CLSI – A One Health Perspective on Susceptibility Testing

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Zoetis, Inc
WHAT IS ONE HEALTH?

One Health Initiative

• The One Health concept is a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment.

CDC

• The goal of One Health is to encourage the collaborative efforts of multiple disciplines-working locally, nationally, and globally-to achieve the best health for people, animals, and our environment.

AVMA

• One Health is the integrative effort of multiple disciplines working locally, nationally, and globally to attain optimal health for people, animals, and the environment.

USDA

• The health of animals, people and the environment is connected. The "One Health" approach is the collaborative effort of the human health, veterinary health and environmental health communities.
The world's total population is expected to exceed 9 billion by 2050 and will require the food supply to double.

As our population expands, the contact between human and wild animal habitats increases, introducing the risk of exposure to new viruses, bacteria and other disease-causing pathogens.

The human-animal bond continues to grow throughout societies.

It is estimated that at least 75% of emerging and re-emerging diseases are either zoonotic or vector-borne.

Vigilant protection of our food and feed supplies from food-borne diseases, contamination, and acts of terrorism is critical for human and animal health.

Contamination by personal care products and pharmaceuticals has been detected in the environment.
The Role of the Veterinarian in One Health

The One Health Triad

- Embedded in Veterinarian’s Oath
  - Protect Animal Welfare
  - Promotion of Public Health
  - Advancement of Medical Knowledge
- Healthy Food Supply
  - Responsible for insuring that healthy animals enter the food chain
  - Responsible for food inspection
- Veterinarians impact human health at every meal!
• Diseases Management is the foundational process
  – Prevention
    • Hygiene
    • Biosecurity
    • Vaccinations
  – Responsible Use of Antibacterials
• What are the common connections between the medical and veterinary communities?
  – Companion Animals
  – Food Producing Animals
    • In herds/flocks, large number of young, healthy individuals in close proximity
    • Disease Prevention is key
    • Rapid response to disease outbreaks
Classification of Antibacterials by Importance in Human Health is the Basis for Microbiological Risk Assessments in Animal Health

<table>
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<th>Human Use Only</th>
<th>Critically Important$^2$</th>
<th>Highly Important</th>
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<td>Chloramphenicol</td>
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$^1$Based on FDA-CVM Guidance #152; Minor differences from WHO Categorizations

$^2$No CIA antibacterials are available as feed or water medications in the US.
Formation of the V-AST in 1993 marks the entry of CLSI into the One Health Area
   – AST members played a key role in early veterinary standard development and continue to contribute
   – First V-AST clinical breakpoint presentation was for a human compound
     • AST and V-AST share same basic process for setting clinical breakpoints
   – Human breakpoints were initially the only breakpoints available for veterinary use
Co-development of a *Campylobacter* test method
• Reporting methods for Methicillin-resistant *Staphylococcus aureus* and Methicillin-resistant *S. pseudintermedius*
• M100/VET08 Table alignment
CLSI Methods and Surveillance Programs

• CLSI standards have played a key role in surveillance programs
  – Only human-veterinary standards that provide equivalent test methods
  – Allows for direct comparison of MIC test data
  – Allows for merging MIC datasets for shared organisms (e.g. *E. coli*)
• Standard for reporting of surveillance data
  – Joint Medical/Veterinary Subcommittee
  – XR-08/VET-05R

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<th>Human Origin Bacteria</th>
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Future CLSI ONE HEALTH INITIATIVES

- Improve communication and collaboration between AST and VAST
- Improved/Expanded Clinical Breakpoints
  - Generic compounds
  - Less frequently encountered pathogens
  - Topical agents
- Insure that CLSI methods and breakpoints are used in Surveillance Programs
- Develop Best Practices for Antimicrobial Stewardship Programs
- Joint Promotion of AST/VAST Documents
CLSI Educational Workshop
January 14, 2017

One Health - One Medicine
CLSI Veterinary Antimicrobial Susceptibility Testing Subcommittee (VAST)
CLSI Educational Workshop

How VAST Develops Breakpoints for Generic Drugs (and how/why they differ from M100 breakpoints)
Examples of How Antibiotic Resistance Spreads

Animals get antibiotics and develop resistant bacteria in their guts.

Drug-resistant bacteria can remain on meat from animals. When not handled or cooked properly, the bacteria can spread to humans.

Fertilizer or water containing animal feces and drug-resistant bacteria is used on food crops.

Drug-resistant bacteria in the animal feces can remain on crops and be eaten. These bacteria can remain in the human gut.

George gets antibiotics and develops resistant bacteria in his gut.

George stays at home and in the general community. Spreads resistant bacteria.

George gets care at a hospital, nursing home or other inpatient care facility.

Resistant germs spread directly to other patients or indirectly on unclean hands of healthcare providers.

Resistant bacteria spread to other patients from surfaces within the healthcare facility.

Patients go home.
We are “One Health”
CLSI Interpretive Categories

- Resistant
- Intermediate
- Susceptible
VET01-A4

Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Fourth Edition

This document addresses the required and recommended criteria and quality control for veterinary antimicrobial agents.

