Division of Microbiology

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Microbiology Executive Summary

Introduction

The Division of Microbiology at NCTR serves a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology. The Division of Microbiology also responds to microbial surveillance and diagnostic needs for research projects within NCTR and FDA. The Division of Microbiology has a multidisciplinary staff including 14 research scientists and 17 research support staff, postdoctoral fellows, undergraduate and graduate students, visiting scientists, and program support specialists. The Microbiology Division has the staff, the know-how, and the facilities to help address the scientific challenges encountered by FDA and other government organizations. Some examples of the research projects within the Division and collaborative research with scientists from other NCTR Divisions, FDA Centers, academic institutions, and industry are described below. Projects are based on FDA priorities and programmatic expertise. The research program is divided into five focal areas: 1) food safety, food biosecurity, and methods development; 2) antimicrobial resistance; 3) gastrointestinal microbiology and host interactions; 4) environmental biotechnology; and 5) microbiological surveillance and diagnostic support of research.

FY 2006 Accomplishments

Division of Microbiology scientists are engaged in research addressing a variety of critically important issues in food safety and biosecurity relevant to the missions of FDA and other regulatory agencies. Recent outbreaks of *E. coli* O157:H7 and *Salmonella* underscore the necessity to address the threat of foodborne pathogens in our food supply. For example, in collaboration with FDA's Center for Food Safety and Applied Nutrition (CFSAN) and United States Department of Agriculture's Agricultural Research Service (ARS), microarray biochips are being developed and validated for rapid and accurate identification of multiple virulence and antimicrobial resistance genes in *Salmonella* serovars. In collaboration with CFSAN, the Division has characterized *Salmonella* and *Vibrio* spp. isolated from seafood samples using the most current state-of-the-art-molecular techniques.

Scientists in the Division of Microbiology collaborate with microbiologists at the FDA's Arkansas Regional Laboratory (ARL) to survey imported fish samples for antibiotic-resistant bacteria. The genetic determinants conferring antibiotic resistance in these bacteria are also being characterized.

In collaboration with NCTR ARL Bioterrorism Mass Spectrometry (MS) programs, methods are being developed for the rapid detection of bacterial foodborne pathogens using pyrolysis and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS) technology with multivariate statistics and artificial neural-network pattern recognition. Division scientists have also developed and completed research for an interagency agreement (IAG) with the USDA and the Department of Homeland Security for a project on the survivability of *Bacillus anthracis* in foods.

FDA is increasingly being called upon to make regulatory judgments on safety questions related to the gastrointestinal microfloras of humans and animals. The Division of Microbiology research staff has the expertise and long-standing interest in assessing the risks to the gastrointestinal microflora of humans of antimicrobial compounds ingested in food residues, probiotics, and dietary supplements. Division scientists provide guidance and expert advice to FDA, other national regulatory agencies, and the World Health Organization on the potential human-health risks associated with the use of antimicrobial agents, competitive exclusion products, probiotics, and dietary supplements in veterinary and human clinical medicine.

Microbiology scientists have developed a series of molecular techniques that can measure perturbations of the intestinal microflora population resulting from exposure of the consumer to antimicrobial residues, probiotics, and dietary supplements. Research has also been undertaken to address the role of intestinal microflora in the metabolism of xenobiotic compounds. In addition, experiments are being conducted on antimicrobial resistance mechanisms, such as efflux pumps, found in commensal organisms in the gastrointestinal tract. The Division is also evaluating the effects of fluoroquinolones on resistance development in bacteria from the human intestinal tract.

Division scientists have entered into a Cooperative Research and Development Agreement (CRADA) with Pfizer Animal Health to study the degradation of the veterinary antimicrobial ceftiofur by the normal bovine intestinal microflora. Results from the investigation will allow FDA to evaluate the long-term use of this antibiotic on the development of antimicrobial resistance and may give guidance for understanding the drug characteristics that minimize resistance development. An FDA Office of Women's Health project has been initiated to investigate the protective effect of *Lactobacillus* against *Staphylococcus aureus*-mediated toxic shock syndrome. This represents a new area of research for the Division in potential probiotic issues in women's health. Another new field for investigation in the Division of Microbiology is determining the critical role that the normal microflora of the human skin has in the metabolism of tattoo pigments and topically applied colorants. A comprehensive study has been completed on the enzymatic mechanisms of azo dye degradation by skin microflora. Microbiology scientists are developing proteomic approaches to identify in *Staphylococcus aureus* the extracellular proteins responsible for staphylococcal pneumonia.

