National Center of Toxicological Research Scientific Advisory Board Subcommittee Program Review of the Division of Microbiology Response to the Subcommittee Report

We appreciate the thorough examination and review of the Division of Microbiology research and surveillance diagnostic programs by the Science Advisory Board Subcommittee (SAB) and liaison FDA members on July 23-25, 2007 chaired by Dr. Anthony L. Pometto III and the consultants Dr. Thomas W. Federle, Dr. Michael G. Johnson, Dr. Susan F. Kotarski and Dr. Lynda Collins Kelley. We would also like to thank the FDA representatives Dr. Patrick McDermott (CVM), Dr. Dennis J. Kopecko (CBER), Dr. Ellen Olson (ORA) and Dr. Mary Lou Tortorello (NCFST). The overall positive constructive nature of the comments and recommendations in the draft report, will guide us in evolving new strategies and research initiatives to strengthen our role in supporting the FDA mission. Our responses to comments made by the SAB are listed below.

Response to General Comments and Recommendations

We appreciate the comments from the committee about the "talented scientific leadership and strong administrative skills of Dr. Cerniglia and high level of research diversity and publication record of the Principal Investigators in the Division of Microbiology".

<u>Comment</u>
Improve scientific communication and exchange with FDA Centers/ORA. Project relevance to the FDA mission.

<u>Response</u>
We fully agree with the SAB comments that "one challenge the Division of Microbiology faces is lack of scientific exchange with FDA Centers/ORA". This is an Agency/NCTR issue. Each FDA Center regulates widely divergent products under unique legal and regulatory requirements which make it difficult for the NCTR to coordinate research across the Agency. It is extremely trying for scientists at the NCTR to maintain a constant dialogue with other FDA Centers and ORA Laboratory researchers, reviewers and policy makers to determine the research regulatory needs and knowledge gaps of the Agency. However, certain mechanisms are in place at the NCTR Director, Division and research scientist levels at the NCTR and the Agency to enhance scientific exchange.

For example:

1) Before NCTR scientists develop full proposals, the NCTR Director solicits input from other interested FDA Centers as to the importance of the research objectives in a concept paper to meet the regulatory concerns of the FDA. Once the concept paper is approved, then a full protocol (research proposal) is written, which is also circulated to the FDA Centers to determine relevance and impact to the FDA mission.

2) The hiring of the Associate Director for Regulatory Activities who is located at FDA Headquarters has also enhanced our communication with the different FDA Centers. A

current example is a research meeting with other FDA Centers and ORA on research priorities for the Food Protection Plan at the NCTR in July, 2008.

3) The recent appointment of FDA Chief Scientist will enhance communication with other centers within the agency to identify top priority scientific and regulatory questions for intramural research of the Agency.

4) Division of Microbiology scientists are also trying to coordinate research projects and write proposals that support the FDA Food Protection Plan, Import Safety Action Plan, Critical Path Initiatives and the Office of Women's Health.

5) Members of the Division of Microbiology have participated in FDA Science Symposiums in 2007 and 2008.

   - Living the Future: Global Changes and Public Health
   - Emerging Foodborne Pathogens
   - Environmental Reservoirs of Antimicrobial Resistant Bacteria
   - Genome Analysis in Risk Assessment

6) Members of the Division of Microbiology presented lectures at various FDA Centers including the CVM Staff College.

7) Members of the Division of Microbiology participated in special interest/intercenter work groups.

   - FDA Amendment Act Steering Committee
   - FDA Antimicrobial Resistance Project Team
   - FDA Pharmaceuticals in the Environment
   - FDA Commissioner Committee for the Advancement of FDA Science (CAFDAS) Committee
   - FDA Intercenter Live Microbial Working Group
   - FDA Food Advisory Committee on Probiotics
   - FDA Intercenter Working Group on Human Microbiome Project

8) Members of the Division of Microbiology are participating in the newly established NCTR Science Training and Exchange Professional (STEP) Development Program to facilitate and strengthen the sharing of laboratory expertise, tools and technology across the FDA Centers. A scientist from the Pacific Regional Laboratory, ORA will be conducting research in August/September 2008 on the microarray detection of *Salmonella, E. coli,* and *Shigella* in foods.

9) The Division of Microbiology has updated a research facilities core capabilities flyer.

10) A Web Ex service is available at the NCTR to increase communication with ORA and FDA Centers.

Comment
"Adequate support staff and supply funds are not available to this division. More discretionary funds for each Principal Investigator are necessary (i.e., laboratory supplies, expendables in the order of $20-25k per year."

