Components 7-Problem Statements.

In developing the ***2021-2025 Action Plan***, we also approached the task appreciating that there are many special challenges, some of which were outlined in detail in the NFSRP 2020 Retrospective Review Document. These challenges include first and foremost: balancing stakeholder and partner needs with the Program's limited fiscal and personnel resources. This is a critical issue since over time, and for a variety of reasons, there has been a reduction in actual research funds; and projects for and within the Program. A brief analysis shows that in 2005 the NFSRP had 77 appropriated funded projects with an approximate budget of $105 million and 250 scientists. As of now, for the ***2021-2025 Action Plan***, we anticipate about 45 projects, a proposed budget of approximately $109 million, (adjusted for inflation is ~$82M) and an un-determined number of scientists (anticipating 110-125). Regretfully, the Program cannot sustain such reductions and losses.

Thus, the NPL's have informed our major stakeholders that we must reduce and or eliminate certain areas of food safety research simply because we do not have the capacity or capability to conduct them. The Program will focus on the things we can do and can do well; ensuring that the research provides accomplishments that have impact, and they can be rapidly translated into practice through technology transfer. Any collaborations nationally and internationally will also focus on relevant issues to the Program, thus ensuring that targeted areas are addressed. Needless-to-say we will continue where possible and appropriate to ensure that the Program has the capability to respond when requested to unexpected research needs and/or issues from our stakeholders and partner. Finally, the Action Plan remains a living document subject to review and realignment (at any time); and when and where required and/or appropriate.

Component 1. Foodborne Contaminants

The production, processing, and distribution system for food in the U.S. remains a diverse, extensive, and easily accessible system. An open system is vulnerable to the introduction of contaminants through natural processes and global commerce, and by intentional means. Thus, the food supply (food system) must be protected from pathogens, toxins, and chemical/physical contaminants that cause disease or harm to humans and/or animals/plants within that system. The NFSRP seeks ways to assess, characterize, control and/or eliminate potentially harmful food contaminants. The NFSRP conducts research and provides scientific information and technology to producers, manufacturers, regulatory agencies, to support their efforts to provide a secure, affordable, and safe supply of food, fiber, and industrial products to consumers.

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 the 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, their ability to thrive or alter their genome. An example of identifiable area of study would include biofilms; their architecture, (antimicrobial) resistance, quorum sensing and control mechanisms.

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 provides a framework for integration of microbial genomic data with disease, enables the development of improved detection methods, 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, the evolution of virulence/pathogenicity; and the/any role/influence of commensals. Where appropriate, a more focused "omics" approach (in its broadest sense) to selected research areas will be undertaken 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 pathogens live is critical to the development of control and intervention strategies.

We will continue, but at a reduced level, an emphasis on mechanisms that allow pathogens to persist in animals and the related environment. This will drive development of mitigation and prevention strategies, as well as guidelines, policy and regulation.

Research Needs

General (animal and plant)

- Improved approaches/designs for microbes based on population-based studies, detection of emerging pathogens, and supplying data for identified data gaps.
- Improved sampling collection protocols to maximize the probability of describing the exceedingly large number of diverse organisms that inhabit ecological communities.
- Data on particular ecological niches or reservoirs for specific pathogens.
- Research to determine why serotypes decrease and re-emerge in production and processing environments.
- Data on factors which enhance or reduce fitness characteristics related to survival and growth and persistent colonization.
- Improved methods that allow evaluation of the impact of intervention or management strategies on microbial contamination throughout the entire food chain from field to plate.

Specific (plant)

- Data on the complex interactions between fungus; crop; environmental factors; and production practices.
- Data on how changes in climate impacts pathogen growth, persistence, pathogenicity and/or virulence.

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.
- Continued knowledge for developing appropriate intervention or management 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 omic- or meta- data with infection and/or disease outcome.
- Descriptions of genetic traits associated with colonization, persistence, and the development and evolution of virulence/pathogenicity, including the development and movement of resistance genes, and the role of commensalism and synergies in resistance gene acquisition.

Potential Benefits/Impact

- Improves and enhances knowledge of how microbial populations in agricultural system can potentially affect and impact food safety and 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 colonization, persistence, survival, and growth allowing for future development of improved/alternate (environmentally compatible) intervention and/or control strategies.
- How climate change impacts the complex interactions of plant-pathogen infection, growth, persistence, pathogenicity and/or virulence, especially among food crops critical for human and animal consumption; for example, grains, tree and ground nuts, and fresh and dried fruits.

Problem Statement 2. Systems Biology

Systems biology involves an integrated, multidisciplinary, multifaceted approach to study the complexities of components within biological/food systems, a central problem to food safety. Within any system, identifying the players, that is, any bacterial, viral and fungal microorganisms and/or food-borne parasites is critical. Once identified, their role or interactions within the system can determined through analyses of their genetic makeup, gene expression and products (for example toxins) produced, which directly relates to the microorganism's survival, growth, persistence etc. In order to study systems biology, quantitative technologies such as "omics" [genomics, proteomics, transcriptomics, metabolomics, and metagenomics] combined with bioinformatics can be directly applied. There is an increased need for data gained from systems biology studies to be directly applied for both pre- and post-harvest food safety. For example, whole genome sequencing (WGS) efforts have increased and allowed regulatory agencies and industry to identify and resolve for attribution purposes, some foodborne illness outbreaks. The problem is however, that WGS is being done under the GenFS umbrella on thousands of microorganisms in large part simply to have the genomic data. ***https://www.aphl.org/conferences/proceedings/Documents/2018/GenomeTrakr/Brown_2018GENFS_GTmeetingv1.pdf.*** WGS for sequencing sake, is not a research effort for the NFSRP.

All-to-frequent outbreaks of industry related microorganism contamination emphasizes the continued need to examine pathogens in actual food systems in order to lessen the foods public health risk. Thus, our goal is to use omic-technologies and apply them to the study of foodborne pathogens in complex food systems. For example, the Program will obtain and relate data directly to the biology of the microorganism, in order to understand how they fit and/or interact within the systems complexity. Areas of concern include assessing their prevalence as part of elucidating how they cause disease; their fitness (ability to survive in diverse environments); their pathogenicity (ability to infect and cause disease) and virulence (the severity of disease). The prevalence and patterns of contamination in food sectors may vary considerably and needs to be assessed and evaluated carefully. Understanding, fitness, pathogenicity and virulence are 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. Differences in microbial prevalence, pathogenicity and virulence are observed across different food production and processing systems, at different sampling times, and by using various methods. Contamination patterns reveal variation in fitness, pathogenicity and virulence; the presence of persistent or sporadic strains; evidence of bacterial transfer from production environments to processing, and from processing environments to food.

A new issue of concern is the effect that environmental stressors (pre-/post-harvest, both extrinsic and intrinsic, have on genetic variation. Previous studies for example, ***https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.2001477*** concluded that "environments influence genetic variation as well as selection"; however, there has been no systematic investigation into any linkage between various food environments, foodborne bacteria and mutational outcomes. This could potentially be a critical issue especially in pathogenic foodborne bacteria, for example, those with short generation times, limited extraneous DNA, and high mutation rates.

