U.S. FOOD & DRUG ADMINISTRATION

Division of Microbiology

Carl E. Cerniglia, Ph.D.

Director

Steven L. Foley, Ph.D.

Deputy Director

Division Staff

- Government Positions (26 FTEs)
  - Research Scientists and Staff Fellows : 19
  - Support Scientists : 4
  - Administrative : 4
- ORISE Post Docs, Graduate Students, etc.: 7

- Total = 34

Outreach

- Collaborations with:
  - All FDA Centers and NCTR Research Divisions
  - National Toxicology Program
  - USDA, CDC, Arkansas Health Department
  - Universities: Local, National, and International
- Global/National Outreach:
  - WHO Committees: JECFA (food additives), JMPR (pesticide residues), VICH (veterinary drugs)
  - International Working groups: HESI Microbiome Steering Committee
  - Societies: American Society for Microbiology, American Academy of Microbiology
  - Science Advisory Boards
  - Journal Editorial Boards
  - U.S. Government Panels: USDA, EPA, NOAA, Microbiome Interagency Working Group on Federal Strategic Plan, Interagency Risk Assessment Consortium
  - Visiting Scientist/Guest Worker Programs
  - FDA-wide Expert Committees, Working groups with FDA Centers

Division of Microbiology

Mission

To serve a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology in areas of FDA's responsibility in toxicology and regulatory science.

Vision

Strive to be a valued resource in advancing regulatory science research in microbiology for FDA.

Strategies to Meet Our Mission

Contribute to FDA Guidelines and Regulations

- Understand the regulatory process to identify issues
- Integrate research program into the FDA infrastructure
- Contribute to the NCTR/FDA mission

Enhance FDA Research Interactions

- Assess the needs of FDA
- Conduct research critical to the FDA regulatory science mission
- Expand collaborative relationship with other FDA Centers & ORA

Strengthen Research Program Management

- Focus research priorities in consultation with regulatory colleagues
- Establish benchmarks of scientific excellence
- Communicate research in plain language
- Upgrade research facilities and infrastructure

Microbiology Research Areas

- Evaluating the impact of antimicrobial agents, food contaminants, food additives, nanomaterials, and FDA-regulated products on the microbiome.
- Developing methods to detect and characterize microbial contaminants in FDA-regulated products.
- Determining antimicrobial resistance and virulence mechanisms of foodborne and other pathogens.
- Conducting research to aid FDA in the areas of women's health, tobacco products, and nanotechnology.
- Improving risk assessments of FDA-regulated products, including by integrating systems biology approaches.

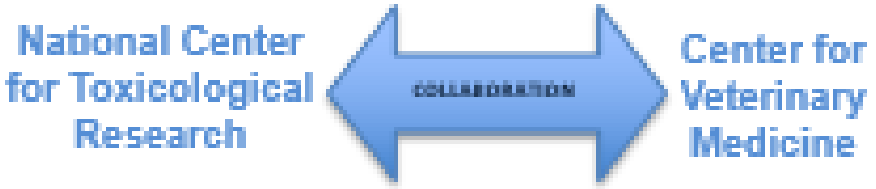
Top Three Accomplishments During the Last 5 Years

- Developed and utilized approaches for the evaluation of the plasmid-associated antimicrobial resistance and virulence in *Salmonella.* (CVM)
- Detected microbial contaminants including pathogenic mycobacteria in tattoo inks. (CFSAN)
- Conducted host-microbiome assessments to evaluate the effects of FDA-regulated products on the microbiome. (CVM, CDER, NanoCore, CTP, NTP/NIEHS)

Plasmid-Associated Antimicrobial Resistance and Virulence in *Salmonella enterica*

Study Objectives

- Assess the impact of plasmids and their genes on *Salmonella* colonization and virulence.
- Evaluate the dynamics of the host immune response to infection by strains containing virulence determinants to identify the mechanisms of pathogenesis.
- Evaluate differences in the expression of genes which are important for the regulation of plasmid transfer in *Salmonella* strains.