Response
The FY 2008 discretionary budget allocated to the Division of Microbiology by the NCTR Director did allow each of the Principal Investigators to purchase all the necessary supplies, expendables and equipment to carry out the goals and objectives of their research protocols. Funds for travel to scientific meetings were also allocated. Additional funding was also obtained by several of the Principal Investigators from the Office of Women's Health, from CRADAs, and as collaborators in grants with universities. In addition, we will identify specific needs and additional resources as part of the NCTR planning for the 2009 budget process.

Comment
"The review team believes that high-impact research needs more permanent support staff. Since several Principal Investigator's do not have ORISE Post-Docs, we would like to see them supported, especially those in Food Safety/Food Biodefense arena."

Response
We agree with the committee that more ORISE Post-Docs and support staff are essential, especially in Food Safety and Food Protection projects. The potential for increased funding to the NCTR in FY 2009-2011 for the Food Protection Plan may allow us to expand our base of support and recruit ORISE Post-Doc's and support staff. This issue has been discussed with the NCTR Director.

Comment
"The Deputy Division Director position still has not been addressed. It would be very difficult to replace Dr. Cerniglia visionary leadership and organization skills. Therefore, succession planning should occur within the next review cycle. Don Paine, team leader of the Surveillance Program, is eligible for retirement and continuity in skills and leadership is needed there also."

Response
Careful consideration will be given to prepare for a transition in leadership to maintain stability for the Division of Microbiology should the Division Director or Team Leader in the Surveillance Diagnostic Program decide to leave NCTR.

Recruitment procedures will follow the Office of Personnel and Management (OPM) guidelines to fill positions in the Division of Microbiology, including if vacancies occur at the Division Director level or Team Leader position in the Surveillance Diagnostic Program. Advertisements will be placed in the most appropriate media to obtain the best qualified candidate for specific vacancies in the Division of Microbiology. After candidates are evaluated and ranked, the most qualified person will be selected.

On the comment of why the Deputy Division Director position was not addressed since the last site visit, it is due to supervisory level of authorities and OPM policies, in which lines of

approval/authority for a Deputy Division Director position are not possible. Furthermore, there are no Deputy Division Directors for any of the research division positions at the NCTR.

Comment
"The expertise in the division can be an extremely valuable component of collaborations with CFSAN, CVM, and ORA method development and validation studies. It is recommended that the division pursue leading, participating, and advising in development and collaborative studies of regulatory methods."

Response
We will continue our communication with the FDA Centers and ORA to determine research needs/gaps in the development of methods to enhance the regulatory mission.

Comment
"Food safety research for produce foods (fruit, vegetables, salad crops, etc.) needs to be increased, as this is a growing area of concern for the public."

Response
We fully agree with the committee's comments about food safety research for produce since recent foodborne outbreaks associated with contaminated produce have heightened concerns about the adequacy of control measures for the safe production of fresh fruits and vegetables. We will attend a meeting July 8-9, 2008 in College Park, Maryland, sponsored by Interagency Risk Assessment Consortium (IRAC). The objective of this meeting is to identify and prioritize research gaps for conducting a risk assessment of *E. coli* 0157:H7 foodborne illness from the consumption of leafy green vegetables from the farm-to-the-fork continuum. The information obtained from the conference will guide us in new research initiatives on food safety research for produce.

Comment
"We suggest recruiting a senior microbiologist in the food safety arena with a background in enteroviruses."

Response
We agree that by hiring a food microbiologist with research experience in enteroviruses would fill a gap in the FDA in this important area of food safety research. Hiring a senior microbiologist in the food safety arena would also provide leadership skills to the program for the food safety research projects in the Division of Microbiology. This recruiting issue has been discussed with the NCTR Director.

The other comments about improved communications among the different FDA Centers and greater focus in risk assessment have been addressed in other sections of this response to the SAB report.

Response to specific comments made by the SAB committee for each of the focal areas in the Division of Microbiology

Surveillance Program

Strengths and recommendations:

This program is highly important, not only for the division but also for the larger NCTR community. The major concern with this program is the vacancies/lack of support staff. The Surveillance Microbiology Program is a bit at a crossroads in terms of staffing - they need one additional person that could do both research and surveillance. It may also be a good time to consider contracting out this service (Don Paine is eligible to retire). The program already contracts out the Virology/Serology Service to the University of Missouri. What is left is the Bacteriology Service. Organizationally speaking, the Surveillance Program could stay in the Division of Microbiology or be moved to the Veterinary Services Program at the NCTR. This would eliminate the issue of the research program discretionary budget underwriting some of the costs for surveillance, which is an NCTR center-wide function.

