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 (27 FTEs)
  - Research Scientists & Staff Fellows : 19
  - Support Scientists : 4
  - Administrative : 4
- ORISE Post Docs, Graduate Students, etc.: 12

- Total = 39

Outreach

- Collaborations with:
  - FDA Centers and NCTR Research Divisions
  - National Toxicology Program
  - USDA, CDC, State Health Departments
  - 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, MCBIOS
  - 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, GEN-FS Genomic Biomarkers Workgroup
  - Guest Worker Programs, ASM International Professorship
  - 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 & Regulations

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

Enhance FDA Research Interactions

- Assess the needs of FDA
- Conduct research critical to the FDA regulatory science mission
- Expand our collaborative relationship with 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 the integration of systems biology approaches

Focus Areas of Accomplishment

- **Evaluating the impact of antimicrobial agents, food contaminants, food additives, nanomaterials and FDA-regulated products on the microbiome**
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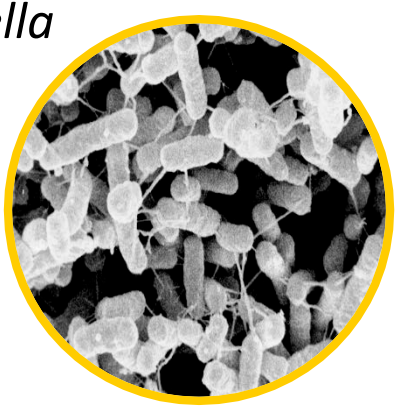
Antimicrobial Resistance and Virulence-associated Accomplishments

- Multiple ongoing studies are evaluating the genetic bases of antimicrobial resistance and virulence mechanisms of *Salmonella enterica* (CVM)
- Developing and optimizing a *Salmonella* virulence gene database and tools to utilize the database to evaluate DNA sequencing results (CVM)
- Utilizing multiple approaches to evaluate antimicrobial resistance associated with antibiotic-coated medical devices (CDRH)

Antimicrobial resistance and virulence studies in *Salmonella enterica*

- Assessed the impact of plasmids and their genes on *Salmonella* virulence
  - Showed that IncFIB plasmid-encoded iron acquisition factors contribute to infection under low-iron conditions
  - Working to establish three-dimensional tissue culture models for intestinal epithelial and macrophages to better assess virulence mechanisms

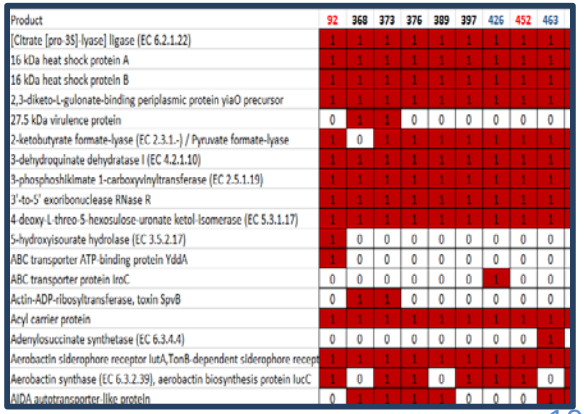


- Demonstrated that antimicrobial exposures can impact plasmid transfer dynamics in a dose dependent fashion
- Evaluated efflux pump activities in multidrug resistant strains and found differences across strains that are currently being evaluated in depth

Collaborative projects with CVM

Database and analysis tool development to evaluate virulence and plasmid-associated genes in *Salmonella*

- Established database with 520 virulence or putative virulence genes cataloged from representative *Salmonella* strains
  - Developed and deployed simple matching algorithms to predict the presence of virulence genes from whole genome sequence data
- Developed tools to allow the comparison of virulence gene profiles between different *Salmonella* strains
  - Working to validate whether the factors predicted to be associated with virulence are associated with differences in *Salmonella* virulence
- Currently working on a plasmid characterization database and related analysis tools