The environmental biotechnology research program in the Division of Microbiology supports FDA in its assessment of potential environmental impacts of regulated chemicals during their lifetime in the environment. To this end, the research program is incorporating the new omics technologies to elucidate the microbial degradation of polycyclic aromatic hydrocarbons (PAHs) found in industrial, agricultural, and municipal effluents and in contaminated sediments. The use of systems-biology approaches for elucidating the metabolic pathways of PAH degradation will further help FDA understand the fate of these biohazardous compounds in the environment and in the human body. Research on the degradation of antimicrobial agents, such as fluoroquinolones, to biologically inactive products continues to be a major emphasis in the Division of Microbiology. This investigation has increased our understanding of the ability of microorganisms to degrade residues of antimicrobial drugs, thereby reducing the pressure for the selection of drug-resistant strains of bacteria.

The continuing primary mission of the Surveillance and Diagnostic Program is to provide the assurance that NCTR research data is not compromised by the use of infected or unhealthy experimental animals. During FY 2006, the Surveillance/Diagnostic Program prevented

pathogen introduction by quarantined animals by 1) detecting bacterial pathogens in the cage water from commercially purchased mice; 2) monitoring the health, environment, and food of the animals in the established NCTR breeder colonies; 3) working closely with the Division of Veterinary Services to monitor the success of a bacterial pathogen eradication program in the NCTR breeder mouse population; and 4) expanding the use of molecular techniques to detect the presence of difficult-to-culture pathogenic bacteria. The Division provided microbial cultures to researchers in the United States and foreign countries as well as to researchers at NCTR. Microbiology scientists provided technical support to NCTR researchers by 1) supplying and maintaining microbial cultures used for research; 2) supplying culture media and reagents; and 3) providing culture identification and antibiotic sensitivity testing.

FY 2007 Plans

Food Safety and Biosecurity, Food Biodefense, and Methods Development

1) In collaboration with CFSAN and USDA, two *Salmonella* biochips are being developed and validated for rapid and accurate identification of multiple virulence and antimicrobial resistance genes in *Salmonella* serovars. The joint effort between the FDA Centers and USDA will be useful in transferring microarray technology from the research stage at NCTR to the FDA field laboratories and law enforcement mobile laboratories.

2) In collaboration with the ORA ARL Bioterrorism Mass Spectrometry project, researchers in the Division of Microbiology are continuing to develop and validate methods for rapid detection of multiple bacterial pathogens using pyrolysis and MALDI-ToF MS technology with multivariate statistical and artificial neural-network pattern recognition.

3) *Salmonella* and *Vibrio* spp. isolated from seafood samples will be characterized by pulsed-field gel electrophoresis (PFGE), multiplex PCR (polymerase chain reaction) ribotyping, ERIC (enterobacterial repetitive intergenic consensus)-PCR, multilocus seqencing, and RAPD (randomly amplified polymorphic DNA) methods. Multidrug-resistant bacteria, including sulfonamide and ciprofloxacin-resistant *Salmonella* spp., will be characterized for integrons and plasmids. The amplified integrons will be sequenced to determine any unique characteristics.

4) The Division will continue working on a project in collaboration with USDA on the survivability of *Bacillus anthracis.*

Antimicrobial Resistance

1) The *van* operons from 17 human vancomycin-resistant enterococcal isolates were amplified and tested for differences by a PCR-RFLP (restriction fragment length polymorphism) method. These isolates had differences in the operons. The *van* operons from these isolates will be cloned and sequenced to determine differences at the sequence level.

2) *Aeromonas* spp. isolates obtained from FDA's Center for Veterinary Medicine (CVM) will be analyzed by PFGE, PCR, and other molecular biology-based typing methods.

