Microbiology

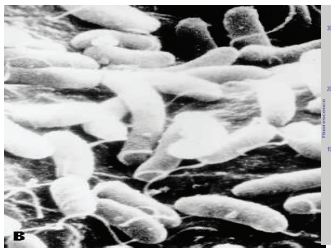
Food Safety and Biosecurity

We have the capability:

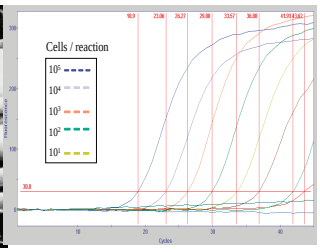
- to develop a comprehensive genomic database to identify and assess the biological threat of foodborne pathogens in the United States from imported food and feed products using conventional and state-of-the-art molecular tools, such as microarray bio-chip technology and real-time polymerase chain reaction (PCR).
- to investigate the ecology, epidemiology, virulence and molecular characteristics of foodborne pathogen populations and select agents for source tracking, delineating transmission pathways and better identifying targeted control measures in poultry, cattle, aquaculture and clinical environments. Molecular typing methods, such as pulsed-field gel electrophoresis, antibiogram patterns, multi-locus sequence typing, PCR-restriction fragment length polymorphism, ribosomal rRNA operon typing and ribotyping, are used to create databases of bacterial DNA fingerprints.
- to develop intervention strategies (physical, chemical or biological) to reduce the frequency, incidence and levels of multi-drug resistant microorganisms and other key pathogens in the U.S. food supply.
- to develop rapid and sensitive detection methods (conventional and molecular) and advance existing methodologies for isolating and identifying microbial agents and toxins in fresh and processed foods that might be used in biological warfare and for proactive counterterrorism.
- to develop, validate and transfer new and improved commercial test kits used for the detection of foodborne pathogens, select agents and toxins in complex food matrices.
- to examine isolation and detection protocols for pathogens associated with foodborne outbreaks.
- to detect mechanisms of resistance to antimicrobial agents in foodborne pathogens.

- ***E. coli* strains can cause diseases, such as diarrhea and urinary tract infections.** We surveyed food samples obtained from supermarkets and shops selling ready to eat foods in Argentina for pathogenic *E. coli*. The isolates were characterized for virulence genes by multiplex PCR targeting toxin genes. This investigation illustrates the need for careful monitoring of sources of foodborne pathogens in the food supply since ready-to-eat foods could represent an important reservoir of virulence genes.

- ***Vibrio parahaemolyticus* is a marine bacterium that causes enteritis in humans through consumption of seafood.** Outbreaks of *V. parahaemolyticus* gastroenteritis in the U.S. emphasized the need to develop molecular methods for identification and differentiation of these pathogens. A multiplex PCR method was developed by targeting genes to identify specifically these virulent *Vibrio* O3:K6 strains in seafood samples. We have cloned and sequenced a phage-related/chemotaxis-related gene from *Vibrio parahaemolyticus* O3:K6 strains that render these strains pathogenic.



Vibrio parahaemolyticus

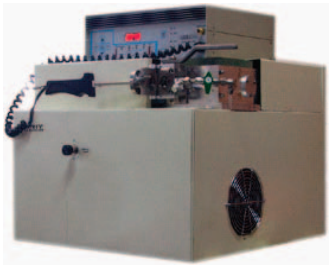


Real Time PCR

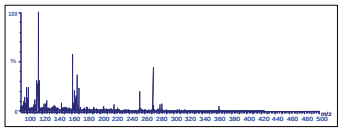
Collaboration with Dr. Susan McCarthy, Seafood Lab, CFSAN on validation and development of Real Time PCR methods

FOOD BIOTERRORISM

Characterization of *Salmonella enterica* and *Vibrio* strains by pyrolysis metastable atom bombardment mass spectrometry with multivariate statistical and artificial neural network pattern recognition.

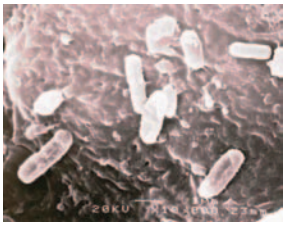


Matrix-assisted laser desorption/ ionization time-of-flight spectrometer detection of food-borne pathogen biomarker proteins.