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 Problem Statement is the need to clearly differentiate between microorganisms that are relevant to agriculture versus food safety and/or public health.

Research Needs

- Whole genome sequencing (WGS) of specific pathogens to provide data to develop high resolution genotyping and molecular serotyping methods; for identifying virulence attributes and elucidating the differences between pathogens and non-pathogens.
- Identification and characterization of pathogen virulence and fitness factors; how they contribute to pathogen persistence and emergence across the food continuum.
- Data to determine if and/or how virulence is directly related to the infective dose.
- Data on pathogen adaptive responses to intrinsic and extrinsic food stressors such as pH, a_w, temperature, O_2, and determine their role in pathogenicity and/or persistence.
- Data on the influence on stress-induced mutagenesis; whether there is a mutation rate bias or is it random with respect to for example: adaptation, or virulence/ pathogenicity.
- Data to determine if resistance genes affect virulence, pathogenicity and/or persistence.
- Identification and characterization of fitness and virulence attributes; and the responses of specific pathogens to their environment relative to changes in immunogenicity in the host.
- A detailed investigation of food production and processing environments for bacterial pathogens; and a determination of what genetic and/or environmental factors might contribute to the presence of persistent or sporadic strains and cycle re-emergence of some strains.
- Data on the impact of changing management, production and intervention practices on the incidence of parasites as it relates to foodborne risk.
- Identification and characterization of unique fungal genes for specific biological and physiological functions. For example, how mycotoxin synthesis is transcriptionally regulated during the fungal growth cycle.
- Data on the effect of climate change on mycotoxin production in food crops. How environmental stress factors interact to affect plants, fungal growth, and subsequent mycotoxin synthesis.

Anticipated Products

- Identification 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 foodborne stressors affect mutation rates and bacterial adaptability and pathogenicity.
- Information to explain why some strains are persistent while others are cyclic or sporadic in occurrence.
- Information relating how stress factors such as climate change affect pathogen gene expression and fitness.

Potential Benefits/Impact

- Provides knowledge of which genes are required for a microorganism to become a pathogen.
- Generates data on mutation rates, and genes that contribute to variations in pathogenicity/virulence; and how gene expression is involved in adaptation, and/or persistence 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 post-harvest). 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 research will focus 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 stressed 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 independent academic, industry, and/or government sectors. Studies that suggest minimal outcome or impact will be terminated, and alternate approaches formulated.

A recent issue of concern is the effect of different interventions on bacterial physical structures, and the potential for false negative enumeration. It is known that in response to various stressors some Gram-positive bacteria undergo sporulation, where the developed spores can survive for extended periods of time until germination and outgrowth. Recent data has shown that some Gram-negative bacteria (Salmonella and Listeria) survive by changing into the viable non-culturable (VNC) state. In this state, using classical enumeration methods it has been shown that potential disease pathogens can avoid detection. Consequently, a false negative detection, leading to a false sense of pathogen free foods, an obvious regulatory issue and potential industry nightmare, with outbreaks, illnesses and potential litigation.

Research Needs

- Sampling protocols to maximize the probability of detecting contaminants especially when combined with innovative approaches to sample processing.
- Sample recovery methods with attention to sample preparation as different matrices may present unique problems.
- Methods that do not have a sample or detection bias.
- Methods that reliably differentiate between viable and non-viable agents.
- Methods that negate the issue of both false negative and false positive detection.
- Methods that address bacterial spore germination and detection of VNC microorganisms.
- Methods that are quantitative rather than qualitative or presumptive.

- Technologies that have applications in surveillance systems, for monitoring the food supply and for food defense.
- Technology development that has uniformity of application in both pre- and postharvest production and processing system.

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.
- Fulfilling requests from stakeholders (USDA-FSIS/industry) for qualitative methods, highly accurate methods with no concerns of false detection.
- Quantitative information that provides useful data for undertaking risk assessments.

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.
- Allows for greater accuracy of research and results. Data can be transformed into usable statistics that can be imparted as part of government agency's regulatory role, or by industry to show that process or product is within regulatory specifications.

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

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 NFSRP'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.

Both toxicological and toxinological studies will also be conducted; where ***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.

A new area of study under is evaluating the role and effect of mycotoxins in animal feeds. This results from reports of a high prevalence's of certain mycotoxins in feeds. Fusarium toxins such as deoxynivalenol and zearalenone, and others such as ochratoxin, a mycotoxin produced by different Aspergillus and Penicillium species are often detected in feeds, often co-occurring with many less well-known mycotoxins, or those that are masked. Development of identification; and intervention and control strategies have now become a critical international food safety, and food trade issue. Research is now specifically directed towards this concern.

Research on the development of biocontrol technologies, and crop/fungal/toxin relationships is part of this area and may also affect Problem Statement 2 (Systems Biology).

Several new areas are included in the new Action Plan: specifically: sensing technologies and their application in detecting foreign materials and food adulteration/fraud.

The development of specific (autonomous) sensing technologies, for example, nondestructive spectral sensing technologies, whole-surface [in-line] hyperspectral imaging or Raman spectroscopy are option for reducing the risks of contamination or food adulteration. Developments in this area are critical since regulatory agencies have placed more of the burden of inspection responsibility on producers and processors. Plants are also responsible for meeting other consumer protection issues as determined by regulatory agencies. An example of a recent food contamination issue is the detection of microplastics. Although no concern has been expressed by U.S. regulatory agencies, other international food safety agencies see the issue as a problem. The NFSRP is being proactive in that more studies are needed to detect/monitor these types of foreign materials; determining the risk they pose to human health and food safety/security; and if necessary developing methods to eliminate them from within the food supply.

Food adulteration, and or food fraud are issues of critical global concern, estimated to cost hundreds of billions, and projected to cost trillions with the next decade. Currently, detection technologies are focused on determining levels of target (known) contaminants. While these technologies can detect know contaminants for example melamine, the real issue is the detection (and characterization) of non-targeted (unknown) contaminants. There are a series of challenges related to these studies, which are appreciated; for example, what is considered an adulterant; developing non-targeted methods that have regulatory approval; and obtaining authentic samples for validation.

Accomplishments and promising technologies within this Problem Statement 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 will require multidisciplinary approaches to meet the challenge, and accomplishments may have far reaching effects regarding food biosecurity, food adulteration/fraud, government regulations and trade issues.

Research Needs

- Accurate, rapid, and easily used analytical detection methods: single/multiclass, single/multi-contaminant analytical methods; lab and field-based methods and instruments for analytical screening.
- Mechanism/action-based bioassays for laboratory and field use.
- Multi-task on/in-line inspection technologies that detect contaminants and quality attributes simultaneously functioning in or near real-time.
- Assays for assessing the efficacy of various processing methods to reduce or eliminate the toxicity in contaminated foods for human/animal consumption.
- Assays that have efficacy in toxico/toxinological studies.
- Intervention methods [bioremediation] to reduce bioavailability.
- Data on the fate and transport of contaminants and their derivatives in food systems and the environment for use in risk assessment by regulatory agencies.
- Provide parameters for regulatory agencies on biological residue depletion and withdrawal rates in animals.
- When requested, develop technologies that have a critical use in food defense.
- Data for use by regulatory agencies on the dose-response relationships and tissue specificity of biological toxins.
- Developing methods for the detection and identification of mycotoxins; and evaluation of mycotoxin toxicity and mechanism of action.