Response

There is a very strong and valuable working relationship between Microbiology Research and the Surveillance/Diagnostic program. The Surveillance/Diagnostic program provides bacterial cultures, research support and bacterial identification to the research staff, as well as the media, reagents and equipment for media preparation. In return, the Surveillance/Diagnostic Program has access to a wealth of expert guidance and consultation from the microbiologists on the research staff. Moving the program into Veterinary Services or contracting out the service is an option but at this time there is no compelling reason to do so since the NCTR Director has provided sufficient funds to the Division of Microbiology Surveillance Program to assure that the experimental animals are healthy and free from infections for on-going studies at NCTR. Therefore, the separate funding of the Surveillance Program does not impact the Division of Microbiology research program discretionary budget.

As soon as an approval for hiring into one or more of the vacancies is obtained, a wide-area search will be instituted to find a replacement for Don Paine. In addition to strong clinical microbiology skills, this person ideally will have experience in molecular biology techniques such as PCR and microarray to further modernize the Program.

Food Biosecurity, Food Safety, and Methods Development

Survival of *Bacillus anthracis* in processed liquid eggs

Strengths:

This program has done interesting work on the effects of egg white lysozyme on *B. anthracis* at the request of Homeland Security via FSIS –USDA, establishing an excellent collaborative relationship with FSIS-USDA in the process.

Weaknesses:

The program needs to be careful about any claims of a unique approach of lysozyme, since much has been published earlier about the ability of this enzyme to trigger spore germination and outgrowth for Gram-positive bacteria.

Response

We appreciate the comments of the SAB review committee. The committee suggested being careful in making statements about the role of lysozyme in the inactivation of *Bacillus* spores. We carried out further investigations that indicated that after inhibition of lysozyme with 5 mM chlorambutol, *Bacillus anthracis* was not inactivated and started growing. As the committee

pointed out that lysozyme has been shown to help in spore germination, we are investigating whether it is species-specific or does so when used alone or when present in its native environment, the egg white. The suggestions of the committee are also being followed by employing other food matrices (milk and beef) and determining the role of lysozyme in the inactivation of *Bacillus anthracis* spores. We are planning to study the differences in the expression profiling of various genes in three different forms of anthrax by using skin, intestinal and lung epithelial cells.

Molecular Epidemiology and Microbial Source Tracking of Antimicrobial-Resistant *Salmonella* Serovars in Preharvest Poultry Environment.

Strengths

The program established a good collaborative relationship with the West Virginia University poultry farm for research. The program also secured cooperation and involvement of multiple federal agencies in looking at an important topic, the persistence of particular serotypes of *Salmonella*, here *S. enterica* serovar Heidelberg, in multiple flocks grown on the same farm. Collaborative relationships were also established with the FDA-CFSAN and FDA-CVM as well as the USDA-ARS for microarray biochip evaluations (developed at the USDA-ARS). This research team evaluated a microarray biochip to detect virulence and antimicrobial-resistant genes of poultry and tomato serovars of *Salmonella* which provides information to build an important database for homeland/bioterrorism defense. Works on *Salmonella enterica* serovar Heidelberg using Pulse Filed Gel Electrophoresis (PFGE) is interesting work, especially showing that resistance to streptomycin and gentamicin can be transferred among strains by mobile genetic elements. The work on *Salmonella enterica* serovar Javiana is important, because this serovar has been associated with recent human salmonellosis cases contracted from consumption of raw sliced Roma tomatoes. More information is needed to understand what the natural reservoirs and alternate reservoirs for such serovars are in nature. This work shows that there was a three fold higher level of antibiotic resistance and resistance to more antibiotics for the food/animal isolates (112 strains) than that for the clinical isolates (144 strains) tested.

Weaknesses

Unequivocal tests in a grow-out area requires quarantine of air, insect, and other animal movement between research plots. A control to consider in the experimental design is a split plot with the same bird type for all treatments, but use no antibiotic plus usual coccidiostats for one set, and a second set of birds with antibiotics and usual coccidostats. Look for persistence of antibiotic resistance in both sets. (Note: work on swine *Salmonella* isolates at University of Kentucky research farm indicates persistence of *Salmonella* types even after removal of antibiotics for management scheme.) Consider monitoring dynamics of *Salmonella* isolates as function of age, two-to-three-week-old poultry vs. harvest age of 22 weeks. Watch for confounding effects of genome drift in target *Salmonella* strains as function of time on farm as this can confuse the PFGE pattern interpretations. Also, streptomycin and gentamicin are not important for treating salmonellosis. The PFGE work is duplicative of the NARMS program which has tested many more *S.* Heidelberg strains.