This document provides the currently recommended techniques for antimicrobial agent disk and dilution susceptibility testing, criteria for quality control testing, and interpretive criteria for veterinary use.

A standard for global application developed through the Clinical and Laboratory Standards Institute consensus process.
CLSI VET 02

Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline (VET 02 – A3).
Veterinary Antimicrobial Susceptibility Testing subcommittee (VAST)

- Role of the Generic Drug Working Group (GWG)
3.7 Development of Interpretive Criteria for Generic or Older Compounds

“The development of interpretive criteria for generic or older compounds is problematic due to limited sponsor support for generation of new data.”

(Many of these agents are also used in human medicine.)
Veterinary-Specific Interpretation: Companion Animals

- **Fluoroquinolones**
  - Enrofloxacin, Marbofloxacin, Orbifloxacin, Difloxacin
- **Gentamicin** (dogs & horses)
- **Amikacin** (dogs, horses & foals)
- **Clindamycin** (dogs)
- **Cefpodoxime proxetil** (dogs)
- **Cephalosporins, 1st Gen** (dogs and horses)
- **Ampicillin/Amoxicillin** (dogs, horses)
- **Amoxicillin-Clavulanate** (dogs, cats)
- **Pradofloxacin** (dogs, cats)
- **Doxycycline, Tetracycline** (dogs)
Veterinary-Specific Interpretation: Companion Animals

- Fluoroquinolones
  - Enrofloxacin, Marbofloxacin, Orbifloxacin, Difloxacin
- Gentamicin (dogs & horses)
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- Amoxicillin-Clavulanate (dogs, cats)
- Pradofloxacin (dogs, cats)
- Doxycycline, Tetracycline (dogs)
Veterinary-Specific Interpretation: Large Animals

- Tulathromycin (cattle)
- Ceftiofur (horses, pigs & cattle)
- Danofloxacin (cattle)
- Enrofloxacin (cattle)
- Florfenicol (cattle & pigs)
- Spectinomycin (cattle)
- Tilmicosin (cattle & pigs)
- Ampicillin (horses & pigs)
- Tetracycline (cattle & pigs)
- Enrofloxacin (pigs)
- Penicillin G (horses, cattle, pigs)
Veterinary-Specific Interpretation: Large Animals

- Tulathromycin (cattle)
- Ceftiofur (horses, pigs & cattle)
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- Enrofloxacin (cattle)
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- Spectinomycin (cattle)
- Tilmicosin (cattle & pigs)
- Ampicillin (horses & pigs)
- Tetracycline (cattle & pigs)
- Enrofloxacin (pigs)
- Penicillin G (horses, cattle, pigs)
CLSI-VAST (VET01-S2, 2013) has updated breakpoints for susceptibility testing:

- Cephalosporins (1st gen): $\leq 8 \, \mu g/mL \rightarrow \leq 2 \, \mu g/mL$
- Amoxicillin-Clavulanate: $\leq 8 \, \mu g/mL \rightarrow \leq 0.25 \, \mu g/mL$
- Ampicillin: $\leq 8 \, \mu g/mL \rightarrow \leq 0.25 \, \mu g/mL$
- Gentamicin: $\leq 4 \, \mu g/mL \rightarrow \leq 2 \, \mu g/mL$
- Chloramphenicol: No change ($\leq 8 \, \mu g/mL$)
- Oxacillin (Resistant *Staph pseudintermedius*): $\geq 4 \, \mu g/mL \rightarrow \geq 0.5 \, \mu g/mL$
CLSI-VAST (VET01-S3, 2014)
New breakpoints for susceptibility testing:

✓ Doxycycline: $\leq 4 \mu g/mL \rightarrow \leq 0.125 \mu g/mL$ (dogs and horses)

✓ Amikacin: $\leq 16 \mu g/mL \rightarrow$
  - Dogs $\leq 4 \mu g/mL$
  - Horses $\leq 4 \mu g/mL$
  - Foals $\leq 2 \mu g/mL$
CLSI-VAST (VET01-S4)
New breakpoints for susceptibility testing
(not yet published)

- Minocycline: $\leq 4 \, \mu\text{g/mL} \rightarrow \leq 0.5 \, \mu\text{g/mL}$
- Piperacillin and Tazobactam: $\leq 16 \, \mu\text{g/mL}$
  - Dogs $\leq 8 \, \mu\text{g/mL}$
- Ciprofloxacin (dogs): $\leq 0.06 \, \mu\text{g/mL}$
  (Human breakpoint is $\leq 1 \, \mu\text{g/mL}$; therefore, recommended no listing.)
How Do We Create Standards?
Where does the dose come from?

• Established consensus documents.
  - ACVIM Consensus Statements
  - ISCAID (International Society of Companion Animal Infectious Diseases) guidelines
Where does the dose come from?

- Food Animal Residue Avoidance Databank (FARAD) files
  - Off-label uses
  - Off-label doses
- The Working Group **avoids** the use of single-author handbooks, guidelines, or review articles.
Microbiological data

- Generated using CLSI standardized testing methods, including the proper use of QC organisms, and should be limited to clinically relevant isolates appropriate for the class of compound being evaluated.