- Examine the negative impacts of mycotoxins on gut composition, functionality and safety in poultry, stressing the particular effect of mycotoxin combinations.
- Develop automated, low cost, accurate, on-line and hand-held, computerized inspection [sensing] systems.
- Evaluating whether current targeted technologies can be modified to detect non-targeted contaminants. If not, develop non-targeted detection technologies which can undertake the chemical/physical analysis of the entire food matrix.
- Collaborate to determine the efficacy of non-targeted detection in terms of risk.

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.
- Automated sensing systems operate with minimum human intervention and can function despite changes in physical plant structure, and environmental conditions.
- Development of specific food fingerprints which can be used for comparison to questionable products. This will directly assist in reducing food adulteration and food fraud through non-compliant inspections.

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.
- On-line, computerized sensing-systems placed or used strategically will assist and improve the regulatory and in-house inspection system; minimizing the problems of human error and variability; and increasing commercial processing productivity and profitability.
- Ensuring the safety/security and integrity of the U.S. and international food supply.

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 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. The “hurdle concept” is still considered a viable strategy for intervention and control and should be used if possible.

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; including biocontrol intervention technologies for food crops contaminated by mycotoxins, such a tree nuts, corn and grains. Several new areas of interest and/or concern will be addressed: First, the production of fresh produce, using hydroponic technology. There are many advantages to its use including: vertical stacking systems that take up less space, produce can be grown year- round, use of less water, lack of use of pesticides and other chemicals, and better control of production systems. Since the technology is relatively new, it behooves an evaluation of any production issues that might express a food safety concern. Second, an increased emphasis on active and intelligent food packaging. This decision is based on changes in consumer preferences for natural or limited processed or preserved foods, and the emergence of various packaging innovations. These innovations can increase both the safety and quality of food products and may significantly contribute to shelf-life extension.

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 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, hosed at National Center for Agricultural Utilization Research (NCAUR), Peoria, IL. If a specific strain is not available in the collection, ONP will facilitate researchers obtaining the appropriate isolate.

It is stressed that 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.

Research Needs

- Interventions that prevent colonization or modulate pathogens in the gut; target specific metabolic endpoints; decrease shedding of zoonotic pathogens at the time of slaughter.
- Data on the role of transportation and lairage, slaughter/processing methods, and equipment on pathogen survival, transfer, post-harvest processing and storage.
- Data on the effect of intrinsic (pH, a_w, Eh, nutrient content, antimicrobials, structure) and extrinsic (temperature, RH, O_2) parameters in the production, processing, handling, preparation, and storage of foods. This need includes food preparation and handling for, or by food service operators and/or consumers.
- Data that elucidate the mechanism(s) of pathogen introduction, survival and persistence in shellfish.
- Production and processing intervention/control strategies for pathogen reduction in shellfish.
- Novel antimicrobials for use in poultry and meat processing.
- For plant crops (fresh produce), obtaining data on the role of extrinsic and intrinsic factors on pathogen internalization and/or attachment; and pathogen occurrence and movement.
- For plant crops (fresh produce), obtaining data on the role and/or influence of commensals and/or non-pathogens.
- Identification of the critical control points in both production and processing of fresh produce, plant crops (grains/tree nuts) that can be mitigated through the development and implementation of intervention and control strategies.

- Evaluating hydroponic produce production as a viable technology in terms of the growth and internalization of pathogens into the produce itself through the nutrient rich water; the potential need to use fungicides to control fungal growth in high moisture systems; the potential need to use insecticides due to insect contamination. The latter may be avoidable by using of hepa-filtration systems or specialized ventilation.
- Biological control strategies to reduce mycotoxin production and contamination of food and feed crops such as corn/maize, cotton seeds, grains and tree nuts. Any new or modified effective biocontrol organisms and delivery systems must not introduce other toxic factors; for example, for the biocontrol of aflatoxins there should be no introduction or expression of the CPA or fusarium toxin genes.
- Data that assesses the role of chemicals that might act synergistically to enhance accepted interventions.
- Technologies/methods to prevent the growth of pathogenic and spoilage microorganisms in minimally preserved, brined, and fresh-cut foods.
- Use physical, chemical, or electronic systems to develop active and/or intelligent storage or packaging technologies, which have both safety and quality performance measures. Where possible link these technologies to sensor systems that can be actively monitored.
- Data on the effect of single and/or combinations of intervention technologies on pathogen reduction. Validate these data through laboratory, pilot-plant processing and commercial processing facilities.
- Data on whether combinations of non-thermal technologies can be incorporated in the hurdle concept; and determine whether single or combinations of non-thermal technologies are more effective if used in combination with traditional interventions.
- Data that evaluates the outcome/impact of intervention options for small and very-small, regulated plants.
- Data in intervention effectiveness to be for use in the development of Quantitative Microbial Risk Assessments (QMRAs).
- Data determining the effect of intervention technologies on sensory/quality deterioration, and accumulation of toxic chemical by-products.

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 will 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 interventions will include development of active and/or intelligent packaging/storage technologies that can enhance and monitor the safety, quality and shelf-life of a food product.
- 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.

- Addressing consumer needs and requirements for increased safety, quality and extension of shelf life of natural or minimally processed food products.

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 including industry, 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 U.S. and other countries producing and exporting food to the U.S. 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 use the inherent ARS expertise and modeling systems mechanisms.

Data Acquisition and Information Storage:

ARS in association with the University of Tasmania (UTas), develops and maintains a publicly available global food safety database, **ComBase-**a Combined dataBase for predictive microbiology. This resource is the number one web-based resource for quantitative and predictive food microbiology. ComBase also includes associate members, sponsors, and advisory groups (including a specific scientific advisory group) ***https://www.combase.cc/index.php/en/.*** ComBase's 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.

There are some specific (new) issues regarding ComBase to be addressed in the new Action Plan. At present, users cannot produce probabilistic estimations of microbial behavior based on data and models in ComBase. If the internet fails, there is currently no means to access ComBase. Under FDA-FSMA food companies are increasingly responsible for up-and down-stream nodes that influence food safety. Currently, ComBase has no feature that allows models to be 'linked' to predict outcomes of food processes along a simulated supply chain. Use and interpretation of ComBase requires a level of technical expertise that, is generally lacking by ComBase users, especially industry. This can be remedied, in part, by integrating training modules in ComBase that increase understanding and skills. Finally, a long-term issue is still data acquisition. Most data in ComBase were donated by ComBase Partners, ComBase Associates, and via specially funded projects, however, this does not keep pace with the needs of industry and government.