Research recommendations:

General suggestion for poultry project: PI should consider expanding the base of work to include working with other PIs on the bigger picture. There is a question as to how environmental stresses of elevated temperatures, decreased pH, and other factors will affect the

level of expression of the antibiotic-resistant genes and of virulence genes. Microarray data has the most utility and meaning when it is tied to whole-cell physiological conditions the pathogens are exposed to in real-world conditions. Work toward expression microarrays would be a useful approach. While detection of antibiotic resistance spread is essential, quantitative assessments would be useful to developing approaches for risk management.

Response

We concur with the committee members that an experimental (split plot) design would be helpful in understanding the effects of antimicrobial/coccidiostats and age of birds on the prevalence dynamics of *Salmonella*. However, it should be noted that the sample collection and molecular characterization of *Salmonella* strains in the turkey facility were mutually exclusive projects. The prevalence dynamics of *Salmonella* in turkey flocks were studied by the PI as part of his doctoral dissertation. Prior to our studies, there was limited information on the prevalence of *Salmonella* in turkey production facilities. The turkey production facility was not a conventional "farm," but a part of the West Virginia University Experimental Station. These strains were characterized for their fingerprint and antimicrobial susceptibility profiles (AMS) after the PI joined NCTR. The main objective of this study was to use the fingerprint data to delineate the *Salmonella* transmission pathways in the turkey flocks, which were maintained under high biosecurity protocols. Furthermore, the AMS profiles were evaluated to study the intrinsic drug resistance in *Salmonella* strains isolated from these turkey flocks that were not treated with any antimicrobials and on a production facility that was not populated with any food producing animals for 20 years prior to this study.

We concur with the members that streptomycin and gentamicin are not important in treating salmonellosis. However, these antimicrobials were part of the NARMS testing panel and the antimicrobials included in the CMV1AGNF standardized testing plate (Trek Diagnostics, Inc.), when this study was conducted.

Although we could not delineate the "original" source of *S.* Heidelberg, the DNA fingerprinting experiments showed that this serovar was first isolated from the birds at end of the brooding period (2 weeks) and identical fingerprint profiles of this serovar were subsequently isolated from birds within the same pen; birds in other pens; and litter, air, and swab samples during the grow-out period. This suggested possible horizontal transmission of the *S.* Heidelberg across the facility. In other words, the birds appeared to be the original source of *Salmonella*. The inability to determine the exact source should be put in perspective with regards to the logistics of sample collection and analysis. There were ~1000 day-old chicks during each flock placement and it was impossible to sample every day-old bird to generate a baseline data of initial *Salmonella* contamination of the facility. We had sampled only about 1% of the incoming birds after consultation with USDA-ARS researchers and found these day-old birds tested negative for *Salmonella*. This suggests that either the 1% of the chicks sampled were inadequate and the hatcheries may be the original source of contamination, or extraneous contaminants/vectors, such as fomites, mice, rats or insects, may potentially be responsible for colonization of *Salmonella*. This could also, in part, explain why positive pens became negative. It is simply possible that *Salmonella* remained undetected in the samples that were collected from those specific pens during the sampling period. Quixotically speaking, it would have been nice to sample every bird in every pen for every flock.

Our study on evaluating the farm-to-fork dynamics of *S.* Heidelberg populations is not duplicative of the NARMS program as suggested by the committee members. While we

have included some strains of *S.* Heidelberg from the NARMS collection (veterinary and retail ground turkey samples), strains from "on farm" facilities and processing plants were also included in this study.

Antimicrobial Resistance

1. *Role of bacterial efflux pump on resistance:*

Strengths:
The basic research work on efflux is "cutting edge" and is resulting in important contributions to our understanding of the role of efflux pumps in both resistance and bacterial physiology. It is clear that efflux pump inhibitors, if safe and effective, would be a major advancement in anti-infective therapy.

Recommendations:
The researchers are encouraged to identify additional aspects of this research to fill data gaps relevant to regulatory issues. One suggestion is to perform the *AcrAB* studies in *Salmonella typhimurium* or *S. enteritidis*, with comparisons among other serovars and foodborne enteric pathogens. The *AcrAB* efflux pump is associated with β-lactam antibiotic resistance. Also, apply microarrays to study expression changes following exposure to clinically relevant antimicrobial agents.

Response
We appreciate the reviewers' comments on the nature of this area of research considering the therapeutic impact and benefit that may potentially result from additional study of efflux systems as antibiotic resistance mechanisms. We agree on expanding this research to include not only *E. coli* but other gram-negatives with pathogenic potential including *Salmonella* and *Shigella* species and quite possibly *Campylobacter* species. In addition, we envision targeting our research efforts in this area to known regulatory gaps in the Agency's mission.