Plasmid-Associated Antimicrobial Resistance and Virulence in *Salmonella enterica*

Major Accomplishments:

- Demonstrated that certain antimicrobial exposures impact plasmid transfer dynamics in a dose-dependent fashion.
- Showed that IncFIB plasmid-encoded factors likely contribute to infection under low-iron conditions.
  - Sit and aerobactin iron acquisition operons were up-regulated during infection
- Identified that approximately half of IncI1 plasmids encode bacteriocins that may impact the ability to outcompete commensal bacteria.

Future Directions:

- Identify underlying mechanisms impacting dissemination of different plasmids.

Detection of Microbial Contaminants Including Pathogenic Mycobacteria in Tattoo Inks

Study Objectives

- Survey tattoo inks to determine whether microorganisms are present in the commercial tattoo and permanent makeup (PMU) closed inks available on the US market.

- Develop culture-based and molecular methods for rapid detection and monitoring of pathogenic mycobacteria, including *Mycobacterium chelonae*, in tattoo inks.

Detection of Microbial Contaminants Including Pathogenic Mycobacteria in Tattoo Inks

Major Accomplishments

- Completed a survey of 85 unopened, sealed tattoo and PMU inks, purchased from 13 companies available in the US, for microbial contamination
  - 42 inks (49%) were contaminated with microorganisms, often more than 10^3 CFU per mL
  - Including *Dermacoccus barathri* and *Roseomonas mucosa,* which have been associated with skin infections
- Follow-up study showed that 12 (44%) were contaminated and many samples contained high level of bacterial endotoxin
  - Research has contributed to OCAC/CFSAN for their regulatory actions: 6 tattoo ink products from 4 companies were recalled

Future Directions

- Continuing survey of tattoo inks for microbial and endotoxin contamination

Microbiome-Associated Accomplishments

- Evaluated the effects of residue levels of antimicrobial agents on the intestinal microbiome. (CVM)
- Assessed the effects of nanoparticles and nanodrugs on the intestinal microbiota and immune function. (CDER, NanoCore)
- Evaluated the effects of smokeless tobacco products on the oral microbiome. (CTP)
- Conducted host-microbiome assessments of the effects of NTP-nominated compounds on the microbiome to inform public health. (NTP/NIEHS)

Impact of Long-Term Exposure to Residual Levels of Antimicrobials on the Human Intestinal Microbiome

Major Accomplishments

- Demonstrated there was interpersonal variability with the microbiome structure and the effect of tetracycline exposure on the gut microbiome
  - Relative increase in *Bacteroides*, *Clostridium* XI and *Fecalibacterium* with tetracycline exposures
  - There were interpersonal variability in the abundance of tetracycline resistance genes *tetO, tetQ, tetW* and *tetX* following tetracycline exposure equivalent to or above the ADI of 25ug/kg bw/day
- Showed fecal binding of tetracycline was related to fecal and antibiotic concentrations
  - Fecal binding occurred rapidly and remained constant at 58% over a prolonged period
- Detected that higher concentration of tetracycline above established ADI residue levels could compromise intestinal barrier functions

Future Directions

- Evaluation of the impact of erythromycin on the intestinal microbiome

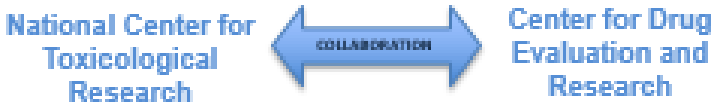
Evaluation of Nanoparticle and Nanodrug Impact on the Intestinal Microbiota and Immune Function

Major Accomplishments

- Assessed the *in vitro* dissolution profiles for nanosuspension and spray-dried crystalline nanodrug showed superior dissolution rate compared to the parent drug Zileuton.
- Developed an *in vitro* intestinal epithelial-cell culture model for the evaluation of toxicity of nanocrystal drugs, active pharmaceutical ingredient (API) mixtures, excipients, and parent drugs.
- Examined intestinal-barrier integrity by evaluating the gene expression profile of permeability-related genes.
  - Exposure of Zileuton physical-mixture (API with three excipients) to T-84 cells showed decreased in trans-epithelial resistance after 24 and 48 h compared to control cells.

Future Directions

- Assess the cell cytotoxicity of Zileuton nanodrug, API, and excipients by exposing them to intestinal epithelial cells.



