Microbiology

Antimicrobial Resistance

We have the capability:

- to isolate, characterize and identify, using cultural and molecular methods, pathogens from processed foods, aquaculture, clinical, and environmental samples.
- to conduct antimicrobial susceptibility testing of bacterial isolates using standardized methods, including Sensititre, micro-broth dilution assays, disk-diffusion assays and E-tests.
- to assess the molecular genetics of pathogens isolated from clinical, food, aquaculture and environmental sources.
- to detect the presence of multiple antibiotic resistance genes in bacteria from different ecological backgrounds by the use of microarray and recombinant DNA technologies.
- to evaluate the mechanisms of antimicrobial resistance of pathogens isolated from clinical, food and environmental sources.
- to evaluate the antimicrobial resistance properties of probiotic products.

Reports of antimicrobial-resistant bacteria from farms, animal carcasses and aquaculture facilities are raising concerns that antimicrobial use in food-producing animals may play a role in the emergence of antibiotic resistance. The research and regulatory issues on antimicrobials used in food-producing animals are of great importance to the FDA. Through collaborative efforts with other FDA Centers, we conduct research on the emergence and dissemination of antibiotic-resistant bacteria.

Disk Diffusion Asssay

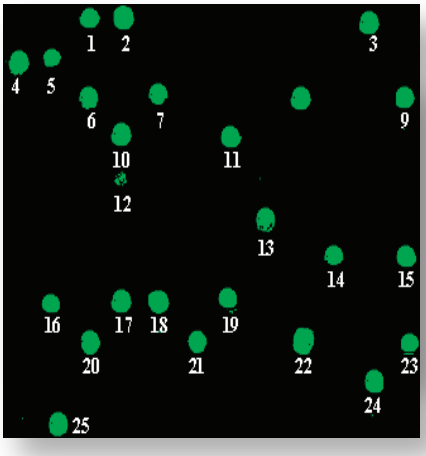
Contributions to the antimicrobial resistance research in the Microbiology Division:

- **The use of fluoroquinolones in poultry has been proposed to result in the emergence and dissemination of fluoroquinolone-resistant *Campylobacter* and *Salmonella* strains.** PCR-based methods have been developed to screen for fluoroquinolone-resistance genes in isolates from chicken and turkey farms. An oligo-based microarray method has been developed for the detection of antibiotic resistance markers representing a broad spectrum of antibiotics.


Molecular methods were developed to screen fluoroquinolone-resistance genes in isolates from chicken and turkey farms

- **Ecological surveys have been conducted to monitor the frequency of multi-drug resistant foodborne pathogens, such as *Salmonella* and *Campylobacter*, in the pre-harvest poultry environment.** These baseline studies are used by the FDA in developing public policies for the use of antimicrobials in food-producing animals in the U.S.

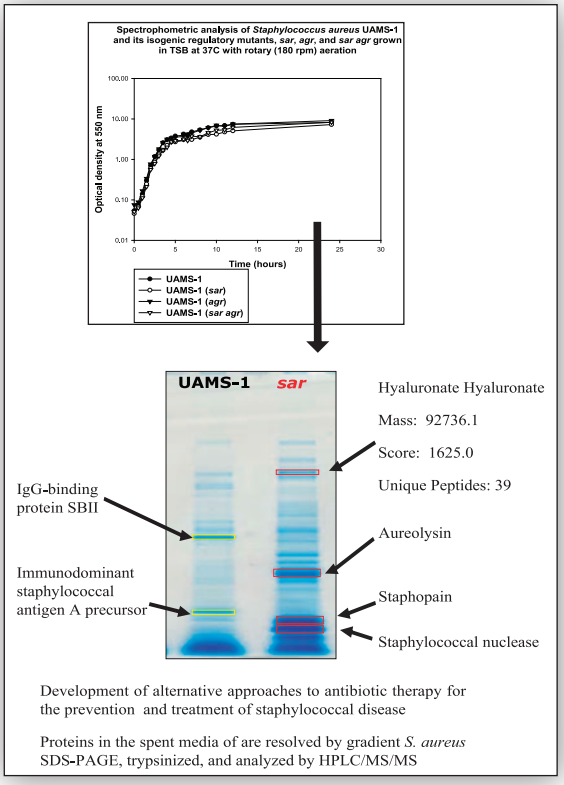
- **We have developed molecular protocols to individually detect all the possible known vancomycin resistance genes in *Enterococcus faecalis*.** The most dangerous forms of enterococcal infections are those caused by *vanA*-type vancomycin-resistant enterococci. They exhibit a high-level of resistance to vancomycin and other antibiotics. To expedite their detection, we have developed a multiplex-PCR assay to concurrently detect the genes *vanA, vanB, vanC1, vanC2* and v*anC3*.