3) The Division plans to study host-pathogen interactions, especially the expression of host and pathogen genes, by microarray analysis. Human intestinal epithelial cells and foodborne pathogens will be used as model systems.

4) The Division will conduct conjugation experiments to determine the rate of *tet* gene transferability from *tet*-resistant *Aeromonas* spp. to *tet*-sensitive *E. coli.* Similar studies will be done with *tet*-resistant *Citrobacter* isolates. In addition, *tet*-resistant *Citrobacter*, *E. coli* and *Pseudomonas* isolates will be characterized from aquaculture samples.

5) The Division will continue evaluation of the effect of fluoroquinolones on resistance development in bacteria from the human intestinal tract. Analysis of the fluoroquinolone-resistance mechanisms in anaerobic bacteria from the human intestinal tract and other sources will also be determined.

6) Our study of efflux-mediated drug resistance will address the physiological role of multidrug efflux pumps in the *Escherichia coli* model system using microarrays to monitor changes in the transcriptome upon their knockout (deletion), inhibition, or substrate challenge. This will be done through collaboration with the NCTR Center for Functional Genomics.

7) In light of the grave potential outcome of an influenza pandemic, research efforts in FY 2006 included collaboration with St. Jude Children's Research Hospital in Memphis, Tennessee. Preliminary data resulted in the submission of a proposal titled "Interaction of influenza and *Staphylococcus aureus* in the mouse and ferret models of severe pneumonia" to the National Institutes of Health. While not funded on its initial submission, the animal models of influenza and bacterial superinfection that have been established in mice and ferrets will be used to identify virulence factors in the virus and bacteria that contribute to the synergism. Genes encoding suspect virulence factors will be altered or deleted and the effects on disease will be examined.

Gastrointestinal Microbiology and Host Interactions

1) Vancomycin-resistant enterococci (VRE) have become a major food safety issue. We recently discovered that a vancomycin-resistant *Lactococcus lactis* that we isolated from a competitive exclusion product used in poultry could transfer the *van*A gene to *Staphylococcus aureus*, which is often found in humans. The Division of Microbiology will continue to explore the potential for bacteria in competitive exclusion and other probiotic products to transfer potentially hazardous genes to human gastrointestinal (GI) tract-associated bacteria.

2) *Lactobacillus* serves as an important indicator of GI and vaginal tract health and is heavily used by consumers, either intentionally as probiotic supplements or unintentionally in microbially fortified foods. Less complicated studies, such as assessing growth fitness or identifying metabolites of endogenous steroid molecules (hormones and bile acids), will be conducted in *Lactobacillus*, as will studies assessing the effects of microbicides and spermicides. In vaginal health, *Lactobacillus* microarrays will be used to address questions on efflux pump function.

3) The Division will analyze degradation products of azo dyes by skin and intestinal microorganisms, using specific enzymatic treatments, high-performance liquid chromatography (HPLC), and LC-MS/GC-MS methods. Since the three-dimensional structure of the azoreductase from *Enterococcus faecalis* has been solved, site-directed mutagenesis will be employed to study the enzyme activity center and flavin mononucleotide (FMN) binding motif and to characterize properties of the mutant

proteins. Microbiology scientists will continue to use DNA probes and antibodies from *E. faecalis* and *S. aureus* azoreductases for screening similar genes in skin and intestinal microflora to determine the distribution of the azoreductase genes among predominant bacteria and the enzyme expression levels in these microorganisms. The Division of Microbiology will also continue to study structure and function of the azoreductases from *S. aureus* and environmental microorganisms.

4) Entering into the second year of the CRADA with Pfizer Animal Health, we will continue our study of the metabolism of the third-generation cephalosporin ceftiofur by the bovine intestinal microflora.

5) In FY 2006, our proposal "Protective effect of vaginal *Lactobacillus* species against *Staphylococcus aureus*-mediated toxic shock syndrome" was funded by the FDA Office of Women's Health. In FY 2007, the Division will carry out this research to investigate whether *Lactobacillus*, when supplemented in tampons, has the capacity to exert probiotic benefits by decreasing either *S. aureus* proliferation and/or TSST-1 exotoxin production.