Evaluation of the Impact of Smokeless Tobacco Products (STPs) on the Oral Microbiome

Major Accomplishments

- Analyzed metabolic alterations in oral bacteria as a result of STPs exposure.
- Demonstrated with *in vitro* studies that STPs affected the growth and viability of some oral bacterial species in a concentration-dependent manner.
  - Among the bacterial species in which the growth rate was enhanced by STPs, some could serve as opportunistic pathogens.
- Evaluated the effects of STPs on oral microbiota in a Syrian Golden hamster cheek-pouch carcinogenesis model and found that the use of STPs significantly disrupted the oral microbiota.

Assessment of the Role that the Microbiome May Play in the Toxicity of Xenobiotics

Major Accomplishments

- Conducted capability-building efforts to standardize sample collection and data-analysis methodologies for gut microbiome and gut mucosa-associated immune responses.
  - Carried out efficiency testing and comparative analyses for mouse- versus rat-intestinal microbiome experiments during vehicle versus water-gavage exposures.
  - Established 16s rRNA sequencing approaches to facilitate assessment of: animal model species, sample anatomical collection sites, exposure vehicle, and toxicological relevance to human disease.

Ongoing and Future Directions

- Assessing the xenobiotic toxicity (adult toxicity: silver nanoparticle or developmental toxicity: Arsenic, Aloin, Bisphenol AF and Triclosan) in rodent models.

Additional Representative Projects

Comparative methods study for the detection of *Burkholderia cepacia* complex from pharmaceutical products (CDER)

- Developed a resuscitative step and enrichment technique for *B. cepacia* complex (BCC) recovery.
  - Demonstrated that diluted TSA and TSB media, and R2A and R2AB showed better recovery efficiency than TSA and TSB.
  - Diluted TSA and R2A media exhibited greater efficiency of recovery from the chlorohexidine gluconate (CHX) and benzalkonium chloride (BZK) solutions than full strength TSA or R2A.
  - Demonstrated that strains could remain viable in CHX and BZK for 28 days.

- Developing a rapid PCR-based detection method for BCC from water and other pharmaceutical drug manufacturing raw materials.

Additional Representative Projects

Exploration of Fecal Transplant Mechanisms: Pro-inflammatory Responses of Intestinal Epithelial and Dendritic Cells to *Clostridium difficile* and Commensal Bacteria (CBER)

- Established 2D and 3D intestinal epithelial (IEC) and dendritic (DC) cell models to evaluate fecal transplant mechanisms.
- Challenged cell models with *C. difficile* and bacterial strains representative of disease agonists and antagonists to evaluate IEC and DC cellular responses.
  - Some commensal strains were shown to impact the host cell response.
- Evaluated sample microbiome effects of *C. difficile* and commensal challenge *in vitro.*
  - Microbiome challenge experiments showed that commensal bacteria inhibited growth of *C. difficile* in an additive manner.
- Orally challenge mice with *C. difficile* and commensal strains showed *C. difficile*-associated cytotoxicity was decreased additively by commensal bacteria.

Additional Representative Projects

Using *in vitro* continuous culture of the human-intestinal microbiota to evaluate risk associated with bacterial contamination of fecal microbiota transplantation (FMT) samples (CBER)

- Optimized conditions for establishing and maintaining complex microbial populations in bioreactors representative of the human intestinal microbiota.
  - Evaluated multiple media formulations for the ability to maintain complex microbiota
  - Established *C. difficile* growth parameters for bioreactor study
- Determining the level of *Clostridium difficile*, provided alone or as part of a simulated FMT sample, that is capable of establishing itself within the complex microbiota community.
- Determining if standard tests used to screen fecal donations for *C. difficile* can detect this pathogen at this level of contamination

Additional Representative Projects

Comparative study to evaluate molecular assays and culture-based reference methods for the detection of toxigenic *Clostridium difficile* and to evaluate storage conditions on the recovery of *C. difficile* in clinical stool specimens (CDRH)