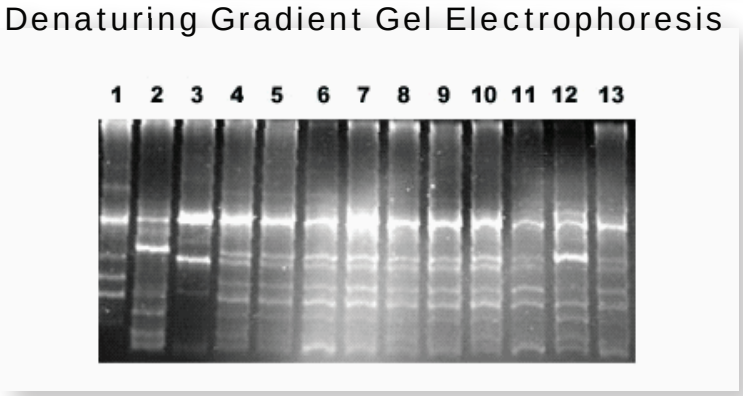


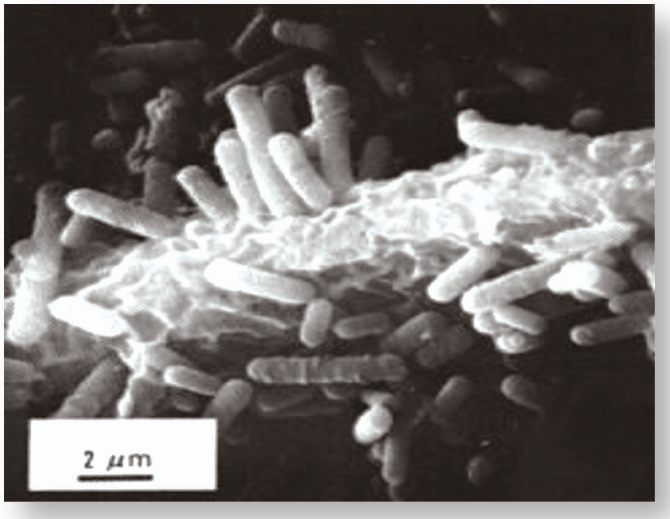
Development of microarray probes to rapidly screen for a wide variety of antimicrobial resistance markers.

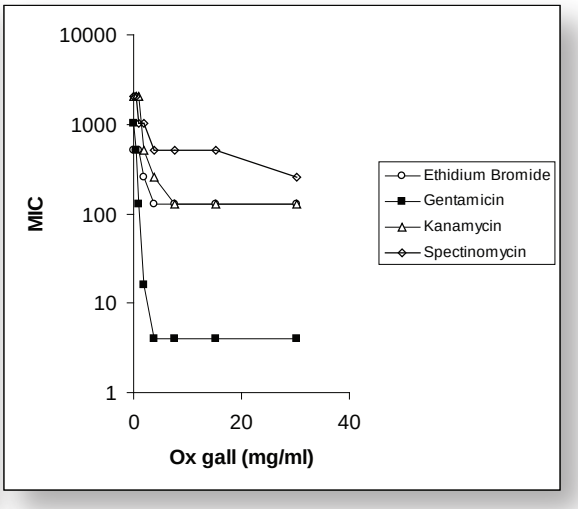
- **We have evaluated the structural variations in fluoroquinolones which result in their effectiveness and the prevention of mutant development in gastrointestinal microflora normally resistant to these compounds.** The various mechanisms that bacteria employ to evade bactericidal activities of antimicrobial agents are being studied.