Environmental Biotechnology

1) The Division will conduct transcriptomic and proteomic analyses to understand PAH metabolism in *M. vanbaalenii* PYR-1. The results will be integrated with metabolomic and genomic data, which will give us a deeper understanding of how the bacterium responds to its environment and degrades PAHs at a systems-biology level.

2) To identify microorganisms that degrade fluoroquinolones, samples from soils and wastewater treatment plants to screen for the presence of fluoroquinolone resistance genes and for bacteria that degrade or transform *N*-phenylpiperazine and fluoroquinolones will be obtained. The Division will prepare DNA and sequence the 16S ribosomal RNA genes from any bacteria that appear to produce metabolites from either *N*-phenylpiperazine or fluoroquinolones, and will submit the 16S rRNA sequences of bacteria to the GenBank.

Microbiological Surveillance and Diagnostic Support of Research

The goal for FY 2007 is the development and implementation of new techniques for the detection and identification of pathogenic microorganisms.

Contribution to FDA's Strategic Goals

The Division of Microbiology uses an interdisciplinary approach to be responsive to FDA-regulatory needs. The Microbiology Division staff has a number of projects in conjunction with other FDA Centers to provide critical research to address FDA's strategic goals. Division of Microbiology scientists provide research, guidance, and expert advice to other FDA Centers, national regulatory agencies, and the World Health Organization for emerging public health issues. In addition, Division scientists respond to FDA-regulatory research needs in food safety, antimicrobial resistance, gastrointestinal microflora, and host interactions and environmental biotechnology.

A significant portion of the research in each focal area in the Division of Microbiology is focused on addressing specific FDA high-priority issues.

In food safety, researchers in the Division of Microbiology have developed diagnostic microarray assays and other rapid molecular techniques for high-throughput screening of virulence and antibiotic resistance genes in foodborne pathogens to assess the threat of bacteria in foodborne outbreak investigations.

The Division of Microbiology developed an interagency agreement with USDA to study the growth and inactivation of a surrogate strain of *Bacillus anthracis* in liquefied egg products at storage, permissive, and nonpermissive temperatures. The research in Food Biosecurity and Bioterrorism in the Division of Microbiology not only relates directly to the FDA's mission but also shows the adaptability of the scientists to develop liaisons with other federal agencies to deal with perceived threats of bioterrorism through the food chain.

The intestinal bacterial population plays an important role in human health acting as a barrier to infection as well as contributing to the digestion of dietary components and metabolism of drugs. Scientists have developed the methods to predict and monitor changes in this complex bacterial population. In addition, research is being conducted on the importance of human intestinal microflora in the metabolism and conversion of food additives, food supplements, and antimicrobial agents which have altered biological activities from those of the parent compounds resulting in activation or detoxification. These approaches will allow FDA to gain a clearer understanding of how drug residues, probiotic products, dietary supplements, and xenobiotic substances affect the intestinal microflora and how changes in this population may affect human health. This research supports the FDA's Strategic Goal 3 (Increase access to new medical and food products) and increase access to safe and effective veterinary products and to safe and nutritious food products, including products for unmet animal and human needs).

Before a veterinary drug can be granted approval for marketing, FDA requires that the drug be subjected to environment risk assessment. Research being conducted in the Division of Microbiology on the environmental fate of FDA-regulated drugs is highly relevant to the prevention of microbial antibiotic resistance. Drug residues that persist in the environment may select for resistance strains of bacteria. If the drugs can be metabolized to inactive products, they will no longer select for bacterial resistance. The research conducted by the Microbiology Division is increasing our understanding of the metabolism of these compounds and will further help FDA understand the fate and environment effects of antimicrobial compounds.

Microbiology Ongoing Research Projects

NCTR's Strategic Goal 1 — Advance the scientific approaches and tools to attain personalized nutrition and medicine for the American public

PI: Chen, Huizhong, Ph.D.