Product	92	368	373	376	389	397	426	452	463
(Citrate [pro-3S]-lyase) ligase (EC 6.2.1.22)	1	1	1	1	1	1	1	1	1
16 kDa heat shock protein A	1	1	1	1	1	1	1	1	1
16 kDa heat shock protein B	1	1	1	1	1	1	1	1	1
2,3-diketo-L-gulonate-binding periplasmic protein yiaO precursor	1	1	1	1	1	1	1	1	1
27.5 kDa virulence protein	0	1	1	0	0	0	0	0	0
2-ketobutyrate formate-lyase (EC 2.3.1.-) / Pyruvate formate-lyase	1	0	1	1	1	1	1	1	1
3-dehydroquinate dehydratase I (EC 4.2.1.10)	1	1	1	1	1	1	1	1	1
3-phosphoshikimate 1-carboxyvinyltransferase (EC 2.5.1.19)	1	1	1	1	1	1	1	1	1
3'-to-5' exoribonuclease RNase R	1	1	1	1	1	1	1	1	1
4-deoxy-L-threo-5-hexosulose-uronate ketol-isomerase (EC 5.3.1.17)	1	1	1	1	1	1	1	1	1
5-hydroxyisourate hydrolase (EC 3.5.2.17)	1	0	0	0	0	0	0	0	0
ABC transporter ATP-binding protein YddA	1	0	0	0	0	0	0	0	0
ABC transporter protein IroC	0	0	0	0	0	0	1	0	0
Actin-ADP-ribosyltransferase, toxin SpvB	0	1	1	0	0	0	0	0	0
Acyl carrier protein	1	1	1	1	1	1	1	1	1
Adenylosuccinate synthetase (EC 6.3.4.4)	0	0	0	0	0	0	0	0	1
Aerobactin siderophore receptor IutA,TonB-dependent siderophore recept	1	1	1	1	1	1	1	1	1
Aerobactin synthase (EC 6.3.2.39), aerobactin biosynthesis protein IucC	1	0	1	1	0	1	1	1	0
AIDA autotransporter like protein	0	1	1	1	1	0	0	0	1

Collaborative project with CVM

Approaches to evaluate antimicrobial resistance associated with antibiotic-coated medical devices

- Characterized *P. aeruginosa* phenotypes when grown with antibiotic-coated catheters in a biofilm reactor
  - Growth rate, biofilm formation, and HeLa cell invasion potential of *P. aeruginosa* grown with antibiotic-coated catheters were higher than those grown with control catheters
- Investigated the transcriptomic and proteomic profiles of *P. aeruginosa* following *in vitro* continuous culture with the antibiotic-coated catheters
  - Bacteria from antibiotic-coated catheter culture demonstrated increased expression of antimicrobial resistance (β-lactamase production, efflux pump, porins) and virulence (type III secretion, iron uptake, motility) genes compared to those from control catheter culture
- Ongoing efforts focused on identifying antimicrobial resistance markers associated with antibiotic-coated catheters using integrated transcriptomic and proteomic analyses

Collaborative project with CDRH

Xenobiotic-Microbiome-associated and Host Interactions Accomplishments

- Evaluating effects of acute and chronic exposure of residue levels of tetracycline and erythromycin on the gastrointestinal tract (CVM)
- Continuing efforts to evaluate the interactions of nanoscale materials used in sunscreens on the skin microbiome (CDER)
- Conducting studies to understand the potential impact of xenobiotic compounds (arsenic, BPAF, triclosan, aloin, silver nanoparticles) on gastrointestinal microbiomes and immune responses (NTP/NIEHS)

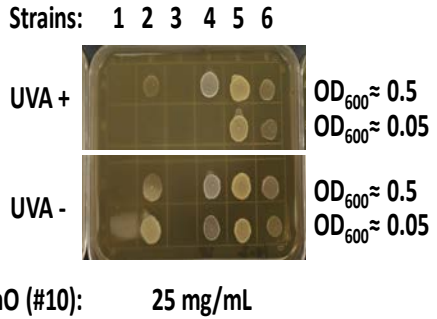
Impact of exposure to residual levels of antimicrobials on the human intestinal tract

- Investigated the effects of tetracycline and erythromycin after acute or chronic exposures on the permeability of human colonic epithelial cells
  - Model mimics a susceptible intestinal surface devoid of commensal microbiota
  - Higher concentrations of the antimicrobials were able to compromise intestinal barrier functions
- Identified expression changes in immune system molecules that may be related to the immunoregulatory activity of erythromycin or compensatory mechanisms due to physiological stress due to increased permeability
- Future studies will assess the impact acute or chronic erythromycin exposures on the intestinal microbiome

Collaborative project with CVM

Assessment of the interactions of nanoscale materials used in sunscreens on the skin microbiome