The Food Safety Research Information Office (FSRIO) is part of the NFSRP based at the National Agricultural Library ***(https://www.nal.usda.gov/fsrio/about-fsrio-0).*** FSRIO's mission is to provide to the research community and the general public information on publicly funded, and to the maximum extent practicable, privately funded food safety research initiatives for the purpose of preventing unintended duplication of food safety research; and assisting the executive and legislative branches of the Federal Government and private research entities to assess food safety research needs and priorities.

Genomics is a functional and critical part of omic-technologies and is now considered-in-part as a replacement for culture-based techniques. Under the previous Action Plan food safety regulatory agencies planned to implement the increased use of genomics, partial and/or WGS for both regulatory monitoring, attribution, and potentially for revising risk assessments. Implementation of such a redirection required developing a coordinated system of genomic sequencing technology for routine testing. Critical to the Program was 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 was made available, through the Microbial Culture Collected based at NCAUR, Peoria, IL. Additional data from isolates studies obtained from national and international collaborations was also incorporated, along with meta-data descriptors.

This research will continue as part of both the National Initiative "Interagency Collaboration on Genomics for Food and Feed Safety (Gen-FS)" [agencies within the Department of Health and Human Services and the USDA] and an international initiative "Global Microbial Identifier (GMI) latter 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.

Research Needs

Modeling

- Models that include an emphasis on probabilistic modeling to balance the deterministic approaches. This includes the influence of challenge strain(s); assessment of a model's performance; predictive value on extrapolation; and efficacy especially in complex food matrices where the intrinsic and extrinsic parameters may change.
- Data that examines and determines if growth/no-growth interface models predict the probability of growth occurring when a population faces more than one stressor/constraint.
- Models that have utility for risk assessment from both the producer and consumers perspective. There are distinctly different consequences of conservative (over) vs. non-conservative (under) prediction of growth or risk.
- Data that determines if changes in the microorganism(s) themselves occur, due to up/down regulation of genes; quorum sensing; or transfer of genetic information between species.
- Models that predict pathogen and non-pathogen behavior in complex food systems utilizing inactivation data. These types of studies are fundamental to developing Hazard Analysis Critical Control Point (HACCP) systems and regulations. Process risk models for industry that derives predictions for Critical Control Point (CCP) assessment.

- Data that demonstrates how models can be integrated more fully into supply chains [nodes], thereby increasing utility to industry and risk assessors.
- Develop or refine cooking and cooling models; in-particular for Staphylococcus, Bacillus and Clostridium species.
- Develop of refine dose-response curves for E. coli STEC, Listeria and Salmonella pathogens.
- Models that determine the effects of food safety interventions, for example carcass and produce sprays; and physical and chemical interventions, for example: radio frequency, heat, cold and Generally Recognized as Safe (GRAS) chemicals.

Database/Models

- Compile modeling data into a shared informational database through national/ international efforts.
- Write program code linking ComBase records to online databases. This feature will collect attributes for individual records, such as journal article title, abstract, authors, and institution.
- Data collection for specific organism-food combinations, enhance the value for the food systems community. Prioritization will be given to data needed to fill current database gaps, as well as records most sought after by ComBase customers.
- Derive and provide relevant data to regulatory agencies for use in HACCP programs, risk assessments, labeling, persistence, and issues relative to international trade.
- Develop the following as new ComBase initiatives:
- Producing probabilistic predictions for specific environmental conditions.
- A stand-alone version of ComBase.
- Allowing users to link a series of models for process risk estimates.
- Developing training modules for users with different skill levels.
- Collecting and archiving new data for high priority pathogen-food combinations.
- Examining new means to obtain additional data (especially from industry).
- *Continue to develop/update FSRIO website resources; conduct literature* reviews supporting food safety, enhance the FSRIO research projects database and publications feed; continue to provide “Meet the Experts” pages; develop and update Website Analytics and Outreach; and work closely with the FSRIO Working Group to ensure material within the website is useful and informative.

Genomics

- Conduct sequencing and annotation efforts on pathogens of concern that fall under research efforts in various Problem Statements.
- Continue development of a genomic database for identification of microorganisms or development of an identifier of microorganisms as a platform for storing data.
- Develop user friendly systems to aggregate, maintain, share, mine and translate genomic data for microorganisms, for example: the identification of relevant genes or for the comparison of genomes to detect outbreaks and emerging pathogens.
- Implementing an increased focus on bioinformatics (computational biology) as more sequence data becomes available, and the complexity of both the data and questions being asked becomes more sophisticated.

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.”

- The global community of food risk managers and researchers will use ComBase more to design and validate food safety risk management plans. Large, small and very small food companies will develop increasingly accurate risk-management plans via access to a larger and more relevant database, models and associated features.
- FSRIO acting as central location to provide a publicly accessible and searchable research projects database that enables access to current food safety research efforts, outcomes, and the latest up-to-date scientific information.
- 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.
- For Gen-FS include: (1) Coordinating research designed to incorporate cutting edge technology to improve identification and sequencing of pathogens obtained from clinical, food, feed, environmental, and animal sources; (2) Developing, documenting, and assessing protocols for comparing genomic sequences; and developing, sharing, and validating methods used to compare sequences and characterize pathogens; for example, single nucleotide polymorphisms (SNP) and whole genome multi-locus sequence typing (wgMLST); (3) Sharing findings that support detection and response to outbreaks, regulatory actions, recalls, and other public health interventions, and research; and (4) Coordinating inter-agency efforts to define genomic determinants for antimicrobial resistance, define mechanisms for transmission within foodborne pathogens, and coordinating activities among agencies in support of the National Action Plan for Combating Antimicrobial Resistant Bacteria (CARB). ***[Extracted from the Gen-FS Charter]. https://www.aphl.org/conferences/proceedings/Documents/2018/GenomeTrakr/Brown_2018GENFS_GTmeetingv1.pdf***

Potential Benefits/Impact

- Generating data on the responses of microorganisms to both defined and changing environmental conditions and translating these data into mathematical models and user-friendly software tools available on the internet at no cost.
- Policy makers and risk assessors will produce more robust risk management plans with greater certainty. These will be used by national and international regulatory and public health agencies, and industry, to assist in ensuring the safety of the food supply.
- FSRIO assists the research community to assess food safety research needs and priorities; allows exploration of current research activities to prevent duplication of efforts allowing for efficient use of research dollars; fosters increased cooperation among individuals and agencies engaged in food safety research; and brings awareness of these services by presenting at scientific conferences and other food safety related meetings.
- 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 U.S. and globally. The increase in occurrence 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 2019, the CDC released an AMR threats report identifying AMR bacteria of urgent concern ***(https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf).*** In 2020, a new National Action Plan for Combatting Antibiotic Resistant Bacteria (CARB) will be released. Both highlight the need for additional research to prevent the spread of AMR. Even though the USDA is not the lead agency for antibiotic use and AMR, USDA-ARS and the NFSRP are an important part of the solution.