In an effort to address these concerns, we have subsequently submitted a concept (E0729201) entitled "Methodologies to assess intrinsic structural mechanisms of bacterial antibiotic resistance and virulence in *Enterobacteriacae* and their dissemination in food and veterinary sources" in response to a call for Agency-wide Critical Path-based projects. This project involves methods development that will hopefully integrate the contribution of these systems as major resistance mechanisms into a regulatory framework. To our knowledge, this area has been relatively unexplored or inadequately tracked within this Agency or others and represents a novel attempt to fill a known regulatory gap. Currently, this concept is being developed into a working protocol document that specifically addresses NCTR Strategic Research Goal 3, Objective 3.1. Furthermore, our current plans include utilizing microarrays to study a variety of factors that may influence expression of efflux pumps and their associated systems including antimicrobials but also other non-antimicrobial therapeutic compounds, biocides, and other environmental agents. Extensions of this research may include assessing the ability of these compounds to influence antibiotic resistance development via pump pathways.

2. *Microarray research:*

Strengths:
The work on the microarray with USDA is a logical method development approach and is leading to novel findings. These discretionary tools are very powerful.

Weaknesses:
The link between pre-harvest *Salmonella* in turkeys and counter-terrorism was not well made. The pre-harvest turkey study did not identify the source of *S.* Heidelberg and did not explain why positive pens became negative or whether the spread was due to multiple introductions. It seems that the array needs more controls. How these tools can be used for biosecurity surveillance needs to be expanded. This is an important area of research.

Response
Our intention was not to generate a link between preharvest *Salmonella* in turkeys and counter-terrorism, but to emphasize the potential usefulness of the microarray technology to be used as a tool for front line defense or as a database to compare future accidental/deliberate outbreak strains and analyze patterns of emerging genotypes and drug-resistant phenotypes. In our microarray virulence biochip, we have used appropriate positive and negative controls (both bacterial and gene).

3. *Bacterial contaminated feed research:*

Isolation and Characterization of *Salmonella* spp. from Imported Seafood and Orange Juice

Strengths:
Studies to characterize *E. coli* and *Salmonella* from feeds and animals have clear benefits to the FDA. The methods applied are appropriate. *Salmonella* strain typing for seafood is a new procedure for tracking intentional and non-intentional foodborne outbreaks.

Weakness
On *Salmonella* antibiotic resistance of isolates from imported seafood, the program needs to first establish that this food source has been a major source of foodborne illness – evidence of which we are not sure exists (i.e., a risk-based approach to prioritizing research). On this second project, the program should consider the effects of exposure of *Salmonella* strains to 3% salt on gene expression.

It was less clear how the information on the S*almonella* from seafood would be used to assist the FDA. There were some technical questions on the orange juice studies, such as the strategy to use PCR to look for the *flo* and *int* in strains that lacked the corresponding phenotypes.

Response
Our research on the isolation and characterization of *Salmonella* spp. from imported seafood and orange juice is in collaboration with ORA (PRL-SW). The objective is to generate a database of imported *Salmonella* isolates for antibiotic resistance, PFGE, and plasmid profiles. This information can be used by FDA to track sources or to monitor the pattern of antibiotic resistance. ORA (PRL-SW) routinely screens seafood samples for *Salmonella* and other foodborne pathogens. This study was initiated to fingerprint the bacteria and screen for antibiotic resistance patterns in these strains. Our research data suggests that *Salmonella* spp. isolated from twenty-six different countries during 2001-2005 contains several multi-drug resistance genes both on plasmid and chromosome. We have isolated several multi-drug resistant *Salmonella* strains from imported seafood, which suggests that it is important to continue the surveillance of foodborne bacterial pathogens from imported foods. We are

planning to study the gene expression of *Salmonella* strains in 3% salt concentration as suggested by the panel.

To characterize the orange juice outbreak *Salmonell*a strains, we used our multiplex PCR method (Khan, AA, et al., 2000, FEMS Microbiology Letters, 182:355-360), which can simultaneously detect four genes, namely, integron, florfenicol, *spvC* plasmid and invasive gene. Although we only found invasive genes in orange juice outbreak *Salmonella* strains, this method is useful to screen other important virulence and antibiotic resistance genes in one simple PCR reaction.

4. *Tetracycline resistance in Aeromonas from catfish:*

Strengths:
Technically sound and thorough.

Weaknesses:
There was concern about the decision, in the face of limited resources, to focus on tetracycline resistance in *Aeromonas* from catfish. Neither the human nor the veterinary health hazards posed in tetracycline resistance in this genus was made clear, nor was the importance to the FDA mission.

Recommendations:
We suggest rethinking the research direction or refining the current studies to address public health priorities. We recommend the researchers move away from tetracycline to fluoroquinolones.

