In the Beginning....
- Colony Morphology
- Growth on selective media
- Biochemical reactions
  - Oxidase
  - Catalase
  - Sugars
  - Amino Acids
  - Others

Partners in Capacity
- MALDI-TOF MS bacterial identification
- Antimicrobial susceptibility testing and resistance monitoring

MALDI-TOF Mass Spectrometry
In the Middle...

- Biochemical test optimization with miniaturized, automated identification systems
  - API
  - Trek
  - Biolog
  - Others

Issues: Traditional Culture Methods

- Dependence on bacterial growth at each step
- Identification limited by numbers of biochemical tests maintained
- Slow reporting

The Future is Now: MALDI-TOF MS in Bacterial ID

- What is this very long acronym?
  - Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry
  - Becoming the standard for bacterial identification in both human and veterinary medicine

>6000 types of bacteria in database
MALDI-TOF MS in Bacterial Identification

• >95% agreement with traditional ID methods

<table>
<thead>
<tr>
<th>Score Range</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3 – 3.0</td>
<td>Highly probable species identification</td>
</tr>
<tr>
<td>2.0 – 2.299</td>
<td>Secure genus identification, probable species identification</td>
</tr>
<tr>
<td>1.7 – 1.999</td>
<td>Probable genus identification</td>
</tr>
<tr>
<td>0 – 1.699</td>
<td>Not reliable identification</td>
</tr>
</tbody>
</table>

The Benefits of MALDI-TOF MS

• Reduction of time to ID
  - >90% reduction (post-isolation)
  - 12 hours vs. 48-72 hours
• Reduction in subjectivity of some biochemically-based testing
• Improves lab to lab consistency
• Development of Custom Veterinary Databases
  - Addition of Veterinary pathogens for accurate ID
  - Further characterization of bacteria of veterinary importance

Why do we test?

• Little picture
  - To define effective antimicrobial therapy for a bacterial infection
  - To monitor effectiveness of antimicrobial therapy
• Big Picture
  - To assess trends or emergence of mutational resistance
  - To promote antimicrobial stewardship and reduce the development of resistance
  - To monitor effectiveness of interventions to reduce or prevent resistance development

Susceptibility Tests: Kirby-Bauer Disk Diffusion

• Disk impregnated with antimicrobial drug
• Measures diameter of growth inhibition
• Qualitative measure of susceptibility
• Requires standard method
  - Agar plate – media and volume
  - Concentration of drug
  - Inoculum
  - Quality Control

Antimicrobial Susceptibility Testing and Resistance monitoring

Susceptibility Tests: Broth Microdilution

• Two-fold dilutions of antimicrobial drug
• Measures inhibition of growth in well
• Quantitative measure of susceptibility
• Requires standard method
  - Media
  - Drugs
  - Inoculum
  - Quality Control
What is Resistance?

• Clinical Resistance
  - Only likelihood of therapeutic failure is considered
  - Based on pharmacology of drug in the animal
  - May not represent a mutation of the bacteria

Avian Testing - Limitations

• No standard protocols addressing avian species
  - Testing performed as for mammalian species
• One clinical breakpoint available
  - Enrofloxacin for *E. coli*
• Some interest in developing poultry-specific data to predict clinical efficacy of therapy
• Commercial testing systems specific for avian species available

What is Resistance?

• Acquired Resistance
  - Bacteria has acquired a mechanism of resistance
  - From another bacteria
  - Through mutation of chromosome
  - May not correlate with failure of antimicrobial treatment

Understanding both types of resistance are critical for treating disease and combating AMR.

Where do we get our information?

• Clinical efficacy:
  - Clinical and Laboratory Standards Institute
  - Veterinary antimicrobial susceptibility testing method and breakpoints (not so great data!)
  - Eucast
  - Human antimicrobial susceptibility testing method and breakpoints
  - Acquired Resistance
  - “The Literature”

Upcoming: National Antimicrobial Resistance Monitoring

• USDA National Animal Health Laboratory Network (NAHLN)
  - Monitoring resistance across the USA using data generated by VDLs
  - Cattle
  - Swine
  - Poultry
    - *Salmonella*
    - *E. coli*
  - Horses
  - Small Animals
Conclusion

• WADDL has complimentary capacity and works with AHFSL to ensure accurate test results for bacteria of significance
• MALDI-TOF MS enhances our ability to correctly identify pathogens quickly and as a potential method for refined characterization/interpretations
• AMR is a epic challenge for the health of animals and humans and WADDL/AHFSL provides resources for testing and interpretation of susceptibility data
• Contributing to the understanding of AMR impacting poultry in Washington and nationally

Thank you!