- A $\text{CO}_{\text{WT}}$ (ECV) should be proposed.
3.7 Development of Interpretive Criteria for Generic or Older Compounds (VET 02)

- Requests for establishing veterinary-specific breakpoints and/or interpretive criteria for older compounds must include PK-PD data.
3.7 Development of Interpretive Criteria for Generic or Older Compounds (VET 02)

**Pharmacokinetic Data**

- Literature search of published papers
- Sponsor’s data
  (original sponsor or generic company)

**PK-PD Targets**

- Published consensus documents
- Guidelines provided in VET02
Pharmacokinetic-Pharmacodynamic (PK-PD) Analysis

- **C<sub>MAX</sub>**
- **AUC**:MIC Ratio
- **Fluoroquinolones**
- **Tetracycline**
- **Macrolides**
- **Chloramphenicol**
- **Aminoglycosides**
- **β-Lactam antibiotics**
- **MIC**
- **Time above MIC**
- **AUC**
- **Time (Hours)**
- **Plasma Concentration (mcg/ml)**
Monte Carlo Simulations

- Simulations integrate interpatient variability in drug exposure – based on analysis of pharmacokinetic studies
- Incorporate *in vivo* exposure targets predictive of positive therapeutic outcomes (*AUC/MIC, T>MIC, C_{MAX}/MIC targets*)
- Generate the Probability of Target Attainment (PTA) tables and graphs to assist committee decisions
PK-PD Calculation (T > MIC)

Determination of T > MIC

\[
\% \ T > \text{MIC} = \\
\ln \left( \frac{\text{Dose}}{[\text{VD} \times \text{MIC}]} \right) \times \left( \frac{\text{T} \frac{1}{2}}{\ln2} \right) \times \left( \frac{100}{\text{DI}} \right)
\]

- VD = volume of distribution
- ln2 = natural logarithm 2
- T \( \frac{1}{2} \) = half-life
- Dose
- DI = dose interval
Determination of AUC / MIC

\[
AUC/MIC = \frac{fu \cdot F \cdot 24 \text{ hr} \cdot \text{Dose}}{CL \cdot \text{MIC}}
\]

- Clearance (CL)
- Fraction absorbed (F)
- Protein binding (fraction unbound, \(fu\))
- Dose
- MIC
Probability of Target Attainment (PTA) for doxycycline administered to horses

Probability of AUC/MIC > 25 in Horses

- 10 mg/kg Once Daily
- 10 mg/kg Twice Daily
- 20 mg/kg Twice Daily

Human Breakpoint
Probability of Target Attainment (PTA) for ciprofloxacin administered to dogs

Human Breakpoint

Certainty % vs. MIC (µg/mL) for different dosages:
- 10 mg/kg q24h
- 25 mg/kg q24h
- 50 mg/kg q24h
Why are some veterinary breakpoints lower than human breakpoints?
Interpretive Categories (Breakpoints)

Why are they different?

- Bacteria: Are they different?
  - Wild-type distributions tend to be similar
- Pharmacokinetics
  - Often much different in animals than people
  - Shorter half-life (important for T>MIC drugs)
  - Oral absorption (F) tends to be lower
- Protein binding
  - High for many veterinary drugs
  - eg, doxycycline 90% protein binding
What are the implications from establishing veterinary breakpoints lower than human breakpoints?
Many Veterinary Breakpoints are Lower than Human Breakpoints

• Some human drugs are used in animals inappropriately
  ♦ Unlikely to be effective for intended use
• Reduce “routine” use of human drugs in veterinary medicine
• Requires education of veterinarians
  ♦ Encourage more susceptibility testing
  ♦ Inform veterinarians of inappropriate uses
Thank You!

Any Questions?
Contact Information

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‘One Health’ in a Clinical Microbiology Laboratory Practice

Thomas R. Fritsche
Division of Laboratory Medicine
Marshfield Clinic
Goals

- **Message:** *how we took a lab-in-a-lab and created one lab to increase value*
- **Who We Are** (Marshfield Clinic Health System)
  - Sets the stage for the interdisciplinary model
- **Current Challenges in Clinical Microbiology**
- **Prior and Current Methods/Instrumentation**
- **Case Studies**
- **Conclusions**
Marshfield Clinic Health System

- Founded in 1916 by six physicians

- Today, a system of care:
  - Staff: >780 physicians, >6,500 employees
  - Clinics: 50 plus 12 Dental Clinics
  - Hospitals: 2, soon to be 4
  - Insurance Plan: Security Health
Laboratory Operations

- Clinical Laboratories
  - 18 MD Pathologists, 5 PhD Clinical Scientists
  - 385 Staff in 29 locations

- Veterinary Services
  - Formed in 1991 at request of veterinarians for regional testing (dairy state!)
  - 12 DVM Pathologists
  - 50 Staff in 4 lab locations

- Human & Vet Accounts: 48 states, 5 countries
- Integrated microbiology operations
The Paradigm of Clinical Bacteriology: 106 Years in the Making (1860-1966)

Louis Pasteur 1860
Robert Koch 1882
Hans Christian Gram 1884
William Kirby and Al Bauer 1966

Germ Theory → Culture and ID → Gram Stain → Standardized Disk Susceptibility Testing
But What’s Wrong with this Paradigm?

