WADDL Bacteriology:
Additional Resources for Combating Avian Disease

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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
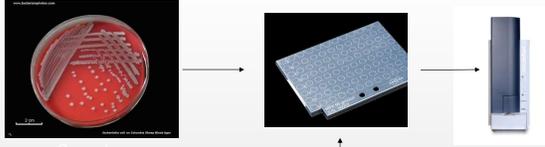

MALDI-TOF Mass Spectrometry

In the Middle…

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

~One colony

Apply matrix

~5 minutes to ID

Issues: Traditional Culture Methods

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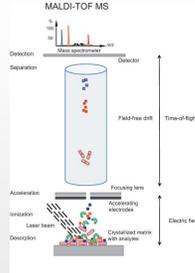- Slow reporting

MALDI-TOF MS

Fig 1. Lavigne et al. 2013

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

- What is this very long acronymn?
  - Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry
  - Becoming the standard for bacterial identification in both human and veterinary medicine
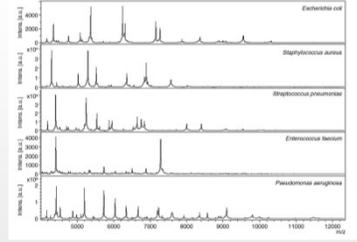
Fig 1. Carbonnelle et al. 2011

>6000 types of bacteria in database

MALDI-TOF MS in Bacterial Identification

• >95% agreement with traditional ID methods

| Score Range	Description
2.3 – 3.0	Highly probable species identification
2.0 – 2.299	Secure genus identification, probable species identification
1.7 – 1.999	Probable genus identification
0 – 1.699	Not reliable identification

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

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

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
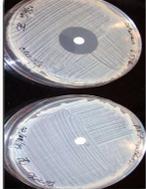
## Antimicrobial Susceptibility Testing and Resistance monitoring

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

Decreasing concentration of drug →
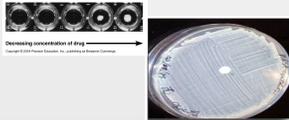
Avian Testing - Limitations

- 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

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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

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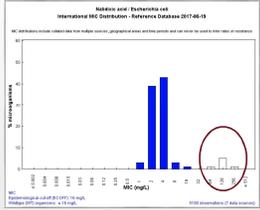
Understanding both types of resistance are critical for treating disease and combating AMR.

Plate Code:	AVIAN1F					Plate Type:	MIC					ANTIMICROBICS	
	1	2	3	4	5	6	7	8	9	10	11	12	
A	ENRO 2	ENRO 1	ENRO 0.5	ENRO 0.25	ENRO 0.12	SPE 64	SDM 256	FFN 8	PEN 8	STR 1024	NOV 4	CLI 4	ENRO Enrofloxacin
B	GEN 8	GEN 4	GEN 2	GEN 1	GEN 0.5	SPE 32	SDM 128	FFN 4	PEN 4	STR 512	NOV 2	CLI 2	GEN Gentamicin
C	TIO 4	TIO 2	TIO 1	TIO 0.50	TIO 0.25	SPE 16	SDM 64	FFN 2	PEN 2	STR 256	NOV 1	CLI 1	TIO Ceftiofur
D	NEO 32	NEO 16	NEO 8	NEO 4	NEO 2	SPE 8	SDM 32	FFN 1	PEN 1	STR 128	NOV 0.5	CLI 0.5	NEO Neomycin
E	ERY 4	ERY 2	ERY 1	ERY 0.5	ERY 0.25	ERY 0.12	SXT 2/38	STZ 256	PEN 0.5	STR 64	TYLT 20	NEG	ERY Erythromycin / OXY Oxytetracycline
F	OXY 8	OXY 4	OXY 2	OXY 1	OXY 0.5	OXY 0.25	SXT 1/19	STZ 128	PEN 0.25	STR 32	TYLT 10	POS	AMOX Amoxicillin / SPE Spectinomycin
G	TET 8	TET 4	TET 2	TET 1	TET 0.5	TET 0.25	SXT 0.5/9.5	STZ 64	PEN 0.12	STR 16	TYLT 5	POS	SDM Sulphadimethoxine / SXT Trimethoprim / sulfamethoxazole
H	AMOX 16	AMOX 8	AMOX 4	AMOX 2	AMOX 1	AMOX 0.5	AMOX 0.25	STZ 32	PEN 0.06	STR 8	TYLT 2.5		FFN Florfenicol / STZ Sulphathiazole

PEN Penicillin
STR Streptomycin
NOV Novobiocin
TYLT Tylosin tartrate
CLI Clindamycin

Need to work closely with Poultry Veterinarians and Producers to ensure correct data interpretation.

Where do we get our information?

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

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EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

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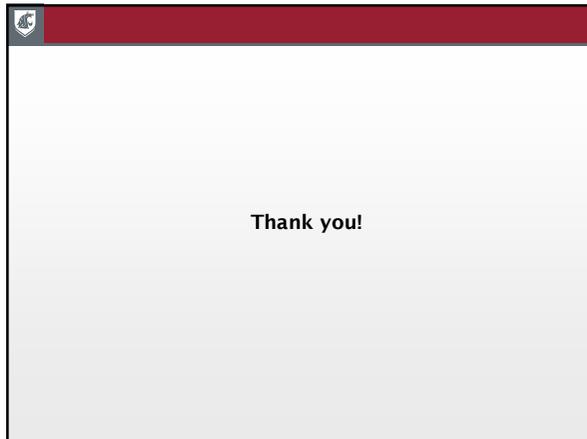## Upcoming: National Antimicrobial Resistance Monitoring

- 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
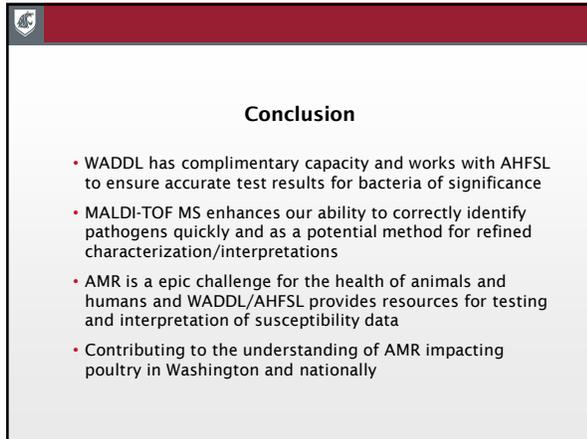
Conclusion

- 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!