- Evaluated the in vitro impact of nanoscale TiO_2 and ZnO in sunscreens on the growth and viability of skin bacteria
  - Sunscreens can contain nanoscale TiO_2, coated ZnO or uncoated ZnO
  - Different bacteria strains have different sensitivity to uncoated ZnO
- Investigated whether the toxicity of nanoscale TiO_2 and ZnO against skin bacteria is affected by physicochemical properties, UV light, and storage time
  - TiO_2 and coated ZnO didn't significantly affect the growth of skin bacteria in either liquid or plate assay with or without UVA exposure
  - Uncoated ZnO had dose-dependent antimicrobial effects in both liquid and plate assay
  - UVA exposure enhanced antibacterial effect of uncoated ZnO on some bacteria strains

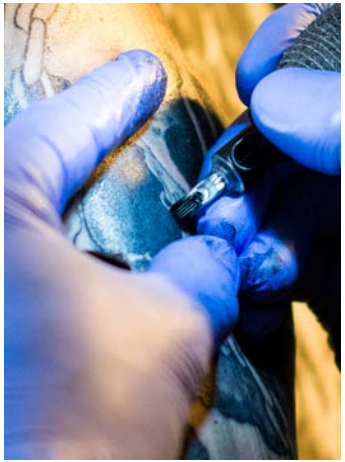


Pathogen Detection-associated Accomplishments

- Studies evaluated the presence of microbial contaminants, including pathogenic mycobacteria, in tattoo inks and permanent makeup products (CFSAN)
- Developed and evaluated of optimized methods for the detection of *Burkholderia cepacia* complex (BCC) from pharmaceutical products (CDER)
- Evaluating the potential health risks associated with contamination of fecal microbiota transplantation samples (CBER)

Detection of microbial contaminants including pathogenic mycobacteria in tattoo inks

- Completed a microbiological survey of 85 unopened tattoo and PMU inks, purchased from 13 companies available in the United States
  - Almost half (49%) were contaminated with microorganisms, including some species that may be opportunistic pathogens
- Carried out follow-up study of an additional 27 tattoo, PMU inks, and ink diluents from 10 companies
  - Ten samples were contaminated, with levels up to 10^8 CFU/ mL
  - Many samples contained high level of bacterial endotoxin
  - Research has contributed to recall of contaminated tattoo inks



- Continuing microbial and endotoxin surveys of different tattoo ink types, country of manufacture and reassessment of previously recalled tattoo inks

Methods for the detection of *Burkholderia cepacia* complex (BCC) from pharmaceutical products

- Developed a resuscitative step and enrichment technique for BCC recovery
  - BBC survived for at least 199 days in the presence of 5-50 µg/ml chlorohexidine gluconate and benzalkonium chloride solutions
  - More dilute media formulations facilitated increased BCC recovery from autoclaved distilled water and antiseptics at 6°C, 23°C and 42°C storage
    - The use of dilute media in a pre-enrichment step was a superior strategy to the compendial test method United States Pharmacopeia (USP) <60> for BC recovery and detection
- Developing a rapid PCR based detection method for BCC from water and other pharmaceutical drug manufacturing raw materials

Evaluation of the risks associated with contamination of fecal microbiota transplantation samples

- 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
- Performed 14-day bioreactor study involving antimicrobial exposure and *C. difficile* challenge – data analyses ongoing
- Determining the level of *Clostridioides 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

Collaborative project with CBER

Division's Future Research Strategies

- Enhance communication channels to reach our stakeholders to develop research projects that help them address their needs to meet FDA's mission
  - Increased participation on FDA workgroups and timely dissemination of research results to stakeholders in other Centers
  - Identify opportunities for increased support for training programs to facilitate research advancement
- Prioritize our research efforts to best serve the Agency 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

Division's Future Research Strategies

- Leverage opportunities with other federal, state and international regulatory and public health agencies, academia and industry
  - Increased participation on interagency and international workgroups to increase awareness of opportunities
  - Enhanced interactions with universities to mentor students and postdocs
    - Assist in overcoming challenges in the recruitment of fellows
- Focusing 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

Division's Future Research Strategies

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

Division's Future Research Strategies

- Develop and conduct research on funded studies in women's health and identify research gaps to address new research initiatives within the Office of Women's Health
  - Developing improved awareness of research gaps and funding priorities and developing projects to address those needs
- 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?
- 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?
  - What 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. Tucker Patterson, Assoc. Director for Science & Policy**
- **Dr. Donna Mendrick, Assoc. Director for Regulatory Activities**
- **Division of Microbiology Staff**

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