Problems historically:
- Too little (in terms of accurate ID results)
- Too late (are we as clinically useful as we think?)
- At too great a cost (decreasing reimbursement)

Answers:
- Provide greater accuracy in identifications, hence better prognostic information
- Improve turn-time: be more clinically relevant
- Provide meaningful susceptibility results
  - MICs and Categorical simultaneously – no call backs
- Be cost-effective: do more with less

Is some or all of this possible?
The Additional Challenge Since 1991:

- Could existing lab services be leveraged to provide both human and animal diagnostic testing in one integrated laboratory system?

- “Between animal and human medicine there are no dividing lines--nor should there be.”
  
  Rudolf Virchow, MD

One Medicine-One Pathology’: are veterinary and human pathology prepared?
Cardiff et al. Lab Investigation 88;18-26;2008
The ‘One Health’ Microbiology Challenge

- Overcome *differences* that exist between human and animal pathogen testing:
  - Different spectrum of pathogens
  - Different identification schema historically
  - Different antimicrobials
  - Different CLSI guidance documents

- How do we provide IDs and AST for both *in an efficient/cost-effective manner*?
Goals to Meet This Challenge

- Reduce methods and platforms
- Improve accuracy
- Improve TAT, increase downstream value
- Expand flexibility
  - Provide IDs for difficult-to-identify groups
  - Provide MIC values on relevant isolates up-front
- Lessen QC activities
- Reduce costs where possible

**Bottom Line:** *Improve client satisfaction*
Laboratory Methods Prior to 2011

Identification Methods
- Spot tests
- Tube biochemicals
- Commercial Strips
- Phoenix (human)
- Vitek Legacy (animal)
- MIDI FAME
- 16/18S rDNA sequencing

Susceptibility Methods
- Phoenix (human)
- Vitek Legacy (animal)
- Kirby-Bauer (both)
- Etest (both)
- Microscan (CF)
Laboratory Methods Since 2011

- **Identification Methods**
  - From 7 to 1
  - MALDI-TOF MS
    - Europe since 2008
    - USA since 2010
    - FDA clearance 2013

- **Susceptibility Testing**
  - From 5 to 1
  - Broth Microdilution AST (dry-form plates)
    - Human- and veterinary-specific drugs
  - MIC values
  - S, I, R results
CLSI M58 Guidance Document in Development

“Methods for Identification of Cultured Microorganisms Using MALDI-TOF MS”

Goals

- Guidance on methods, implementation, verification, QA, reporting, limitations, etc

DDC Members

- Professions – DVM & MD directors, Managers
- Government – FDA, NIH, CDC (US, Canada)
- Industry – leading diagnostic manufacturers

Timeline – 2017
CLSI AST Resource Documents

- Human Testing
  - M02-A12 Diffusion methods
  - M07-A10 Dilution methods
  - M100-S26 Breakpoint Tables
  - M45-A3 Infrequent/Fastidious
  - Others (M24, M11)

- Veterinary Testing
  - Vet01-A4 Dilution and Diffusion Methods
  - Vet01-S3 Breakpoint Tables (to be Vet08)
  - Vet06 (pending) Infrequent/Fastidious
  - Vet04-A2 Aquatic Animals
Identification Methods
MALDI-TOF Mass Spectrometry

- Species-specific riboprotein spectral ‘fingerprints’
- Colonial growth directly from agar used
- <5 minutes/identification
- Reagents are off the shelf consumables
- Large RUO databases, updated >=1x/year
  - bioMerieux Vitek® MS: 279 genera, 1,424 species
  - Bruker BioTyper™: 380 genera, 2,290 species
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<td>C7</td>
<td>0</td>
<td>Bordetella bronchiseptica</td>
<td>2.470</td>
</tr>
<tr>
<td>898715</td>
<td>C8</td>
<td>C8</td>
<td>0</td>
<td>Staphylococcus aureus</td>
<td>2.358</td>
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<tr>
<td>898715-2</td>
<td>D1</td>
<td>D1</td>
<td>0</td>
<td>Staphylococcus aureus</td>
<td>2.452</td>
</tr>
<tr>
<td>114139</td>
<td>D2</td>
<td>D2</td>
<td>0</td>
<td>Staphylococcus pseudintermedius</td>
<td>0.962</td>
</tr>
<tr>
<td>107512</td>
<td>D3</td>
<td>D3</td>
<td>0</td>
<td>Pseudomonas putida</td>
<td>1.876</td>
</tr>
<tr>
<td>114364</td>
<td>D4</td>
<td>D4</td>
<td>0</td>
<td>Pasteurella multocida</td>
<td>2.285</td>
</tr>
</tbody>
</table>
Costs: Johns Hopkins Experience for 952 Isolates Annualized to 47,845 Isolates (279 spp.)*