Genomic Approaches to Determine the Role of Skin Microflora in the Metabolism of Tattoo Dyes (E0717901)

Collaborating Division(s):

Biochemical Toxicology

Objective(s):

To focus on metabolic capacity and enzyme expression in human skin microflora: 1) Biodegradation and bioconversion of pigments used for tattooing and permanent makeup pigments; 2) To determine the effects of the skin microflora on tattoo and topically applied dyes that reside in the dermis; 3) To isolate, clone, and overexpress genes encoding for azoreductases and nitroreductases, which are able to decolorize the pigments; 4) To determine physicochemical properties of the purified native enzymes from the bacteria and/or the expressed recombinant enzymes cloned in *E. coli*; and 5) To elucidate the role of the microflora with potential genotoxic effects of tattoo and permanent makeup pigments.

Novel Molecular Approaches for the Detection and Analysis of the Predominant Bacterial Species in the Human Gastrointestinal Tract (E0711901)

Objective(s):

1) To develop a rapid method for quantification of intestinal bacteria; 2) To perform qualitative analysis of the communities for several major genera and discovering the species which are noncultivated; 3) To isolation and identify the bacterial species from probiotics used for human or animal health; and 4) To develop a microarray method for the detection of intestinal bacteria.

PI: Elkins, Christopher, Ph.D.

Assessment of Membrane-Associated Antibiotic Resistance Mechanisms in *Lactobacilli* (E0718001)

Collaborating Division(s):

Systems Toxicology

Objective(s):

1) To evaluate of intrinsic drug resistance of *Lactobacillus* isolates from various sources; 2) To achieve functional identification of *MDR* genes from currently sequenced *Lactobacillus* genomes using genomic and proteomic approaches; and 3) To achieve epidemiological profiling via pulsed-field gel electrophoresis (PFGE) and microarray technology for resistance determining factors (RDFs).

Protective Effect of Vaginal *Lactobacillus* Species Against *Staphylococcus Aureus*-Mediated Toxic Shock Syndrome (E0725501)

Objective(s):

To determine whether probiotic administration of *Lactobacillus* can thwart *S. aureus* TSST-1 production if supplemented in women's tampons. Alternatively, bacteriophage therapy may be investigated as a multifaceted approach to strengthening probiotic introduction for such conditions.

PI: Erickson, Bruce D., Ph.D.

Evaluation of the Mechanisms of Inactivation and Degradation of Third Generation Cephalosporins by the Bovine Intestinal Microflora (E0721901)

Objective(s):

1) To evaluate the ability of the bovine intestinal microflora to inactivate ceftiofur using pure culture isolates and mixed fecal cultures; 2) To identify primary metabolites of ceftiofur degradation; 3) To isolate ceftiofur-resistant bacteria and determine the primary mechanisms of drug inactivation; 4) To investigate the metabolic potential of anaerobic fungi isolated from bovine fecal samples to degrade ceftiofur; and 5) To compare the metabolism of ceftiofur with the human third-generation cephalosporin, ceftriaxone.

PI: Hart, Mark E., Ph.D.

Development of Proteomic Approaches to Identify *Staphylococcal aureus* Extracellular Proteins Responsible for Staphylococcal Pneumonia (E0717501)

Collaborating Division(s):

Systems Toxicology

Objective(s):

1) To develop a proteomic approach of identifying proteins by first fractionating proteins in spent media using isoelectric focusing followed by nonporous, reverse phase HPLC; and 2) To generate a proteomic profile for *S. aureus* RN6390 and its *agr* and *sar* isogenic mutants.

PI: Khan, Saeed A., Ph.D.

Development of a Microarray Chip for the Detection of Multiple Antibiotic Resistance Markers (E0715101)

Objective(s):

To develop a microarray-based method for the detection of 150 genes associated with 22 antibiotics; some of which are used to promote growth in poultry and animal farming while others are used to treat infections in both humans and animals. The data generated by the use of the chip in monitoring and tracking the spread of resistance markers may be helpful for FDA in making regulatory decisions that require banning and/or approving the use of certain antibiotics in poultry and farm animals.

PI: Paine, Donald D.

Microbiological Diagnostic Methods: Development, Testing, & Evaluation (E0026200)

Objective(s):

To improve diagnostic and epidemiological capabilities in bacteriology, parasitology, mycology, virology, and serology as applicable to NCTR programs and projects.