Solution oriented research is needed to understand the factors that promote the development and spread of AMR and to identify solutions to reduce the impact of foodborne AMR bacteria on human health outcomes. Areas of concern include quantifying and assessing the fate of AMR bacteria in production and processing environments with an emphasis on foodborne pathogens. In addition, developing alternative strategies to optimize 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 antibiotic use on the food animal gastrointestinal microbiome and the development of AMR bacteria is not well defined. Research is needed to determine the impact AMR bacteria associated with food animals on public health outcomes and to identify foodborne pathogens with AMR that are of critical concern and to develop strategies to reduce their occurrence. In addition, there is a critical need for the development of alternatives to antibiotic to reduce the need for antibiotics as well as development of mitigation strategies for foodborne AMR bacteria in food producing animals. These areas are cross-linked with Problem Statements 1 and 2 within this Action Plan.

Research Needs

- Multidisciplinary approaches to identify reservoirs of AMR bacteria and to understand the development, persistence, and transmission of antimicrobial resistance.
- Identification of mechanisms (fitness factors, genetic elements, reservoirs) that enhance or decrease survival and transmission of AMR bacteria in pre- and post-harvest environments.
- Data to fill knowledge gaps in predicting risk associated with the occurrence of AMR bacteria in a given environment and the associated risks to human health outcomes.
- Assist Federal partners (i.e., National Antimicrobial Resistance Monitoring System (NARMS) and USDA's National Animal Health Monitoring System (NAHMS)) through analysis of isolates and associated WGS data to identify critical elements that promote the development/persistence of AMR pathogens and that can be used to combat their impact.
- Development of alternatives to antibiotics including management practices, pre-and probiotics, bacteriophage gene products, lytic enzymes, vaccines and other novel products to reduce AMR in food producing animals, thus reducing the need for antibiotics.
- Elucidating the ecology of foodborne AMR bacteria in terms of gene transfer, the role of the host microbiome in the development and maintenance of AMR, and the role of biofilms in the development of AMR.

Anticipated Products

- Improved detection techniques facilitating the speed, ability, and accuracy of detecting foodborne AMR bacteria in food producing animals and their products.
- Data that fills knowledge gaps in understanding how AMR bacteria develop and spread through the agricultural continuum (production, processing and retail environments.)
- Identification of the most critically important AMR foodborne pathogens and management methods to reduce their occurrence.
- Improved strategies to reduce the need for antibiotics and the occurrence 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.
- Provides data critical for assessing the risk of AMR in foodborne pathogens to human health outcomes.
- Provides data critical for identification and assessment of priority AMR pathogens and development of interventions.

- Improves strategies to reduce the need for 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.

NFSRP Resources/Locations

The following ARS locations have research projects addressing the 7 Problem Statements identified under Component 1

Pacific West Area: Albany, CA; Maricopa, AZ

Plains Area: Clay Center, NE; Fargo, ND; College Station, TX

Mid-West Area*:* West Lafayette, IN; Peoria, IL; Ames, IA

Southeast Area*:* Fayetteville, AR; Stoneville, MS; New Orleans, LA; Athens, GA; Raleigh, NC

Northeast Area: Beltsville, MD; Wyndmoor, PA

(sub locations at Delaware State University & University of Maryland Eastern Shore)

Highlighted Accomplishments

Aflatoxin management with non-aflatoxigenic-based biocontrol products in tree crops. Aflatoxin biocontrol products have been registered for pistachios and other tree nut crops for nearly a decade, but the long-term, areawide effects of biocontrol applications has not been well studied. ARS scientists in Maricopa, Arizona, analyzed soil and crop samples from biocontrol treated and untreated fields in tree nut growing areas of Arizona and California. Biocontrol strains were most prevalent in treated areas and frequencies declined with distance from treatment. However, results indicated that biocontrol strains move long distances throughout the landscape, resulting in areawide displacement of aflatoxin-producing fungi. Since biocontrol applications can reduce aflatoxin contamination risk in non-treated crops, it may not be necessary to treat every orchard annually to achieve adequate aflatoxin control in tree nuts, which will reduce the overall cost of aflatoxin mitigation. (NP108, C1, PSE, 2020-42000-023-000D)

Wild birds carry Shiga-toxigenic Escherichia coli with increased potential for human diseases. Current knowledge about the pathogenesis of Shiga toxin-producing Escherichia coli (STEC) is largely limited to strains of several predominant groups such as serotype O157:H7. Non-O157:H7 serotypes associated with foodborne outbreaks and human infections have recently increased, so more information is needed about virulence and pathogenesis of diverse STEC strains for developing a One Health mitigation strategy. ARS researchers in Albany, California, characterized the pathogenicity potential of STEC strains isolated from wild birds in a major agriculture region in California and revealed novel virulence determinants in the diverse non-O157:H7 avian STEC strains. This information is important for risk assessment in preharvest environments and contributes to developing effective control strategies to ensure the safety of food from farm to fork. (NP108, C1, PSB, 2030-42000-052-000D)

A biosensor to detect Shiga toxin-producing Escherichia coli. Shiga-toxin producing Escherichia coli (STEC) causes a wide spectrum of diseases, including hemorrhagic colitis and hemolytic uremic syndrome (HUS). Current testing methods for STEC generally include enrichment, cell plating, and genomic sequencing, which takes time to complete and delays diagnosis and treatment. ARS researchers in Albany, California, used the Cellular Analysis and Notification of Antigen Risks and Yields B-cell based biosensor technology (CANARY) to develop a rapid (within 3 minutes), sensitive (100 pg/mL), and potentially portable assay that can identify STEC by detecting Shiga toxin (Stx). This technology could be highly useful for environmental and food safety surveillance programs. (NP108, C1, PSD, 2030-42000-053-000D)

Detection of toxic mushrooms. Amatoxins are toxic substances found in several poisonous mushroom species, including the well-known "Death Cap" mushroom and their ingestion can lead to liver failure and death. Current clinical detection methods usually require extracting and detecting amatoxin from the urine of suspected amatoxin ingestion patients. ARS researchers in Albany, California, and collaborators at Saarland University (Germany) developed a method of liquid-chromatography-high resolution mass spectrometry (LC-HMS) that can detect amatoxin in urine at levels as low as 1 ng/mL. The assay is valuable for rapidly and accurately detecting amatoxins in emergencies, since amatoxin ingestion results in symptoms approximately 12 hours after ingestion, after the time when amatoxins might already be eliminated in plasma and concentrated in urine. Rapid detection allows for earlier therapeutic intervention and improved outcomes. (NP108, C1, PSD, 2030-42000-053-000D)

Detecting low levels of Salmonella in surface waters. Recovering antibiotic-resistant Salmonella from water is important for the environmental component of the National Antimicrobial Resistance Monitoring System (NARMS), but a specific or standardized method for recovering Salmonella from surface water has not been available. ARS scientists in California, Georgia, Maryland, and Nebraska collaborated to modify a standard method that consistently recovered low levels of Salmonella from water samples at frequencies equivalent to a gold standard method. Using this modified standard method, the scientists consistently recovered S. Typhimurium from 60 water samples provided by 4 laboratories at 5 different sites. This modified method is now being used in the EPA National Rivers and Streams Assessment survey and findings inform NARMS science as it expands its monitoring antibiotic-resistant Salmonella levels in water. (NP108, C1, PSC, 8042-42610-001-000D)