<table>
<thead>
<tr>
<th>Item</th>
<th>Std Method Cost</th>
<th>MALDI Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent costs</td>
<td>$158,645</td>
<td>$29,614</td>
</tr>
<tr>
<td>Labor costs</td>
<td>$31,324</td>
<td>$26,669</td>
</tr>
<tr>
<td>Fixed MALDI costs</td>
<td>-</td>
<td>$31,272</td>
</tr>
<tr>
<td>Total</td>
<td>$189,969</td>
<td>$87,556</td>
</tr>
<tr>
<td></td>
<td>($3.97/isolate)</td>
<td>($1.83/isolate)</td>
</tr>
</tbody>
</table>

*Bottom line - accuracy 98.3%, identifications 1.45 days earlier and 53.9% cost reduction in 12 months

Benefits of Mass Spectrometry for One Health

- **Better**: large databases, inclusion of environmental and animal pathogens, accurate IDs - number of rDNA sequencing requests greatly reduced
- **Faster**: organism IDs 24-48 hours sooner
- **Cheaper**: Cost effective – directly addresses concerns of ‘value-based care’
- Patients/clients benefit from rapidity and accuracy and decreased LOS
- Results generated aid antimicrobial stewardship
Susceptibility Testing
ThermoFisher ARIS™ System using Broth Microdilution MIC Panels
AST Reporting

- Human isolates: S, I, R results
  - >22,000 panel results/year
  - MICs available on request
  - Separate Hospital/Clinic antibiograms yearly

- Animal isolates: S, I, R and MIC results
  - >21,000 panel results/year
  - Antibiograms by major species biennially
    - Canine, feline, equine, bovine, avian
Case Study Examples

- Comparisons of human-animal antibiograms
  - *E. coli*, *K. pneumoniae*, *P. aeruginosa*
  - *S. aureus*

- Canine Coag-positive staphylococci
  - Oxacillin resistance
  - Mupirocin resistance
## Human/Canine Antibiotics

### % Susceptible

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th>K. pneumoniae</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H 1,881</td>
<td>H 275</td>
<td>H 120</td>
</tr>
<tr>
<td></td>
<td>C 5,380</td>
<td>C 171</td>
<td>C 1451</td>
</tr>
<tr>
<td>GM</td>
<td>93</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>96</td>
<td>78</td>
</tr>
<tr>
<td>AMP</td>
<td>63</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>79</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VEC</td>
<td>-</td>
<td>92</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CRO</td>
<td>95</td>
<td>98</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CPD</td>
<td>-</td>
<td>91</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CIP</td>
<td>85</td>
<td>97</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ENO</td>
<td>-</td>
<td>94</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>LVX</td>
<td>85</td>
<td>98</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MAR</td>
<td>-</td>
<td>94</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>75</td>
</tr>
<tr>
<td>TET (DOX)</td>
<td>81</td>
<td>86</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(90)</td>
<td>(90)</td>
<td>-</td>
</tr>
<tr>
<td>SXT</td>
<td>84</td>
<td>93</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>94</td>
<td>95</td>
<td>-</td>
</tr>
</tbody>
</table>

*Human isolates 2015; Canine isolates 2014-2015*
### Human/Canine Antibiograms

#### % Susceptible

<table>
<thead>
<tr>
<th></th>
<th>S. aureus</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H 621</td>
<td>C 231</td>
<td></td>
</tr>
<tr>
<td>OX</td>
<td>76</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>PEN</td>
<td>21</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>ENO</td>
<td>-</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>LVX</td>
<td>76</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MAR</td>
<td>-</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>TET (DOX)</td>
<td>94</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>SXT</td>
<td>99</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

Human isolates 2015; Canine isolates 2014-2015
# Trends in Ox-R: *S. intermedius* group, *S. schleiferi*, *S. aureus* in Canines

<table>
<thead>
<tr>
<th>Year</th>
<th>SIG*</th>
<th><em>S. schleiferi</em></th>
<th><em>S. aureus</em></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>19.5 (1688)</td>
<td>38.5 (135)</td>
<td>36.5 (96)</td>
<td>21.7 (1919)</td>
</tr>
<tr>
<td>2013</td>
<td>19.7 (2432)</td>
<td>41.4 (239)</td>
<td>18.9 (127)</td>
<td>21.4 (2798)</td>
</tr>
<tr>
<td>2014</td>
<td>19.2 (3140)</td>
<td>37.5 (392)</td>
<td>25.5 (145)</td>
<td>21.3 (3677)</td>
</tr>
<tr>
<td>2015</td>
<td>20.6 (3341)</td>
<td>32.2 (391)</td>
<td>26.6 (137)</td>
<td>21.9 (3869)</td>
</tr>
<tr>
<td>Totals</td>
<td>19.8 (10601)</td>
<td>37.4 (1157)</td>
<td>26.9 (505)</td>
<td>21.6 (12263)</td>
</tr>
</tbody>
</table>