EMERGING FOOD-BORNE PATHOGENS

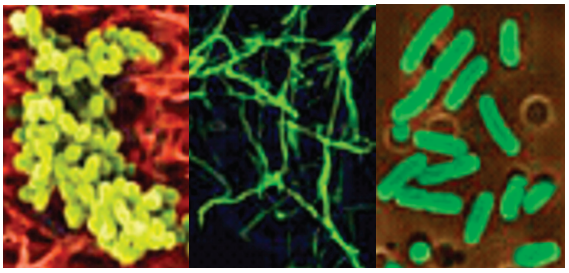
Assessing threats of emerging FBP, such as multi-drug resistant Salmonella serovars from imported food and feed , and their potential to cause infections.



The microbiological safety of food has become an important concern of consumers, industry and regulatory agencies. Rapid identification of pathogenic bacteria is important to protect public health in a suspected bioterrorist attack. We have collaborations with FDA Centers, academic institutions and public health laboratories on a variety of projects that identify and DNA fingerprint bacteria involved in disease outbreaks and adulterated foods. This information will be valuable in augmenting FDA's scientific capability in conducting efficient and effective food safety policies. This approach, which engages the public to make informed decisions with regard to food safety and related issues, is a vital approach in an overall effort to improve food safety.

Through the development of rapid and sensitive methods, we are able to detect foodborne pathogens and determine the mechanisms of pathogenesis. We are committed in developing an integrated approach to food safety and biosecurity consistent with the FDA mission and objectives.

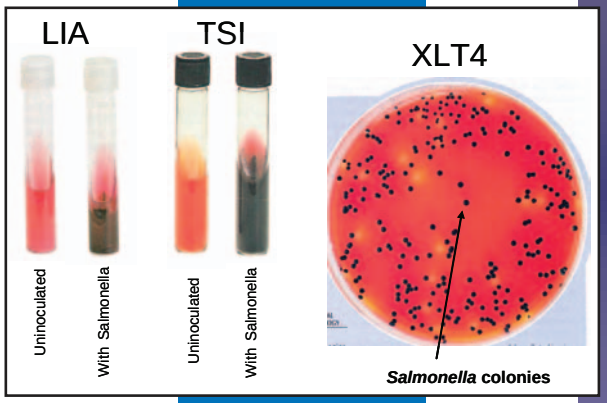
Rapid Identification Of Possible Bioterrorism Agents



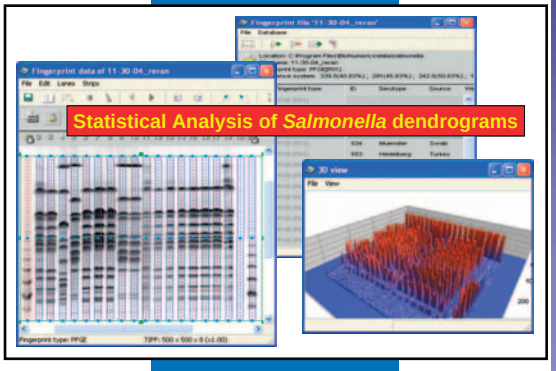
- Rapid identification of pathogenic bacteria is important to protect public health in a suspected bioterrorist attack.
- Some of the bacterial agents to be expected are *Bacillus anthracis, Campylobacter, Clostridium, Escherichia coli* O157, *Listeria, Salmonella, Shigella, Staphylococcus, Vibrio,* and *Yersinia*.
- In collaboration with the Division of System Toxicology chemist, we are comparing novel mass spectrometric methods with cultural methods, serological tests, and molecular methods for rapid identification.