PI: Rafii, Fatemeh, Ph.D.

Elucidation of the Mechanism of Resistance Development in Anaerobic Bacteria from the Human Intestinal Tract (E0709301)

Objective(s):

To evaluate the effects of fluoroquinolones on resistance development in bacteria from the human intestinal tract and analysis of the fluoroquinolone-resistance mechanisms in anaerobic bacteria from the human intestinal tract.

PI: Wagner, Robert D., Ph.D.

Characterization of Antimicrobial Drug Resistance Genes from *Lactococcus lactis* P1-79 (E0716201)

Objective(s):

1) To determine whether the antimicrobial resistance genes are encoded on the bacterial chromosome or on episomes; 2) To screen for the presence of common resistance genes; 3) To clone the resistance genes in *E. coli* and evaluate their DNA sequence; and 4) To evaluate the potential for *L. lactis* P1-79 to transfer antimicrobial resistance genes to *Enterococcus faecium* or *Staphylococcus aureus*.

Measurement of Antimicrobial Drug Concentrations that Inhibit Colonization Resistance (E0708601)

Objective(s):

To adapt an enterocyte culture model of colonization resistance by enteric microbial flora against *Salmonella* sp. colonization/invasion to measure concentrations of antimicrobial drugs as food residues that would inhibit the barrier effect of the consumer's intestinal flora.

Probiotic Effects on Host Defense Against Enteric Pathogens (E0709701)

Objective(s):

1) To establish a model intestinal bacterial population in mice that consists of human intestine-derived bacteria; 2) To observe the fate of members of the model bacterial population when probiotic bacteria are fed to the mice; 3) To observe the fate of the probiotic bacteria fed to the human flora-associated mice; 4) To observe the effects of the human-derived flora on the host protective systems of immunodeficient and immunocompetent mice; 5) To observe effects of adding probiotic bacteria to the human flora-associated (HFA) mouse on immunodeficient and immunocompetent host-protective systems; and 6) To observe the roles of model host flora and probiotic bacteria to modulate host-protective systems of immunodeficient and immunocompetent mice from *Salmonella typhimurium* and *Campylobacter jejuni*.

NCTR's Strategic Goal 2 — Develop science-based best practice standards and tools to incorporate translational and applied toxicological advancements into the regulatory science process to create a seamless bench-to-bedside continuum

PI: Cerniglia, Carl E., Ph.D.

Proteomic Approaches to Elucidate Biodegradative Pathways (E0711801)

Collaborating Division(s):

Biochemical Toxicology
Systems Toxicology

Objective(s):

1) To use a proteomic approach to isolate putative catabolic proteins that are overexpressed when microorganisms are grown in the presence of polycyclic aromatic hydrocarbons; and 2) To use omics approaches to understand the environmental fate of PAHs.

PI: Sutherland, John B., Ph.D.

Microbial Degradation of Fluoroquinolone Antimicrobial Agents (E0722701)

Collaborating Division(s):

Biochemical Toxicology

Objective(s):

To identify microorganisms that either completely degrade fluoroquinolones or modify the fluoroquinolone molecule so as to reduce its toxicity to bacteria and levels in the environment.

NCTR's Strategic Goal 3 — Develop and apply rapid detection technologies and testing platforms to assure food safety, biosecurity, food biodefense, and to combat bioterrorism

PI: Khan, Ashraf A., Ph.D.

Molecular Characterization of *Salmonella* spp. and *Vibrio* spp. Isolated from Seafood and Development of Microarray Detection Method (E0720801)

Collaborating FDA Center(s):

ORA

Objective(s):

To characterize representative isolates of *Salmonella* and *Vibrio* spp. by molecular techniques, such as pulsed-field gel electrophoresis (PFGE), multilocus sequencing, ERIC (Enterobacterial repetitive intergenic consensus), and REP-PCR (Repetitive Extragenic Palindromic) methods. The results of this study will be used as a template for development of a diagnostic gene chip capable of simultaneous detection of multiple foodborne pathogens.