*S. intermedius* group
Trends in MUP-R: *S. intermedius* group, *S. schleiferi*, *S. aureus* in Canines

<table>
<thead>
<tr>
<th>Year</th>
<th>SIG*</th>
<th><em>S. schleiferi</em></th>
<th><em>S. aureus</em>*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>0 (0/261)</td>
<td>0 (0/35)</td>
<td>0 (0/5)</td>
<td>0.0 (0/301)</td>
</tr>
<tr>
<td>2013</td>
<td>0.7 (2/289)</td>
<td>7.7 (3/39)</td>
<td>14.3 (1/7)</td>
<td>1.8 (6/335)</td>
</tr>
<tr>
<td>2014</td>
<td>0 (0/200)</td>
<td>5.6 (1/18)</td>
<td>0 (0/5)</td>
<td>0.4 (1/223)</td>
</tr>
<tr>
<td>2015</td>
<td>0.7 (2/271)</td>
<td>4.3 (1/23)</td>
<td>0 (0/2)</td>
<td>1.0 (3/296)</td>
</tr>
<tr>
<td>Totals</td>
<td>0.4 (4/1021)</td>
<td>4.3 (5/115)</td>
<td>5.2 (1/19)</td>
<td>0.9 (10/1155)</td>
</tr>
</tbody>
</table>

*SIG* | *S. intermedius* group
**2.1% (1/47) Human *S. aureus* mupirocin resistant
Additional Value Possible with Lab Integration

- Participation in National/Global Human-Animal Resistance Surveillance Studies
- Collaborations with researchers and industry
- Interactions with Public Health
  - Tracking of unusual resistance patterns
  - Identifying presence of cross-over pathogens
    - *Streptococcus halichoeri* (GBS)
    - *Wolfahrtiamonas chitinoclastica*
    - *Campylobacter upsaliensis*
Conclusions

- Newer Dx technologies are breaking down barriers between human and animal medicine
  - Providing meaningful results sooner, hopefully with better outcomes and increased value
  - Permitting better assessments of shared and emerging pathogens
  - Allowing insights into types and spread of antimicrobial resistance

Thank you!
Phenotypic MIC Prediction from Whole Genome Sequencing

Ron A. Miller, PhD
Regulatory Review Microbiologist
Center for Veterinary Medicine
Office of New Animal Drug Evaluation
Rockville, MD

Disclaimer
This communication is consistent with 21 CFR 10.85 (k) and constitutes an informal communication that represents my best judgment at this time but does not constitute an advisory opinion, does not necessarily represent the formal position of FDA, and does not bind or otherwise obligate or commit the agency to the views expressed.
Objective

Discuss how whole genome sequencing (WGS) has been used for phenotypic detection of resistance genes, and how it needs to be part of the process to establish ECVs.
Outline

• Terminology
• Historical perspective
• Harmonization
• EUCAST efforts w/ ECOFFs
• CLSI efforts w/ ECVs – limitations, opportunity
• WGS utility – current uses, limitations
• Next steps with CLSI VET05-R revisions
**Terminology**

- **Clinical breakpoints (CBP)**
  - Interpretive categories - S, I, R – established for clinical application, dose dependent
  - Reported as %R, %S etc.

- **Epidemiological cutoffs (ECVs by CLSI; ECOFFs by EUCAST)**
  - Interpretive categories
    - Wild type (WT) – no phenotypically detectable RZ mechs
    - Non-wild type (NWT) – presence of RZ mechs
  - ‘Always’ reported as %R or %S ≡ misleading
Widely reported incorrectly as “%R”

Figure 1. Distribution of MICs and Categorization by Clinical Breakpoints Contrasted to ECVs
Terminology

• Peter Silley argued for an urgent need to harmonize the definitions used in AST.
  – Not all surveillance programs define R in the same way making comparisons across programs very difficult.
  – Trend for R to be defined by the ECOFF rather than CBP and no standard way to define the wild-type cut-off

EUCAST plans to formally propose reporting as %NWT and %WT
Issues Concerning AMR Surveillance

Program directors should understand their program’s limitations and intended scope.

• Are isolates coming from global, regional, national, state-wide, or local sources?
  • Critical issue if cross-jurisdictional AST data comparisons are expected from data → dose variability → potentially different CBPs are needed
Issues Concerning AMR Surveillance...

[ECVs] Are principally used to signal the emergence or evolution of NWT strains. – *CLSI M100-S27*

...the epidemiological cut-off value (ECOFF) is the highest MIC for organisms devoid of *phenotypically detectable* acquired resistance mechanisms. – *EUCAST Discussion Document, Dec 2016*

• Is the goal to detect clinically relevant RZ or the presence of AMR genes that suggest RZ may be emerging?
  • Critical issue if CBPs or ECVs are to be used.

One argument is, “The drug does not ‘see’ the gene, it only sees its product(s), and to detect this we need phenotypic tests.”

