One Health benefits of using pathogen WGS as a tool for herd management

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Overview

1. Pathogen Whole Genome Sequencing (WGS) strategies
2. Source tracking
3. Pathotyping
4. Antimicrobial resistance
1. How pathogens are sequenced

Clinical Goal: provide rapid, low-cost, clinically actionable data in lay terms for veterinarians & producers
Most common strategies for pathogen WGS

- Pure cultures (shotgun)
- Amplicons
- Selective extraction
The Whole Genome Sequencing Process

1. DNA Extraction
   - Scientists take bacterial cells from an agar plate and treat them with chemicals that break them open, releasing the DNA. The DNA is then purified.

2. DNA Shearing
   - DNA is cut into short fragments of known length, either by using enzymes "molecular scissors" or mechanical disruption.

3. DNA Library Preparation
   - Scientists make many copies of each DNA fragment using a process called polymerase chain reaction (PCR). The pool of fragments generated in a PCR machine is called a "DNA library."

4. DNA Library Sequencing
   - The DNA library is loaded onto a sequencer. The combination of nucleotides (A, T, C, and G) making up each individual fragment of DNA is determined, and each result is called a "DNA read."

5. DNA Sequencing Analysis
   - The sequencer produces millions of DNA reads and specialized computer programs are used to put them together in the correct order like pieces of a jigsaw puzzle. When completed, the genome sequence containing millions of nucleotides (in one or a few large pieces) is ready for further analysis.

https://www.cdc.gov/pulsenet/pathogens/protocol-images.html#wgs
National pathogen WGS database: heavily human-focused

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total isolates (10/3/18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>153,255</td>
</tr>
<tr>
<td><em>E. Coli</em></td>
<td>54,679</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>20,879</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>21,838</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>7,774</td>
</tr>
<tr>
<td><em>Staphylococcus pseudintermedius</em></td>
<td>249</td>
</tr>
</tbody>
</table>

Culture-independent detection methods

*Mostly for environmental testing – some commercial clinical tests for humans*

- Targeted amplification (PCR of *multiple* genes)
- Targeted metagenomics (sequencing of *many* genes)
- Shotgun metagenomics (sequences “all” DNA)
DNA blunder creates phantom serial killer

Police admit they wasted 15 years hunting for the 'Woman Without a Face'

She was a mysterious serial killer known as the "The Woman Without a Face" and detectives across Europe spent more than 15 years doing their utmost to bring her to justice for at least six brutal murders and a string of break-ins. Yesterday, however, they were forced to admit that she probably didn’t exist.

The only clues that "The Woman Without a Face" left behind at 40 different crime scenes were DNA traces. These were collected on cotton swabs, supplied to the police in a number of European countries. Now police investigators have established that in all probability the DNA had not been left by their quarry but by a woman working for the German medical company supplying the swabs, who had inadvertently contaminated them.

German police who had been leading the hunt said they had probably been involved in one of the longest and most perplexing wild goose chases in criminal history. "This is a very embarrassing story," admitted police spokesman Josef Schneider.
2. Source tracking

Clinical Goals: pin point specific common sources of outbreak strains, monitor efficacy of autogenous bacterins/vaccines
Linking animal and feed strains
Managing environmental *Salmonella* contamination on dairy farms

Picture credit: www.gea.com
FDA Vet-LIRN *Salmonella* surveillance, 2017
SNP Distance to Human Isolate (n = 54)
Within 10 SNPs
Not Within 10 SNPs
Distance to Human Isolate

Host Type

Proportion
Managing recurring *Klebsiella* mastitis

- Dairy herd of 940 cows
- Bulk milk SCC = 293,000 / mL
- Compared clinical, fecal, and environmental isolates to assess shedding potential
Monitoring new introductions of canine influenza to the USA

China and Korea Emergent

China Enzootic

Korea Enzootic

USA Enzootic

0.003 subs/site

Collaboration with Ian Voorhees and Colin Parish
3. Pathotyping and species ID

Clinical Goal: differentiate normal host bacteria from those of clinical significance
Serotype prediction in bacteria and viruses