Manual sampling mitt for beef manufacturing trimmings for pathogen testing. All raw beef trimmings are sampled and tested for pathogens in federally-inspected meat processing facilities. The samples are collected manually using excision methods or using the non-destructive MicroTally (TM) Swab (MT-Swab). A new method using a MicroTally (TM) Mitt (MT-Mitt) has been developed to improve ease and reliability of sample collection. ARS scientists at Clay Center, Nebraska, performed trials and compared results using the MT-Swab excision with the new MT-Mitt method. They found that manual sampling of raw beef trimmings using the MT-Mitt is equivalent to the MT-Swab and excision methods for recovering bacteria. This indicates the MT-Mitt method provides an alternative sampling method with equivalent bacterial recovery and significant advantages (saves labor and is easier to use) compared to other approved methods for sampling beef trimmings. (NP108, C1, PSC, 3040-42000-021-000D)

Perfluoroalkyl substance (PFAS) depletion in dairy cattle. Poly-and perfluorinated substances (PFAS) are becoming a concern in the United States and other countries due to negative health effects associated with their presence in food and water. In some states, dairy animals exposed to feed and water contaminated with PFAS have become contaminated and their milk could not be sold, which significantly reduced economic returns for producers. ARS scientists in Fargo, North Dakota, characterized the rates of PFAS depletion from milk and edible tissues of cattle sourced from a contaminated farm. Although microscopic evaluation did not reveal tissue abnormalities in exposed animals, sulfate containing PFAS residues remained in milk and most tissues for the duration of the 22-week study. In contrast, carboxylate containing PFAS residues did not accumulate in the cattle. Data generated from the study will aid regulatory agencies in determining the relative risks of consuming milk or meat from cattle inadvertently exposed to PFAS in feed and/or water. (NP108, C1, PSA, 3060-32420-003-000D)

Remediation of contaminated grain. Fungal contamination of the U.S. corn supply can pose health risks. However, insects, including crickets, are increasingly used as an ingredient in livestock feed rations, so ARS researchers in Peoria, Illinois, and Stoneville, Mississippi, examined whether crickets can safely consume corn contaminated with the toxin fumonisin. They found crickets can readily grow on a ration of corn and do not retain fumonisin, so they can be harvested as a safe product to supplement livestock feed. Using contaminated corn for rearing insects can upcycle contaminated corn into a valuable and safe coproduct, provides grain processors with options for using a toxic waste commodity, and provides livestock producers with a safe, high-protein feed ingredient. (NP108, C1, PSD, 5010-42000-052-000D)

An artificial intelligence approach to improve corn safety. Corn is the most important U.S. crop in terms of total production with an average value of $79.5 billion during the past 4 years. Corn quality, safety, and yields are negatively impacted by fungal diseases, toxins, and adverse weather. Understanding how changes in corn proteins affect their functions is necessary to improve corn resistance to diseases, toxin contamination, and adverse weather. ARS researchers in Peoria, Illinois, and Ames, Iowa, used artificial intelligence to develop PanEffect, an interactive online database that allows plant breeders and other scientists to investigate how variations in corn proteins affect their functions. The PanEffect database is hosted by the Maize Genetics and Genomics Database and includes information on approximately 40,000 proteins from 50 different types of corn. The database allows researchers to use computer modeling for investigating the functions of proteins, and then use the resulting information to guide laboratory experiments, including breeding efforts and genetic engineering. The PanEffect database will allow scientists to identify variation in proteins more efficiently and accurately and use the information to develop varieties of corn with resistance to disease, toxin contamination, and adverse weather. (NP108, C1, PSB, 5010-42000-053-000D)

Biomapping analyses for broiler microbiome. Hatcheries represent a commercial broiler management nexus point between egg production and live production. ARS researchers in Athens, Georgia, biomapped general microbial communities and Salmonella populations from a commercial broiler hatchery facility. They found Salmonella in 26 percent of areas mapped; it was highest in floor drain and transport truck swabs (56 percent) and in the hatch and post-hatch hatchery areas (50 percent). They also identified the dominant Salmonella serotypes and found that serotypes most associated with outbreaks made up only 6 percent of all types recovered in the survey. They also found that Typhimurium sequences matched the live vaccine strain applied directly onto the newly chicks. These findings highlight the complex diversity of general microbial and Salmonella populations in commercial hatcheries. They also suggest hatchery managers can focus on facility floor drains and transport trucks as potentially important critical control points for Salmonella mitigation efforts to reduce loads and serotypes entering live production farms. (NP108, C1, PSA & PSB, 6040-32000-012-000D)

Deep learning models for hypercubes from foodborne bacteria. Foodborne pathogens persist as a serious public safety concern in the United States that affects millions of people annually. While combining hyperspectral microscope imaging (HMI) with deep learning (DL) methods presents a potent strategy for the swift and accurate detection of foodborne bacteria, the widespread application of HMI-DL for food safety is somewhat constrained by generalization issues of the DL models for bacterial detection. ARS researchers at Athens, Georgia, developed an advanced artificial intelligence (AI) algorithm to identify and address a persistent problem with the generalizability of current AI models. This new AI approach improves data generalizability by eliminating the need for intricate per-image calibrations, a notable hurdle in the application of darkfield HMI technologies. Results showed that implementing this correction strategy maintained bacterial detectability and boosted the accuracy of the current Fusion-Net AI model from 38-70 percent to 95-99 percent. The enhanced AI model developed by ARS is a critical step towards the seamless integration of powerful HMI techniques into practical food safety investigations, marking a considerable advancement in foodborne pathogen detection. (NP108, C1, PSC, 6040-42440-001-000D)

pH modeling to assure safety of acidified foods. ARS scientists in Raleigh, North Carolina, used publicly available software they previously developed to devise methods for assessing how individual ingredients affect the final product pH of an acidified elderberry syrup. Results from the studies included a measure of total ingredient buffering (defined as tBeta) for the ingredients in the syrup product. These data showed that the buffer modeling methods may be used to guide manufacturers when adjusting formulations to achieve a desired pH for safety and/or product quality. Several large food companies that supply ingredients or manufacture acid and acidified foods have adapted the technology to use with product development and to help meet regulatory compliance for pH and pH stability. The measurement of tBeta developed for this work has been applied to more than 40 ingredients common to other acid and acidified foods and the data have been made available in a public database file on the USDA software download website. The software and data for buffer modeling have also been presented to the Food and Drug Administration as a scientific tool that can help clarify acid and acidified food regulations. (NP108, C1, PSF, 6070-41420-009-000D)

Microgravity simulator for space biology research. As humans move closer to undertaking long-duration space missions, it is critical to develop a deep understanding of how microgravity—both during spaceflight and a reduced-gravity environment on the Moon and Mars—affects humans, animals, plants, and microorganisms. One major challenge in current space biology research is the lack of precise, versatile, and affordable testing platforms allowing simultaneous evaluation of multiple treatments or replications under identical gravitational and environmental conditions (temperature, humidity, atmospheric composition, etc.). ARS researchers at Beltsville, Maryland, designed and prototyped a novel 3D microgravity simulator with multiplatform and multi-spectral modulation capabilities. This invention enables multiple biological and microbiological trials to be conducted simultaneously under simulated microgravity, each at precisely and independently controllable variables of light spectrum, intensity, and photoperiod. (NP108, C1, PSB, 8042-32420-009-000D)