Response
We appreciate the committee's statements on the technical soundness and thorough execution of the tetracycline-resistant Aeromonad investigation. As per the committee's recommendations, we have redirected this research to focus on the isolation and characterization of fluoroquinolone-resistant microflora from imported aquaculture samples. In this regard, we have established collaborative research efforts with several personnel from the ORA labs in Jefferson, AR and Atlanta, GA.

5. *Biodegradation of ceftiofur:*

Strengths:
Biodegradation of ceftiofur is an example of the division's biggest strength in an area of primary importance to the FDA/CVM mission, both in terms of the environmental fate of antibiotics and for the selection pressure place on foodborne pathogens, where cephalosporin resistance is a high priority. Pfizer found it was rapidly degraded by the bovine flora. NCTR interests are to characterize its degradation process.

Recommendations:
The division should take full advantage of their expertise in this area and continue to apply their considerable skills to address important public health concerns.

Response
We appreciate the reviewers' favorable comments about the progress of the research on the "Evaluation of the Mechanisms of Inactivation and Degradation of Third Generation Cephalosporins By the Bovine Intestinal Microflora". We will continue to take advantage of the research expertise in the Division of Microbiology as well as the analytical chemistry facilities and staff at the NCTR.

6. *Probiotic influence on intestinal health and competitive exclusion:*

Strengths:
While not directly related to antibiotic resistance, the work on probiotics, if successful, can reduce our reliance on therapeutic interventions. Much remains to be learned about probiotics and their ability to influence intestinal health and exclude colonization by fecal pathogens.

Recommendations:
We encourage the researchers to extend the probiotic prevention studies to *Campylobacter* in poultry. This is a complex problem that needs more interaction with other NCTR PI and other FDA centers. Seek out some CRADA, since the industry is also interested in probiotics.

Response
We can explore the possibility of CRADA or IAG interactions in the area of probiotics for *Campylobacter* in poultry. The poultry probiotics industry and CVM are currently having discussions over the safety issue of antimicrobial drug resistance in component bacteria. This will continue to be an area where research in the Division of Microbiology could have significant impact on the FDA mission.

7. *Vancomycin-resistant Enterococci:*

Strengths:
With difficulty getting other some primers to work, they are producing their own primers.

Weaknesses:
As noted elsewhere, we believe it is important that epidemiology and risk analysis play an important role in the prioritization of NCTR research. The spread of VRE via food animals is not a major public health issue in the United States, where glycopeptides have never been used in food animal husbandry. In addition, the typing scheme used to characterize the isolates was devised *de novo*, despite the availability of established schemes for typing the van gene cluster in clinical isolates; and it appears that no attempt was made to compare techniques. We question the current relevance to FDA's research needs.

Recommendations:
Need some guidance from CDER. We agree with moving to *Campylobacter,* which is a growing problem.

Response
We agree with the comments of the committee about the spread of VREs via food animals not being a major concern. However, about the comments that we did not compare our results with the established conditions for typing the strains, we would like to point out that we did use the

established techniques, such as PFGE, PCR, RFLP and sequencing, to pinpoint differences in the *van* operons of different VRE isolates. Moreover, considering the facts that VREs have acquired resistance to multiple antibiotics, that vancomycin is one of the last resort antibiotics, that VREs are present in the competitive exclusion product "Preempt™" that is used to stop the colonization of chicken with *Salmonella*, that VRE infections have increased among hospitalized patients, and that the public health falls under the aegis of the FDA, the work on VREs is relevant to the research needs of the FDA and clearly warrants monitoring the antibiotic resistance profile and the development of new trends among VREs.

8. *Staphylococcus aureus antibiotic resistance:*

Strengths:
Staphylococcus aureus methicillin resistance is fatal in healthy individuals. Proteomic studies demonstrated 541 unique protein expressions within 24-hour incubation. Bacterial hyaluronidase is an important invasive enzyme toward human infection. The researchers are proposing to produce hyaluronidase knock-out to determine if this enzyme is important for virulence. Hyaluronidase is a virulence factor for *Streptococcus*.

Recommendations:
Is anyone evaluating heat stable virulence factor in staph as a virulence factor? There are 17 serotypes of Staph enterotoxin. Which proteins are relevant? The protein structure function issue is relevant. This research is very good and very important.

Response
S. aureus growth and enterotoxin production have been studied extensively. In addition, the genetics, including molecular diagnostics and synthesis of staphylococcal enterotoxins, have been reported. In an effort to facilitate collaborative efforts with scientists at other FDA Centers, we have contacted CBER to determine their interest in our *S. aureus* projects.