- Designed and evaluated a composite molecular method consisting of two FDA-uncleared molecular assays for the detection of *C. difficile* toxin genes (*tcdA* and *tcdB*) for the detection of *C. difficile* in human stool samples.
- Evaluated the effects of storage on viability of vegetative cells and spores in stool specimens and showed that all storage conditions adversely affected their viability; however there was no discernible storage effect on *C. difficile* DNA for at least 28 days.
- Carrying out comparative evaluation of our new composite molecular method by comparison to the currently accepted reference method toxigenic culture and to an FDA-cleared nucleic acid test for the detection of toxigenic *C. difficile.*

Additional Representative Projects

Database and analysis tool development and the evaluation the plasmid-associated antimicrobial resistance and virulence in *Salmonella* (CVM)

- Developing improved databases for virulence gene identification and plasmid characterization.
- Developing analytical tools for WGS data analyses and computational tools to determine the virulence genes present within strains.
- Developing improved approaches to identify plasmids present and establish tools to predict the potential for conjugal transfer of the plasmids.
- Validating whether factors predicted to be associated with virulence are associated with differences in virulence using molecular biology approaches and *in vitro* virulence models.

Future Direction of the Division

STRATEGIES

- Enhance mechanisms of communication to reach out to our stakeholders to develop research projects that help them meet FDA's mission.
  - Increased participation on FDA workgroups and timely dissemination of research results to stakeholders in other Centers.

- Prioritize our research efforts by moving away from areas that currently have less need to those more pressing to meet FDA's mission.
  - Engaging colleagues at other Centers early in the research design phase to assess the feasibility and interest in the research.
  - Increasing awareness of the research priorities of the other Centers.

Future Direction of the Division

- Identify improved ways to leverage opportunities with other federal, state and international regulatory and public health agencies, academia, and industry.
  - Increased participation on FDA, interagency and international workgroups to increase awareness of opportunities.
  - Enhanced interactions with universities to mentor students and postdocs.

- Continue emphasis on research studies to better understand the impact of FDA-regulated products on the microbiome and host response to improve toxicology risk safety assessments.
  - Enhancing interactions with other Centers, the National Toxicology Program, and other stakeholders to move microbiome studies forward.

Future Direction of the Division

- Advance new scientific approaches to determine the impact of microbial contaminants in foods and other FDA-regulated products on human and veterinary health.
  - Engaging the other Centers on their research needs for improved methods for microbial detection and characterization.
  - Participating in microbial method validation activities.
- Continue to conduct research to provide data for the safety assessments of human and veterinary drugs through the integration of systems biology approaches.
  - Engaging our stakeholders on their needs and making sure our research meets the needs.
  - Assessing research equipment needs and upgrading technologies to facilitate research success.

Future Direction of the Division

- Develop and conduct research on funded studies in women's health and identify research gaps to address new research initiatives within FDA's Office of Women's Health.
  - Increasing awareness of research gaps and funding priorities and developing a project to address those needs.
  - Building upon previous research successes to develop new projects.
- Develop additional nanotechnology projects in collaboration with the NCTR/ORA NanoCore Facility and FDA regulatory Centers.
  - Continue to engage the NanoCore on their capabilities and their utility to answer microbiological research questions.
  - Participate in FDA-wide nanotechnology funding initiatives.

Feedback Requested

- Is the Division addressing the needs of the FDA Centers?
  - Are there ways to get the research results to the stakeholders in a more rapid timeframe?
    - Additional support of postdoctoral fellows?
    - More timely budget allocations when available?
- How can we do a better job of engaging the Centers to learn about the needs?
  - What emerging sciences/technologies can you advise the Division to pursue?
- What future directions do you recommend for this Division that would impact the FDA?
  - How is the best way to transition to these new areas?
  - How do we best recruit fellows to NCTR?

Thanks

- **Members of the Science Advisory Board**
- **Representatives of FDA Centers and Offices**
- **Dr. William Slikker. Jr., Director, NCTR**
- **Dr. Daniel Acosta, Deputy Director, NCTR**
- **Dr. Donna Mendrick, Assoc. Director for Regulatory Activities**
- **Division of Microbiology Staff**

Division Contact Information:
Carl E. Cerniglia, PhD.
Director, Division of Microbiology
National Center for Toxicological Research
US Food and Drug Administration
3900 NCTR Road
Jefferson, AR 72079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