Forward processing and risk management in romaine lettuce. Enterohemorrhagic E. coli (EHEC) outbreaks from 2017 to 2020 associated with romaine lettuce suggested “forward processing” might be a factor in these outbreaks. Forward processing is an industry practice of shipping raw commodities from production areas in California and Arizona to distant facilities (e.g., East Coast) for processing and regional marketing. ARS researchers in Beltsville, Maryland, collaborated with commercial romaine producers/processors to characterize how forward processing affects EHEC survival and its potential effects on cell physiology and infectivity. Researchers sampled and tracked romaine lettuce originating from Salinas, California, and transported via forward and source processing to Maryland and California. Their findings indicate that the forward processing practice does not enhance EHEC growth or survival. Processors can use the information in risk assessments of fresh produce commodity transportation and management. (NP108, C1, PSB, 8042-32420-009-000D)

Reducing Escherichia coli with biochars and filters. Foodborne pathogens such as Shiga toxin-producing Escherichia coli (E. coli) can contaminate irrigation water, leading to contaminated fresh produce and human foodborne illnesses. ARS researchers in Wyndmoor, Pennsylvania, used multiple types of biochar to construct biochar/sand irrigation water filters for reducing E. coli in irrigation water. Reductions of greater than 4.0 log CFU/ml of E. coli in irrigation water were achieved when biochar/sand filters were optimized by biochar type and preparation, biochar/sand ratios were adjusted, and other factors affecting filtration were adjusted. These results may provide practical ways fresh produce growers can reduce bacterial foodborne pathogens in crop irrigation water to prevent contamination of fresh produce and human illnesses. (NP108, C1, PSE, 8072-41420-022-000D)

Inactivation of avian influenza virus (AIV) in beef patties. Since 2022, approximately 100 million poultry have been lost in the United States alone due to AIV. In 2024, AIV was detected in dairy cows and in raw milk from dairy cattle infected with AIV. As of October 2024, nine cases of bird flu infection had also been confirmed in people exposed to sick or infected dairy cows. Culled dairy cows comprise about 10 percent of U.S. beef production. ARS scientists in Wyndmoor, Pennsylvania, and Athens, Georgia, inoculated ground beef patties (20 percent fat) with high levels of AIV. Cooking inoculated ground beef patties to a rare degree of doneness (about 120°F) reduced AIV by about 300 to 500 fold per gram of patty, while cooking patties to a medium degree of doneness (about 145°F) or to well done (about 160°F and the FSIS-recommended minimum internal temperature for ground beef) reduced infectious virus levels at least 500,000 fold per gram of patty (no infectious virus could be detected). Results from this small study suggest that the current risk for humans becoming infected with AIV from a beef source is negligible when patties are heated to FSIS temperature recommendations. (NP108, C1, PSE, 8072-41420-024-000D)

Lethality process parameters for pathogen inactivation in ready-to-eat (RTE) meat products. Heating processes commonly used for large mass meat products may not meet FSIS recommended lethality (Appendix A) performance guidelines, allowing pathogens to survive during cooking and/or outgrowth during cooling and storage. ARS scientists in Wyndmoor, Pennsylvania, worked with an industry partner to develop and validate processing conditions for cured, restructured, smoked boneless ham and cured pork necks following Appendix A guidelines. Hams and pork necks were inoculated with multi-strain cocktails of Salmonella (Sal) or Listeria monocytogenes (Lm) at about 10 to 100 million cells/product, and then cooked within a commercial smokehouse under different temperature, time, smoking, and relative humidity (RH) cycles. Results populated data gaps related to time, temperature, and RH parameters that are now elaborated in Appendix A. These data established reductions of about 60 thousand to 80 million cells of Lm and Sal in hams and pork necks. These findings will help processors meet current regulatory guidelines for killing pathogens in large meat products. (NP108, C1, PSE, 8072-41420-024-000D)

Pulsed light plus novel sanitizers mean clean lettuce. The produce industry employs chlorine-based chemical sanitizer washes to reduce contamination, but safer and more effective methods are needed. ARS researchers in Wyndmoor, Pennsylvania, determined that high intensity short time pulsed light (PL) combined with an antimicrobial solution (AW) that contains organic acid and Nisin substantially enhanced pathogen control that was synergistic rather than merely additive and killed more than 99 percent of pathogenic Escherichia coli in Romaine lettuce. In addition, the scientists did not observe any resurgence of pathogenic E. coli growth or deterioration in quality either immediately after treatment or after 7 days of refrigerated storage. This combination treatment of PL and AW holds promise as a valuable tool for producers and processors of fresh and fresh-cut fruits and vegetables. (NP108, C1, PSE, 8072-41420-025-000D)

Minimizing chlorine byproducts in fresh produce wash water. The produce industry commonly uses chlorine to sanitize its products and often maximizes its antimicrobial efficacy by adjusting its pH with citric acid. Earlier ARS results indicated that citric acid reacts with chlorine to generate high amounts of trichloromethane, a probable carcinogen, so alternatives to citric acid are needed to minimize the formation of chlorine byproducts. ARS scientists in Wyndmoor, Pennsylvania, evaluated 13 organic and inorganic pH adjusters as potential alternatives to citric acid. Their results showed that inorganic acids and many organic acids other than citric and malic acids maintained effective chlorine levels in wash water and led to minimal formation of trichloromethane (less than 5 parts per billion). These findings suggest the food industry can replace citric acid with inorganic acids and certain organic acids to control pH in chlorinated water, stabilize the chlorine level in wash water, and minimize the formation of undesirable chlorine byproducts. (NP108, C1, PSE, 8072-41420-025-000D)

Prediction of Salmonella consumed from chicken. Salmonella from chicken is a leading cause of foodborne illness in the United States and around the world. This risk to public health depends in part on how much Salmonella is consumed, but it is difficult to predict because different chicken handling practices can affect Salmonella levels. ARS scientist in Princess Anne, Maryland, developed a computer model to better predict how chicken handling practices affect how much Salmonella might be consumed. The model provides a better simulation of actual observations and, compared to older models, predicts less consumption of Salmonella from chicken, so provides a better model for assessing chicken safety. It can also save the chicken industry $10 million per chicken recall and benefit consumers by supporting better nutrition and food security. (NP108, C1, PSE, 8072-42000-087-000D)

Drug residues in cattle “raised without antibiotics (RWA)”. In 2022, a study reported that urine samples from 15 percent of RWA cattle at a U.S. slaughter establishment contained detectable levels of antibiotic residues. ARS scientists in Wyndmoor, Pennsylvania, collaborated with FSIS and analyzed kidney and liver samples from 189 RWA cattle across the United States for 185 veterinary drugs, using sophisticated methods of analysis to identify, quantify, and confirm any of the monitored drug residues in multiple samples. Twenty percent of the RWA animals were found to contain ultra-trace residues of antibiotics commonly used to treat illnesses in cattle. FSIS is following up with producers to further investigate the causes of the findings, and possible changes in RWA policies and production practices are being considered. (NP108, C1, PSD, 8072-42000-088-000D)

