New molecular approaches to AMR testing

Laura Goodman
Cornell University
Animal Health Diagnostic Center &
NY State Veterinary Diagnostic Laboratory
laura.goodman@cornell.edu
Why do we need to genetically “predict” antimicrobial resistance in animal health?
Bacterial **whole genome sequencing** in vet diagnostics

- Performed on **cultures** (costs ~$200)
- Nationally harmonized lab procedures (with FDA/CDC/state health)
- Confirms species, subspecies, isolate relatedness
- Large databases mined to predict features (functional genomics):
  - Serotype
  - Virulence factors
  - Antibiotic resistance gene (ARG) profile

National CARB veterinary surveillance project using WGS

- FDA Veterinary Laboratory Investigation and Response Network
  - 25 vet diagnostic source labs
  - ~2,000 isolates collected in 2017
    - *Salmonella* (all hosts)
    - *E. coli* (dogs)
    - *S. pseudintermedius* (dogs)
    - “Other” (2018)
  - Including whole genome sequencing on a subset (done by 5 additional vet labs) uploaded to NCBI in near real-time
  - NARMS integration
## Metadata protections

<table>
<thead>
<tr>
<th>Data included</th>
<th>Not included</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host species</td>
<td>VDL accession number</td>
</tr>
<tr>
<td>Sample type (e.g. feces, respiratory, wound swab)</td>
<td>Referring DVM</td>
</tr>
<tr>
<td>Collection date</td>
<td>Animal owner</td>
</tr>
<tr>
<td>State of origin</td>
<td>Animal name</td>
</tr>
<tr>
<td>Case type</td>
<td></td>
</tr>
<tr>
<td>Lab methods</td>
<td></td>
</tr>
</tbody>
</table>
Working towards animal health representation in the NCBI database

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total isolates (10/22/19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td>235,207</td>
</tr>
<tr>
<td>E. Coli</td>
<td>87,471</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>44,737</td>
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<tr>
<td>Listeria monocytogenes</td>
<td>29,306</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>17,982</td>
</tr>
<tr>
<td>Staphylococcus pseudintermedius</td>
<td>695</td>
</tr>
</tbody>
</table>

Rethinking the antibiogram: The ARG heat map

Class
- β-Lactam
- Aminoglycoside
- Sulfonamide
- Tetracycline
- Phenicol
- Trimethoprim
- Fosfomycin
- Fluoroquinolone
- Antiseptic

Veterinary *Salmonella*

Ceric et al., BMC Vet 2019
FDA Vet-LIRN, data available at ncbi.nlm.nih.gov/pathogens/
Distribution of ARGs in veterinary *Salmonella* by Distance to Human Cases

FDA Vet-LIRN, data available at ncbi.nlm.nih.gov/pathogens/
Most extreme cases highlight role of companion animals in AMR

Canine lung (2017)
- aac(3)-Iid (gentamicin)
- aadA1 (streptomycin)
- aph(3")-Ib (streptomycin)
  - aph(3')-Ia (kanamycin)
- aph(6)-Id (streptomycin)
- blaCMY-2 (penicillins, amoxi-clav, cephalosporins)
- blaTEM-1 (penicillins)
- catA1 (phenicols)
- dfrA14 (trimethoprim)
- mph(A) (macrolides)
- qacL (disinfectants)
- sul2, sul 3 (sulfonamides)
- tet(B) (tetracycline)
- gyrA mutations (fluoroquinolones)

Canine lung (2018)
- aac(3)-Iid (gentamicin)
- aadA1, A2, A5 (streptomycin)
- aph(3")-Ib (streptomycin)
  - aph(3')-Ia (kanamycin)
- aph(6)-Id (streptomycin)
- blaEC (cephalosporins)
- blaTEM-1 (penicillins)
- catA1, cmlA1, floR (phenicols)
- dfrA12, 17 (trimethoprim)
- Inu(F) (lincosamide)
- mph(A) (macrolides)
- qacL, qacEdelta1 (disinfectants)
- sul1, sul2, sul 3 (sulfonamides)
- tet(B, M) (tetracycline)
Most extreme cases

 Nearly pan-resistant *E. coli* from canines

- 6 *E. coli* isolates with $\geq 15$ ARGs in Vet-LIRN CARB collection ($n = 333$)
  - 3* serotypes (O8:H9, O9:H10, O89:H9)
  - 5 different MLST patterns
  - No shared SNP clusters

*One only had H antigen predicted (H9)
Culture-independent AMR detection

- Week 1
  - Clostridiales

- Week 2
  - Pseudomonales

- Week 3

- Week 4

Collaboration with Kevin Cummings and Janet L. Swanson Wildlife Hospital
Culture-independent AMR detection

Relative Abundance

Week 1  Week 2  Week 3  Week 4

β-lactam  Aminoglycoside  Macrolide  Tetracycline  Phenicol  Other

Collaboration with Kevin Cummings and Janet L. Swanson Wildlife Hospital
Take-home points

1. People and animals share pathogens and pathogens share genes

2. By monitoring ARGs in animal populations, we can better protect both animal and human health
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