PI: Khan, Saeed A., Ph.D.

The Survival of *Bacillus Anthracis* in Processed Liquid Eggs (E0725101)

Objective(s):

1) To determine of the lag phase duration (LPD), growth rate (GR), and maximum population density (MPD) of *B. anthracis* Sterne strain at different temperatures used for storing and cooking liquid eggs; and 2) Inactivation kinetics of spores of Sterne strain at different temperatures.

PI: Nawaz, Mohamed S., Ph.D.

The Fate and Degradation of Antimicrobials, Oxytetracycline (OTC), and Sulfadimethoxine-Ormetoprim (Romet-30) from Aquaculture Environmental Samples (E0707501)

Collaborating FDA Center(s):

CVM

Objective(s):

1) To determine the biodegradation rates and metabolic fate of antimicrobials, oxytetracycline (OTC), and sulfadimethoxine-Ormetoprim (Romet-30®) (SDO) To used in fish farming systems; and 2) To isolate, characterize, and identify OTC- and SDO-resistant organisms from aquaculture sediment and natural environment samples and conduct molecular characterization of the genes that regulate resistance to the drugs.

PI: Nayak, Rajesh R., Ph.D.

Molecular Epidemiology and Characterization of Multiple Antibiotic-Resistant *Salmonella* Isolated from Turkey Production Environment (E0717301)

Collaborating Division(s):

Systems Toxicology

Objective(s):

1) To determine the preharvest sources and/or vectors of horizontal transmission *Salmonella* in turkey flocks: 2) To evaluate the intrinsic resistances of *Salmonella* isolates to multiple antibiotics; 3) To assess the genetic diversity and epidemiological profiles of *Salmonella* strains isolated in a turkey production environment; and 4) To develop DNA-based and microarray assays to detect genes in *Salmonella* isolates that are involved in antibiotic resistance and pathogenicity.

PI: Wagner, Robert D., Ph.D.

Measurement of Antimicrobial Drug Concentrations that Inhibit Colonization Resistance (E0708601)

Objective(s):

To adapt an enterocyte culture model of colonization resistance by enteric microbial flora against *Salmonella* sp. colonization/invasion to measure concentrations of antimicrobial drugs as food residues that would inhibit the barrier effect of the consumer's intestinal flora.

Microbiology Research Projects Completed in FY 2006

PI: Wagner, Robert D., Ph.D.

In Vitro Model and Molecular Analysis of Competitive Exclusion Products (E0704901)

Objective(s):

1) To evaluate individual component bacteria in a defined competitive exclusion (CE) product for exclusion of enteric pathogens from Caco-2 and CRL-2117 cell monolayers; 2) To define the antimicrobial susceptibility patterns of the component bacteria using Minimal Inhibitory concentration measurements; 3) To perform sequence analysis of 16s rRNA polymerase chain reaction (PCR) products from defined culture component bacteria and development of a database containing the sequences for use in subsequent identification of the organisms in undefined CE products; and 4) To apply the 16s rRNA sequence analysis procedure to detect and identify effective CE component bacteria in undefined CE products.

Results:

1) The *in vitro* assay measured the amount of a defined mixture of 29 human bacterial isolates (7.83 log10 CFU) and of the Preempt competitive exclusion product (4.05 log10 CFU) that significantly reduced *Salmonella* invasion of 6.41 log10 Caco-2 human intestinal-like cells and 6.89 log10 of CRL-2117 chicken intestinal cells; 2) Most of the bacteria isolated from the Preempt competitive exclusion product were susceptible to antibiotics; however, there were tetracycline-resistant *Bacteroides* spp., erythromycin-resistant enterococci, and vancomycin-resistant *Lactococus lactis* isolates that should be studied further for abilities to transfer resistance genes to other bacteria; and 3) The composition of the Preempt competitive exclusion product was confirmed for 48% of the strains reported to be present in the product. Of the 20 strains isolated, 48% were not strains reported to be present in the product. The 16S rRNA sequence comparisons with GenBank entries provided more reliable identifications of anaerobic bacteria than fatty acid methyl ester or biochemical profiling techniques.