• We must ask the critical question – Are ‘we’...
  a) More concerned with detection of emerging resistance mechanisms, or
  b) More concerned with detection of emerging phenotypic resistance

I believe the answer is ‘a)’ since ultimately AST data are used to manage risk and if a gene is present it will likely be assumed it translates to a non-wild type phenotype (=elevated risk).
AMR Monitoring and Harmonization

U.S. Presidential CARB Initiative

**Surveillance**: Establish *capacity to detect, analyze, and report antibiotic resistance in order to make information needed for evidence-based decision making available in each country and globally.*

... By 2020 U.S. Federal agencies will:

*Support efforts to harmonize and integrate antibiotic-resistance surveillance data on WHO and CDC priority pathogens generated by WHO regional surveillance networks.*
OIE Efforts
White et al. (2001)
• Introduced the term ‘microbiological breakpoints’

For surveillance purposes, use of the microbiological breakpoint (also referred to as epidemiological cut-off point), which is based on the distribution of MICs or inhibition zone diameters of the specific bacterial species tested, is preferred. When using microbiological breakpoints, only the bacterial population with acquired resistance that clearly deviates from the distribution of the normal susceptible population will be designated as resistant.
To enable standardized, comparable and validated data on AMR to be collected, analysed and shared with countries, in order to inform decision-making, drive local, national and regional action and provide the evidence base for action and advocacy.

Combines patient, laboratory and epidemiological surveillance data to enhance understanding of the extent and impact of AMR on populations.

1.3 Objectives of GLASS

GLASS will collect, analyse and report harmonized data on infected patients, aggregated at national level following the standard definitions described in this manual. The objectives of GLASS are:

- foster national surveillance systems and harmonized global standards;
- estimate the extent and burden of AMR globally by selected indicators;
- analyse and report global data on AMR on a regular basis;
- detect emerging resistance and its international spread;
- inform implementation of targeted prevention and control programmes; and
- assess the impact of interventions.

E. coli
K. pneumo.
A. baumannii
S. aureus
S. pneumo.
Salmonella spp.
Shigella spp.
N. gonorrhoeae
WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) - Terms of Reference

1) Develop harmonized schemes for monitoring AMR in zoonotic and enteric bacteria

…it is recommended that ECOFF values be used when interpreting the results of in vitro antimicrobial susceptibility tests (15). It is also important to consider the clinical breakpoints provided by CLSI or EUCAST in order to evaluate the public health risk associated with the microorganism of interest/mechanism of resistance.

...Being dependent exclusively on microbiological properties, ECOFF values provide a categorization of bacteria relative to antimicrobial susceptibility that is comparable across geographical areas, animal species and over time. Therefore, for monitoring purposes the WHO ... recommends and uses ECOFF values as provided by EUCAST, as the reference standard for all organisms and antimicrobials.

...The results of these [whole genome sequencing] monitoring efforts have been in app. 99% concordance with the phenotypic data and even more precise. WGS combined with bioinformatic tools are now being used to monitor antimicrobial resistance and will most likely be the successor of future integrated AMR surveillance systems...
Who sets/publishes ECOFFs/ECVs?

EUCAST — European Committee on Antimicrobial Susceptibility Testing

- For now focused on human pathogens (VetCAST)
- AST distributions freely available
MIC distributions and ECOFFs

The website gives MIC distributions (and since 2010 inhibition zone diameter distributions generated with the new EUCAST disk diffusion method) for a wide range of organisms and antimicrobial agents, including antifungals.

The distributions are based on collated data from a total of more than 27000 MIC distributions containing more than several million MICs from worldwide sources. The distributions include MICs from national and international studies such as resistance surveillance programs (Alexander, BSAC, ECO-SENS, MYSTIC, NORM and SENTRY), as well as MIC distributions from published articles, the pharmaceutical industry, veterinary programmes and individual laboratories. Histograms display wild type organisms, together with EUCAST clinical breakpoints and epidemiological cut-off values (ECOFFs). The distributions should never be referred to in any epidemiological context since data from many time periods and many countries have been aggregated.
Who sets/publishes ECOFFs/ECVs?

**EUCAST** — European Committee on Antimicrobial Susceptibility Testing

- For now focused on human pathogens (VetCAST)
- AST distributions freely available

**CLSI**

- AST SC - human pathogens
  - *Shigella* spp. and *N. gonorrhoeae* – M100-S27 (freely available)
- Antifungal SC
  - *Candida* spp., *Aspergillus* spp. – M57/M59
- Veterinary AST SC
  - No longer pursuing for foodborne pathogens as of Jan 2017 – VET07-S
  - Aquaculture Working Group
Offers guidance on areas in which harmonization can be achieved in national antimicrobial surveillance programs, with the intent of facilitating comparisons of data among various national surveillance programs...

Currently, there is a lack of standardized methodology describing how the data from these programs are presented in the reports and discussed with regard to the specific program objective...

**Planned Revisions**

Should position the use of CLSI methods as the most appropriate for national monitoring programs. CLSI then can expand its international training and Workshops to include LMICs or organizations such as OIE or FAO.

Emphasize ECOFFs for surveillance and not CBPs

Update ECOFFinder and NRI descriptions

Discuss whole genome sequencing

Solicit AST data for additional ECOFFs to detect emerging resistance mechanisms

i.e. US NARMS – see later slides
How are ECVs currently set?