Gastrointestinal Microbiology and Host Interactions

Strengths:
We complement the investigators on the breadth of their expertise and the applicability of their research programs to aid in addressing safety questions and issues. The scientists of the Microbiology Division are a resource in developing a variety of *in vivo* (gnotobiotic, germ- free, target animal) and *in vitro* model systems (e.g., fecal batch culture, fecal semi-continuous cultures, intestinal epithelial model) and methodologies to examine the effects of exogenous compounds (antimicrobial agents and their residues, probiotics, food and feed additives,) and endogenous host-derived factors on the GI microflora and the host immune system.

Weaknesses:
Scientists are encouraged to evaluate the variability, reproducibility, and predictive value of the model systems used in addressing human-safety questions and the risks of identified hazards. As the scientists are working in an advisory capacity, the issue of the applicability of the model systems in predicting effects on humans and decisions on public health safety becomes more important in applying their experimental work in context of any safety recommendations (see below on recommended work).

Gastrointestinal Microbiology and Host Interactions: Research Recommendations:

1. Given that the division has developed and has expertise in more than one intestinal model (*in vivo* and *in vitro*) and that it is difficult to validate these models for purposes of evaluating human safety questions addressed by the FDA, is it possible to examine the effects of molecules in different models and determine whether the observations (e.g., no-effect concentrations, determinations of changes in colonization barriers, metabolic fate, etc.) are similar in different models? Are the observations reproducible within a model? If the answer is unknown or no for either of these questions, then what is the justification for their use in risk assessments for safety of exposure to test molecules? Are the models predicting no-effect concentrations that are higher or lower than would be expected in humans?

Response

We appreciate the recognition of the committee that the investigators in the Division of Microbiology have outstanding training and expertise in gastrointestinal microbiology and host interactions. Many *in vitro* and *in vivo* approaches have been used to examine the effects of antibiotics, probiotic products, food additives and dietary supplements on the microbiota of the gastrointestinal tract. We agree with the committee that these models should be validated to determine the predictive capabilities of *in vitro* and *in vivo* test systems in determining adverse human health effects. We are currently having discussions with CVM scientists to harmonize approaches and test methods to evaluate the safety of drugs used in food animals for potential human health risks.

2. Do the high levels of enterococci and clostridia (compared to *Bifidobacteria* and *Bacteroides*) on day 14 post inoculation occur in all mice similarly inoculated?

Are there other models/studies that corroborate/validate the observations made in the studies of the probiotic effects on pathogen colonization of the mice? For example, reproducible models of *Campylobacter* colonization in mice are lacking.

Response

The higher percentage of enterococci in HMA mice vs. human colon is the major difference in the model microbiota. It reflects higher percentages of enterococci in murine GI tract microbiota than in humans and confirms that there are species differences. The model is otherwise similar to human microbiota, but much validation work would be needed before regulatory decisions could be made from it. These studies are first steps toward models for prediction of effects on humans. More research is needed to determine if these models are predictive for human safety decisions. We will consult a statistician for future studies.

3. The *Clostridium* studies provide molecular information on fluoroquinolone resistance where basic information is lacking. Have attempts been made to determine whether fluoroquinolone-resistant, clinical (diagnostic) isolates from humans (hospital or community-acquired) or animals (e.g., poultry isolates that may be obtained via the USDA collaboration) have the same mutations as those documented in the laboratory strains? If the mutations are the same in clinical (field) isolates and those generated in the laboratory, such information would provide a rationale for further investigational studies of the laboratory strains and expression of virulence determinants. Comparisons could be made between the isogenic lab strains as well as fluoroquinolone resistant, clinical isolates that have the same (or different) mutations. How prevalent are such mutants

among clinical isolates in humans? If prevalence is low, or the mutations in the lab isolates versus clinical isolates were different, what would be the prioritization/emphasis of future studies on the laboratory strains?

Response

In the literature the Clostridia are described as an important cause of morbidity and mortality in humans and animals. Some of the most common clostridial infections are those of the gut. The primary infections in humans are caused by *C. perfringens* and *C. difficile.*

C. perfringens, in addition to being a cause of food poisoning, is the cause of gas gangrene and nonfoodborne GI illnesses, including antibiotic-associated diarrhea (AAD) and sporadic diarrhea. *C. perfringens* GI diseases are typically more severe and longer-lasting than *C. perfringens* type A food poisoning. *C. perfringens* is also the dominant cause of gastrointestinal infections in animals, including necrotic enteritis in chicken and turkey, which could be a source for food poisoning, directly or through contamination of produce. The FDA Bad Bug Book (http://www.cfsan.fda.gov/~mow/chap11.html) reports *C. perfringens* food poisoning as one of the most commonly reported foodborne illnesses in the U.S. Research conducted on this organism is of interest to FDA, USDA and public health officials.