Replacing the kidney inhibition swab (KIS) method. The KIS test has been used by FSIS inspectors at slaughter establishments since 2012 to screen kidneys from carcasses for illegal levels of antibiotic residues. Although the screening test is inexpensive and easy to perform, it takes 3 hours and leads to high rates of false positives and negatives. To improve performance, ARS scientists in Wyndmoor, Pennsylvania, compared KIS readings by eye with readings from a handheld colorimeter and found the colorimeter did not provide an advantage worth the added cost. A commercial lateral flow immunoassay that merely took 20 minutes was also evaluated, but it was found to be useful at only regulatory levels for a few of the drugs of concern. Based on ARS recommendations, FSIS is reconsidering its KIS instructions to inspectors to reduce the number of false positive samples sent to the labs, and vendors are working to produce better screening tests to meet FSIS needs. (NP108, C1, PSD, 8072-42000-088-000D)

Per- and polyfluoroalkyl substances (PFAS) not routinely monitored. U.S. Environmental Protection Agency (EPA) recognizes that more than 16,000 chemicals in commerce have PFAS chemistry, but many of their identities are unknown, and analytical standards to test for them are not available. As a result, only about 40 PFAS are tested using targeted methods, while many others remain undetected. ARS scientists in Wyndmoor, Pennsylvania, developed non-targeted workflows using high-resolution mass spectroscopy to identify unknown PFAS in food packaging samples. The workflows were then validated to minimize the rates of false positives and false negatives. The results demonstrated the prevalence of both emerging and legacy PFAS in food packaging samples, including reports of some of PFAS identified for the first time that may be added to target lists in the future. (NP108, C1, PSD, 8072-42000-088-000D)

Enhancing the safety of cold smoked salmon. Cold smoked salmon is a high-value seafood product, but its commercial manufacturing does not include a cooking process normally used to kill foodborne pathogens such as Listeria monocytogenes. Instead, cold smoked salmon relies on various antimicrobial compounds in the liquid smoke and refrigeration for food preservation, but this is not sufficient to prevent the growth of L. monocytogenes. ARS scientists at Wyndmoor, Pennsylvania, developed a mathematical model to predict the growth probability of L. monocytogenes in cold smoked salmon as a function of storage temperature and time. The model has a greater than 89 percent accuracy level for predicting no growth of L. monocytogenes and provides a risk-based approach to manage the shelf life of cold smoked salmon and the growth of L. monocytogenes in cold smoked salmon. (NP108, C1, PSF, 8072-42000-092-000D)

Amplified luminescent proximity homogenous assay-linked immunosorbent assay (AlphaLISA). Enzyme-linked immunosorbent assays (ELISAs) are one of the most widely adopted detection platforms for quantifying analytes in biological samples and are known for their simplicity and sensitivity. However, their typical dynamic range is relatively narrow, and their automation is hampered by mandatory wash steps. The "no wash" alternative, known as the AlphaLISA, overcomes these technical hurdles. ARS researchers at Wyndmoor, Pennsylvania, created an innovative platform, known as the oligo-Alpha, that used the same principles as the AlphaLISA and that can detect nucleic acids instead of proteins. This technology has the advantage of a large dynamic range, rapid testing time, and reduced hands-on workflow resulting from the ability to sequentially overlay the reagents, and is readily automated. Despite its recent development, the oligo-Alpha has already been adapted for detecting Listeria monocytogenes and is currently being applied to other foodborne pathogens within the Campylobacter genus. (NP108, C1, PSC, 8072-42000-093-000D)

Turkey vaccination for multidrug-resistant Salmonella. Multidrug-resistant Salmonella enterica serotype Infantis (MDR S. Infantis) has emerged as one of the most common Salmonella serotypes isolated from U.S. poultry. ARS researchers in Ames, Iowa, discovered that MDR S. Infantis can be found in turkey bone marrow, which is not a common site to isolate Salmonella. This discovery in bone marrow is concerning because the pathogen could potentially contaminate bone meal or mechanically separated poultry meat during processing. However, vaccination with a commercially available Salmonella vaccine was found to protect against MDR S. Infantis in bone marrow and reduced Salmonella in the intestinal tract, providing producers with a method to reduce contamination of food products. (NP108, C1, PSE, 5030-32000-227-000D)

Multidrug-resistant (MDR) Salmonella Reading isolates from turkeys and humans. ARS scientists in Ames, Iowa, investigated potential causes for a human foodborne outbreak of Salmonella enterica serotype Reading involving turkey products in the United States and Canada from 2017-2019. They compared the DNA make-up of MDR S. Reading isolates associated with the outbreak to Reading isolates collected before the outbreak period. Unique genetic traits were identified, including resistance to a toxin involved in competing with other bacteria in the intestine (i.e., competitive exclusion). The results provide turkey stakeholders with information on genetic features within the emergent Reading outbreak isolates that could have promoted Salmonella dissemination within the turkey production environment and the subsequent human outbreak from contaminated products. The unique genetic features can be targeted in turkey production and products for detecting pathogens associated with foodborne illness outbreaks. (NP108, C1, PSE, 5030-32000-227-000D)

Differential fitness of Salmonella serotypes in enrichment media. Detecting virulent serotypes of Salmonella during routine food surveillance is needed to ensure food safety, but media typically used to grow and identify Salmonella shows a bias toward different serotypes. ARS researchers in Albany, California, and Clay Center, Nebraska, collaborated with a University of Georgia scientist and demonstrated that Salmonella serotype Enteritidis was more likely to be isolated from Tetrathionate Broth media, while serotype Infantis was more likely to be isolated using Rappaport Vassiliadis Soya Peptone Broth. Additionally, the research showed that the lesser virulent serotype Kentucky was able to outperform the other serotypes in both media when its initial inoculation level was 10 times higher, but not when inoculated at equivalent levels. This observation was critical for FSIS, since it indicated the high recovery of serotype Kentucky from some FSIS samples may be due to high levels of Kentucky on the product and not from better performance of that serotype in standard enrichments. (NP108, C1, PSC, 2030-42000-052-000D)

Quantifying antibiotic resistant Escherichia coli in the environment. Antimicrobial resistance genes in bacteria are easily shared among bacteria and can reduce the effectiveness of antibiotics used for humans. There are concerns these genes can be transferred to humans via water, soil, and air, but measuring them in the environment is difficult, time consuming, and expensive. ARS scientists in Clay Center, Nebraska, partnered with other federal agencies to develop and evaluate methods to detect and count Escherichia coli in water that carry genes for a particularly dangerous type of drug resistance called extended spectrum-beta-lactamase resistance. The effort included a comparison with a global standard method, a modification of an EPA standard method, and a modification of a widely used and commercially available water-quality test. The results provided an effective method needed by the National Antibiotic Resistance Monitoring System (NARMS) for a new national survey of drug-resistant bacteria in surface waters. (NP108, C1, PSA, PSE & PSG, 3040-32000-035-000D)