Nmhealth.org

Nature Reviews Microbiology
More precise speciation

• Many bacteria are not well identified by 16S alone (or by phenotypic characteristics of conventional microbiology)
  – Define/predict which biochemical tests are appropriate

• Many viral isolates go unspeciated due to lack of specific tests
  – Complement other tests such as EM, IFA, PCR, cytotoxicity, chloroform resistance
Bacterial toxin gene detection
Informed wildlife management

- Food production
- Water resources
4. Antimicrobial resistance

Clinical Goals: detect markers that are not covered by species-specific AST panels, make antibiograms to support judicious use initiatives
WGS AMR predictions by gene have good correlation with sensitivity results

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Gen/Phe correlation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella enterica</em></td>
<td>99.7%</td>
<td>Zankari et al. 2013, J Antimicrob Chemother</td>
</tr>
<tr>
<td></td>
<td>99.00%</td>
<td>McDermott et al. 2016, Antim Agents Chemother</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>97.1%</td>
<td>Stoesser et al. 2013, J Antimicrob Chemother</td>
</tr>
<tr>
<td></td>
<td>98.5%</td>
<td>Tyson et al. 2015, J Antimicrob Chemother</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>99.2%</td>
<td>Zhao et al. 2015, J Antimicrob Chemother</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>98.8%</td>
<td>Gordon et al. 2014, J Antimicrob Chemother</td>
</tr>
<tr>
<td><em>Pneumococcus</em></td>
<td>98%</td>
<td>Metcalf et al. 2016, Clin Microbiol Infect</td>
</tr>
<tr>
<td><em>Enterobacteriaceae (B-lacs)</em></td>
<td>100%</td>
<td>Shelburne et al. 2017, Clin Infect Dis</td>
</tr>
<tr>
<td><em>Mycobacterium</em></td>
<td>95.3%</td>
<td>Phelan et al. 2016, Genome Med</td>
</tr>
<tr>
<td></td>
<td>92.3%</td>
<td>Walker et al. 2015, Lancet Infect Dis</td>
</tr>
</tbody>
</table>
MICs can now be predicted for some bacteria
GOAL 2: Strengthen National One-Health Surveillance Efforts to Combat Resistance
Distribution of Antibiotic Resistance Genes (ARGs) in *Salmonella* by Human Distance
Distribution of *Salmonella* ARGs by Host Type
Canine *E. Coli* ARGs by Specimen Type
Most extreme case – from 2017

Nearly pan-resistant *E. Coli* from canine fecal sample

**ECOL-17-VL-NY-FL-0002**

- aac(3)-Iid (gentamicin)
- aadA1 (streptomycin)
- aph(3")-Ib (streptomycin)
- aph(3')-Ia (kanamycin)
- aph(6)-Id (streptomycin)
- blaCMY-2 (penicillins, amoxi-clav, cephlosporins)
- blaTEM-1 (penicillins)
- catA1 (phenicols)
- dfrA14 (trimethoprim)
- mph(A) (macrolides)
- qacL (disinfectants)
- sul2, sul 3 (sulfonamides)
- tet(B) (tetracycline)
- gyrA mutations (fluoroquinolones)
One Health AMR Data Sharing

- Meeting sponsored by NY Integrated Food Safety Center of Excellence held May 2018 with stakeholders from different sectors
- Surveillance from WGS AMR data is more rapidly available than traditional methods
  - NARMS monitors NCBI for emerging resistance threats
  - Also publically accessible to researchers and other agencies
- Importance of data integrity, security, and confidentiality
  - NAHLN study emphasizing secure messaging
- A tiered system with a 3rd party protector of identifiable information proposed as safeguard for confidentiality
Take-home points

1. WGS supports preventative controls in herd management

2. Open data is critical for disease surveillance
Acknowledgments

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