Some examples of the expertise of the team are listed below:

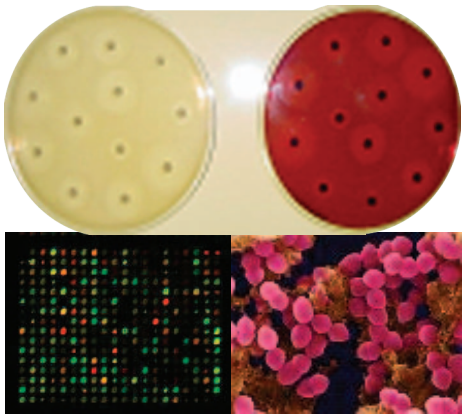
- **Salmonellosis, a disease caused by the foodborne pathogen *Salmonella*, accounts for nearly one-third of the deaths from foodborne illnesses in the U.S..** In collaboration with West Virginia University scientists, we evaluated the prevalence and source/vectors of *Salmonella* colonization on turkey farms, measured the intrinsic antibiotic resistance in *Salmonella* isolates using the disk diffusion assay and minimum inhibitory concentration (MIC) broth dilution methods, and determined the genetic diversity of *Salmonella* isolates using molecular genetic techniques to delineate possible transmission pathways and bacterial source tracking to show how the pathogen moves within the production facility. Based on the data from these studies, the FDA can make recommendations on the use of antibiotics by the turkey industry and develop guidelines for pre harvest control of *Salmonella* serovars in the poultry industry.



- **Methods for the rapid detection of *Salmonella enterica* and *Vibrio* strains by pyrolysis metastable atom bombardment mass spectrometry with multivariate statistical and artificial neural network pattern recognition and matrix-assisted laser desorption/ ionization time-of-flight spectrometer detection of food-borne pathogen biomarker proteins are being developed in collaboration with chemists in the Division of Systems Toxicology.** Research is also being conducted in the use of flow cytometry to facilitate the isolation of bacteria from contaminated food samples for rapid identification.

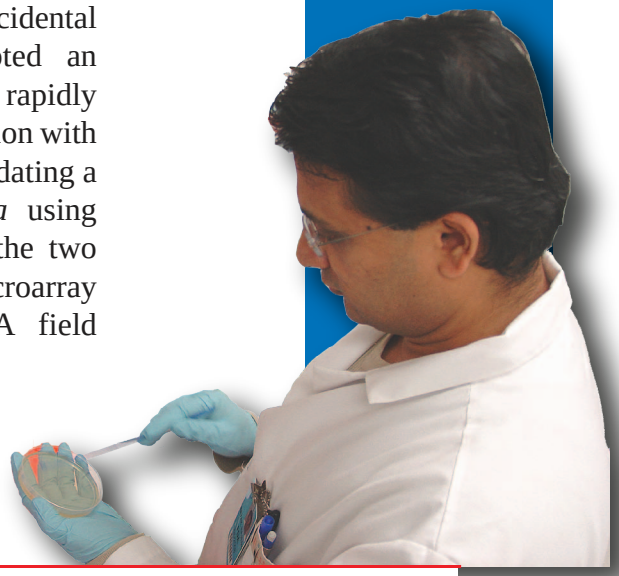


- **Campylobacteriosis, an infectious disease caused by *Campylobacter jejuni* and *Campylobacter coli*, is treated by fluoroquinolone antibiotics in clinical practice.** However, use of these drugs in animal husbandry may select for fluoroquinolone-resistant campylobacters and thereby compromise the clinical treatment of infection. Molecular typing is a powerful epidemilogical tool in characterization of campylobacter responsible for the outbreak of infectious diseases. We used PCR-RFLP and PFGE to characterize fluoroquinolone-resistant campylobacter strains from poultry. In addition, the antimicrobial resistance profiles of these strains were also determined. These data are useful in determining the origin of these bacteria for the subsequent prevention of campylobacteriosis and dissemination of antibiotic resistance genes.



DETECTION OF FOOD-BORNE PATHOGENS
Use of conventional, PCR-based and microarray technology in detection of food-borne pathogens (FBP), such as *Salmonella, Campylobacter, E. coli, Aeromonas, Vibrio, Enterococcus,* and *Staphylococcus*

- **The U.S. imports food and feed products from all over the world, and it is the FDA's responsibility to ensure that these imported foreign foods conform to strict federal standards with respect to product quality and microbial safety.** The threats of either deliberate or accidental contamination of our food supply have prompted an appropriate re-examination of methodologies to rapidly isolate and identify bacterial pathogens. In collaboration with FDA's CFSAN, we are currently developing and validating a biochip to identify virulent strains of *Salmonella* using microarray technology. The joint effort between the two FDA Centers will be useful in transferring microarray technology from the research stage to the FDA field laboratories and law enforcement mobile labs.



www.fda.gov/nctr/science/divisions/micro.htm

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