Antibiotic-associated diarrhea is an important cause of morbidity and mortality, particularly in the hospitalized elderly. Several recent outbreaks of *C. difficile* associated disease have been attributed to the emergence of an epidemic strain with increased resistance to fluoroquinolones. Some of the *in vitro* mutants, developed as the result of exposure of wild type *C. difficile* to increasing concentrations of gatifloxacin and other fluoroquinolones, have resulted in the generation of mutants with gyrase mutations identical to those found in some clinical strains.

Fluoroquinolones have been considered an important risk factor for *C. difficile* antibiotic associated disease due to epidemic strains. Fluoroquinolone-resistant strains of highly virulent *C. difficile* with enhanced toxin production have been found in health care facilities. So, in addition to evaluating the effects of antimicrobial agents on resistance development, it is timely to evaluate the physiological changes that occur in these bacteria as the result of exposure to fluoroquinolones, which may result in increases in virulence. We already have found enhanced toxin production in one out of five mutant strains of *C. perfringens* compared with the wild-type strains. These results have been similar to differences observed in the clinical setting in the correlation between the use of fluoroquinolones and emergence of virulent strains of *C. difficile.* So we agree with the assessment of the committee that the *Clostridium* study provides molecular information on fluoroquinolones where basic information is lacking. Use of isogenic strains with genotypic and phenotypic differences resulting from exposure to antimicrobial agents is a powerful tool for understanding the effects that these compounds have on changing the bacterial physiology in ways that affect human health.

Environmental Biotechnology

Microbial Metabolism of Fluoroquinolone Drugs

Strengths:
This work shows the potential for fluoroquinolone drugs to undergo microbial biotransformation, and resulted in the identification of potential metabolites, which might be present in the environment. This is new information for assessing the fate of these drugs in the environment.

Weaknesses:
The work to date focuses on the use of pure cultures and a grab sample of liquor from a sewage treatment plant. All studies have been done at relatively high concentrations, which facilitate the analytical chemistry, but may not reflect what occurs *in situ*. This work should be viewed as a first step and a move should be made into more realistic systems simulating the actual environment to provide a more accurate picture of the kinetics and extent of metabolism occurring *in situ*.

Response
To increase the relevance of our research to the FDA regulatory needs in Environmental Biotechnology, we have recently participated as members of "The Working Group on Pharmaceuticals in the Environment (PIE)". The following departments and agencies were represented in the PIE Working Group; Department of Agriculture, Department of Commerce, Environmental Protection Agency, National Oceanic and Atmospheric Administration, U.S. Geological Survey, Food and Drug Administration, Centers for Disease Control and Prevention, and National Institute of Health/National Institute of Environmental Health Sciences. The purpose of the PIE Working Group is to develop a research strategy that defines and prioritizes topic areas and data needs for which resolution would improve the ability to assess possible human and ecological risk from pharmaceuticals in the environment and recommend areas for federal collaboration to address those priorities. In addition, our concept papers have been circulated to scientists in Environmental Safety programs at the Center for Veterinary Medicine (CVM) and Center for Drug Evaluation and Research (CDER) for FDA regulatory relevance.

Elucidation of PAH Pathways

Strengths:
This works represents an unparalleled application of genomic, proteonomic, and analytical chemical approaches to document the bacterial biodegradative pathway for a xenobiotic. The completion of this project represents a major achievement in microbial physiology. The quality of this work is validated in the nature of the journals in which it has been published.

Weaknesses:
There is not a transparent link between this work and FDA's mission. However, the work is well funded by EPA and DOE and additional support has been requested from NSF and the Arkansas Biological Institute. The techniques and capabilities developed as a result of this work may have application to future work needed by the FDA.

Response

We appreciate the comments of the SAB committee about the novelty, thoroughness and impact of the research on "Omics" approaches in the "Elucidation of Biodegradative Pathways for Carcinogenic Polycyclic Aromatic Hydrocarbons" (PAHs). We are conducting integrated field and laboratory studies that provide the opportunity for research across the range of disciplines that will increase our understanding of the environmental fate of PAHs. Through our collaborations with other federal agencies and academia, the advanced "omics" methods developed will be employed to address future research needs of the FDA.

There are some editorial and typographical errors that need to be rectified in the SAB report.

p1. Dr. Ellen Olson (ORA) name was omitted as part of the review team.

p2. Paragraph 4, line 1, should be deputy director.

Paragraph 4, line 4, should be Don Paine.

p4. Paragraph 3, Line 1, Replace "….with university of West Virginia poultry...." to "….with West Virginia University poultry"

Paragraph 3, Line 4, Replace "….here S. enterica serovar **Javiana**, in multiple" to "….here S. enterica serovar **Heidelberg**, in multiple.…"

Paragraph 3, Line 5, Replace "….grown on the same Virginia farm." to "….grown on the West Virginia University farm."