Future of WGS and Food Safety

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Future Directions

- Other groups within CFSAN are also considering how to use WGS including Market Basket Surveys, Compliance Sampling Assignments, and Baseline testing programs in CFSAN.

- WGS-based typing approaches are supplanting current microbiological methods (i.e., phage typing – serotyping, PFGE, etc.) and will yield one microbial workflow very soon. Significant lab cost savings with one approach!

- Sequencing spoilage organisms and build targeted sequencing approaches for food industry.

- Functional Genomics to understand markers and diagnosis for preventative controls, such as resistance to heat, cleaners, high salt and or desiccation. Risk predictions may be improved by gene presence/absence for key pathogens.

- The GenomeTrakr database will serve as the primary reference database for transition to culture-independent food testing and surveillance in the near future. Begin MetaGenomeTrakr for shot-gun sequencing proof of concept.
One Data Record - Many Possibilities

- SNP
- Virulence
- Sanitizer Resistance
- Serotype
- wgMLST
- AMR
- Unknown

.....AAGCTTGGAGATCTACGTGTACCTAGTCGAAGCTA....
HGP led to more than just understanding Ancestry!

Human Genome Sequencing

- Drug treatment
- Targeted drug development
- Gene therapy
- Improved human health
- Revealed genetic predictors of disease
- Human genetic variation
- Improved disease diagnosis
The full impact of WGS for foodborne pathogens

- Better agricultural practices
- Safer food
- Improve microbial disease diagnosis
- Targeted drug development
- Improved human health
- Risk assessment
- Microbial genetic variation
Bacterial Toxins
Overview

Ingestion vs Infection

*Bacillus cereus* toxins

Limitations of functional assays

Toxin profiling with WGS
Genomic analysis of Botulinum Neurotoxin (BoNT) producing clostridia and non-clostridiales producing BoNT homologs

Goals:
• Develop reference database development for neurotoxigenic clostridia and phylogenetic analysis
• Understand the phylogenomic competence of infant botulism strains (infectious form) versus food-borne botulism (toxigenic form)
• Risk assessment of BoNT homologs for human health risks and interference with BoNT detection methods

Specific objectives:
• Whole genome sequencing and closing of clostridial genomes (CFSAN repository strains; more than 500 unique strains)
• Genetic and functional analysis of BoNT gene clusters and toxin homologs
Antimicrobial Resistance (AMR)
The National Antimicrobial Resistance Monitoring System: One Health Surveillance in the Age of Genomics

- Highest practical resolution of individuating structural traits among microbial members of an ecosystem
- Accurate prediction of clinical resistance, and perhaps MIC.
- Resistance to compounds not tested phenotypically such as disinfectants and heavy metals, and other potential drivers of resistance
- Deep surveillance into previously hidden associations (e.g., co-resistance with plasmid type, virulence) including determinants of zoonotic transmissibility of resistance
- Animal/food source attribution for more precise intervention
- Retrospective resistance surveillance for new mechanisms
- Metagenomic approaches to comprehensive resistome surveillance
- Look farther with few resources: domestic and wild animal populations, animal feed, environmental transmission & exposure pathways.
- Global resistance emergence and spread can be better tracked and addressed internationally
Environmental Sampling and Good Agricultural Practices
Scenario 1 (pass through)

Field 1

Field 2

Processing facility
Scenario 2 (harborage and persistence)
Why is environmental sampling important?

The GenomeTrakr database relies on a reference set of isolates from known foods and known geographic localities.

• A genetic match of an unknown clinical to a known food or geographic location provides a clue for source-tracking where the contamination has occurred.

• Knowledge of where a contaminant is coming from allows the potential for industry to fix the problem based on scientific evidence (supporting preventative controls)
Salmonella enterica subsp. enterica sv. Newport

- Emergent from livestock (ie., cattle, swine, chickens)
- Many strains resistant to multiple drugs including third-generation (Cephalosporins)
- Third most common serotype of Salmonella associated with foodborne illness
- Diverse range of niches and reservoirs: livestock, variety of produce commodities, and a diverse array of environmental niches
- Hearty environmental bacterium with ecological and geographic signal (ie., JJPX01.0014 – oysters; JJPX01.0061, -0.0030 – Eastern Shore)
Tomato Agriculture

- Fumigation and Plastic mulch
- Drip irrigation
- Seedlings planted by hand
- Filtered irrigation water
- Stringing, staking by hand
- Routine application of pesticides, fungicides

Figure 14 - Age-standardized rate per 100,000 population, by county, 2006 to 2011

Age standardization based on 2011 census
Salmonella strain discrimination using WGS

Salmonella Newport 61 WGS subgroups

Farm A
Farm B
Farm C
Farm D
Illness
Farm E
Farm F
Farm G
Farm H

Farm D Identified specifically as the potential illness reservoir
Metagenomics
(Culture-Independent Sequencing)
GenomeTrakr WGS vs. MetaGenomeTrakr WGS

Whole Genome Sequencing

Metagenomic Sequencing

Sequence genomes of a single type of bacteria

Sequence genomes of an entire microbial community or microbiome
QUASI-METAGENOMIC ANALYSIS FOR SALMONELLA
**TOWARDS A CULTURE INDEPENDENT FOOD TESTING PROGRAM**

*Lm* enrichment (BAM) – characterized every 4 hours to describe genomic coverage of *Lm* and describe co-enriching microbiota.
Sprout Spent Irrigation Water
Metagenomics

Relative Abundance

Sample

Other genera < 3%
Escherichia
Bacillus
Leclercia
Acinetobacter
Pseudomonas
Erwinia
Cronobacter
Pantoea
Klebsiella
Salmonella enterica

T0
T2
T24
T8
T24
T28
T32
T36
T40
T44
T48

10
Sprout Spent Irrigation Water
Using RNAseq for Metabolic Pathways

Metabolism of cofactors and vitamins
Biosynthesis of secondary metabolites
Tracking presence of bacteria in ice cream during preenrichment using Shotgun metagenomics.

Andrea Ottesen, Padmini Ramachandran, Elizabeth Reed, James R. White, Nur Hasan, Poorani Subramanian, Gina Ryan, Karen Jarvis, Christopher Grim, Ninalynn Daquigan, Darcy Hanes, Marc Allard, Rita Colwell, Eric Brown and Yi Chen. Enrichment dynamics of Listeria monocytogenes and the associated microbiome from naturally contaminated ice cream linked to a listeriosis outbreak. BMC Microbiology 2016, 16:275

In 2-3 years FDA regulatory labs will collect this type of data. MetaGenomeTrakr
Genomes from different hours of enrichment (H20, H36, & H40) cluster with WGS of pure isolates from ice cream listeriosis outbreak

quasiMetaGenomic Sequencing...
The full impact of WGS for foodborne pathogens

- Better agricultural practices
- Safer food
- Improve microbial disease diagnosis
- Targeted drug development
- Improved human health
- Risk assessment
- Microbial genetic variation

Microbial Sequencing

www.fda.gov
Next-Generation sequencing faces several large challenges as it deploys to a global public health tool:

- Will all share data?
- Quality concerns and curation?
- Who pays?
- Who owns the IP?
- Administration, coordination, and oversight?
What other issues?
Note:

• These slides are for teaching purposes only and have been collected from images that I have made, from the CDC and FDA, and from around the web.

• The findings and conclusions in this report are those of the author and do not necessarily represent the official position of the Food and Drug Administration.