- **We have evaluated the antimicrobial drug resistance patterns of multiple-antibiotic-resistant bacteria isolated from competitive exclusion products.** Competitive exclusion products consist of mixtures of bacteria originally isolated from chicken intestinal tracts. The bacteria isolated were *Lactococcus lactis* and *Enterococcus faecalis.* These bacteria are resistant to vancomycin, erythromycin, and tetracycline and can transfer their drug-resistance to strains of *Enterococcus* spp. isolated from the competitive exclusion product and to a laboratory strain of *Staphylococcus aureus.* The results of the study indicate the probability of resistance transfer among between bacteria in competitive exclusion products.









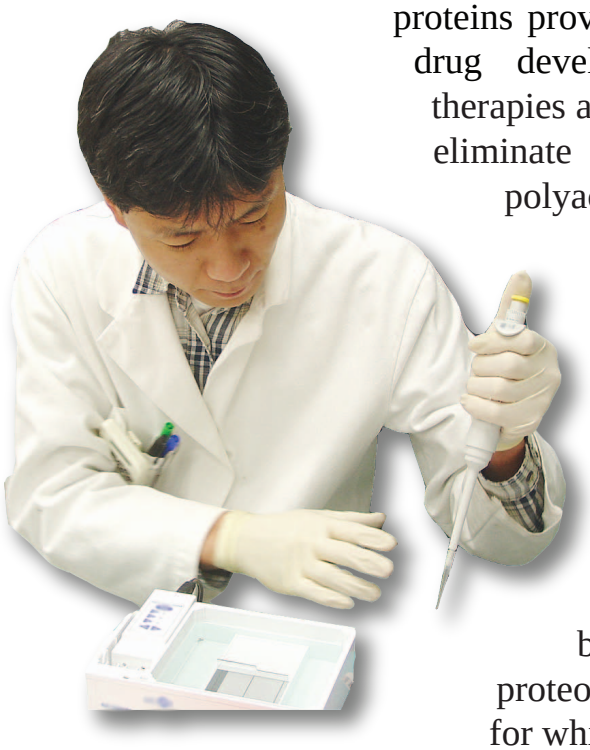
Antibiotic Resistance in GI tract Microbiota: Focus on ***Lactobacillus***

Common GI and vaginal tract microbes
Starters in a variety of food consumables
Resistance studies in vitro versus in GI tract
Consumed as probiotics (living drugs)
Effects of autogenic factors (i.e. bile acids) on resistance phenotype
Contribution of efflux-mediated resistance

- **We have found that the use of erythromycin in poultry and aquaculture industries selects for bacteria with the erythromycin ribosomal methylase (*erm*) genes *ermA* and *ermC*.** Both of these determinant genes were found to be transferable to human *Staphylococcus aureus* strains. Transposition and transposition-assisted plasmid mobilization were the mechanisms of drug-resistance transfer.

- **We have developed a multiplex PCR assay to simultaneously screen for the presence of five tetracycline resistance genes in *Aeromonas* samples isolated from farm-raised catfish.** A 16S rRNA based protocol to identify 14 different species of *Aeromonas* has been developed.

- **Proteomic approaches are necessary to identify *Staphylococcus aureus* extracellular proteins responsible for staphylococcal disease.** The identification of proteins provides a target for anti-staphylococcal vaccine and drug development. This would augment antimicrobial therapies and allow the patient's own defenses to control and eliminate the infection. Research using one-dimensional polyacrylamide gel electrophoresis and liquid chromatography-tandem, mass spectrometry has generated profiles of proteins, both cytoplasmically located and those secreted, from *S. aureus* UAMS-1, an osteomyelitis clinical strain, and its isogenic regulatory mutants *sar*, *agr*, and *sar/agr*. In addition, a time-course study has been conducted showing extracellular protein changes during cell growth. Some 624 proteins have been identified thus far by searching against the proteomic database of *S. aureus* MW2, a clinical isolate for which a proteomic database has been established.



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

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March 10, 2006