ECOFFinder
Visual Inspection
- Observer-dependent & lacks reproducibility, but it is still widely used
- Poor method when overlap exists among WT and NWT MICs

Whole genome sequencing to detect the presence of underlying AMR genes
- Concerns for gene database and management logistics
How are ECVs currently set?

Estimation of ECVs from MIC distributions may be supplemented with molecular tests for known resistance mechanisms, as a form of validation. The detection of a resistance gene per se in strains with MICs at or below the ECV does not necessarily contradict the choice of ECV, unless it can be accompanied by evidence that the gene is being expressed. – CLSI M100-S27

Conditions for setting ECVs are not fully defined or ‘standardized’ by CLSI or EUCAST

- Minimum # of different WT isolates? –likely to be >100
- Minimum # of labs to account for inter-laboratory assay variation? – likely to be ≥5
- Can isolate data from multiple hosts be merged (humans, pigs, cattle, poultry)? –generally believed to be the case
- Use of whole genome sequencing? -major role or supportive?
ECVs Approved by VAST

- VET03/VET-04-S2 Aquaculture supplement
  - *Aeromonas salmonicida*
    - Four antimicrobials - MIC and zone diameter ECVs (Miller et al. 2006) - used Visual Inspection
  - *Flavobacterium psychrophilum*
    - Six antimicrobials – MIC ECVs (analysis by Peter Smith, 2017) – used ECOFFinder and NRI – VAST approved Jan 2017
ECVs Approved by VAST

- VET03/VET-04-S2 Aquaculture supplement
  - *Aeromonas salmonicida*
    - Four antimicrobials - MIC and zone diameter ECVs (*Miller et al. 2006*) - used Visual Inspection
  - *Flavobacterium psychrophilum*
    - Six antimicrobials – MIC ECVs (analysis by Peter Smith, 2017) – used ECOFFinder and NRI – VAST approved Jan 2017

- Since 2015, VAST has approved several ECVs for *Salmonella*, *C. coli*, *C. jejuni*, and *E. coli*........*none published*
  - Based on US NARMS data
    - Interagency program operating since 1996
    - Monitors AMR of foodborne pathogens in animals, retail meats, humans
  - Most in agreement with EUCAST, some new pathogen:drug combination ECVs
# Need More ECOFFs for Foodborne Pathogens

<table>
<thead>
<tr>
<th>Species</th>
<th>Antimicrobial Agent</th>
<th>Interpretive Category Currently Used by NARMS</th>
<th>EUCAST ECOFF available for...</th>
<th>EUCAST ECOFF available for...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella/E. coli</td>
<td>Gentamicin</td>
<td>CLSI bp</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Salmonella/E. coli</td>
<td>Streptomycin</td>
<td>CLSI bp (using GCV)</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Salmonella/E. coli</td>
<td>Amoxicillin-Clavulanate</td>
<td>CLSI bp</td>
<td>No*</td>
<td>No*</td>
</tr>
<tr>
<td>Salmonella/E. coli</td>
<td>Cefoxitin</td>
<td>CLSI bp</td>
<td>No*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Salmonella/E. coli</td>
<td>Ceftiofur</td>
<td>CLSI bp</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Salmonella/E. coli</td>
<td>Ceftriaxone</td>
<td>CLSI bp</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Salmonella/E. coli</td>
<td>Sulfoxazole</td>
<td>CLSI bp</td>
<td>No*</td>
<td>no, sulfameth yes*</td>
</tr>
<tr>
<td>Salmonella/E. coli</td>
<td>Trimethoprim-sulfamethoxazole</td>
<td>CLSI bp</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Salmonella/E. coli</td>
<td>Azithromycin</td>
<td>NARMS bp</td>
<td>No*</td>
<td>No*</td>
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<tr>
<td>Salmonella/E. coli</td>
<td>Ampicillin</td>
<td>CLSI bp</td>
<td>Yes*</td>
<td>Yes*</td>
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<tr>
<td>Salmonella/E. coli</td>
<td>Chloramphenicol</td>
<td>CLSI bp</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Salmonella/E. coli</td>
<td>Ciprofloxacin</td>
<td>CLSI bp</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Salmonella/E. coli</td>
<td>Nalidixic Acid</td>
<td>CLSI bp</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Salmonella/E. coli</td>
<td>Tetracycline</td>
<td>CLSI bp</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Campylobacter jejuni/coli</td>
<td>Gentamicin</td>
<td>EUCAST ECOFF</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Campylobacter jejuni/coli</td>
<td>Telithromycin</td>
<td>EUCAST ECOFF (none set for coli, so jejuni criteria used for both)</td>
<td>Yes*</td>
<td>No*</td>
</tr>
<tr>
<td>Campylobacter jejuni/coli</td>
<td>Clindamycin</td>
<td>EUCAST ECOFF</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Campylobacter jejuni/coli</td>
<td>Azithromycin</td>
<td>EUCAST ECOFF</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Campylobacter jejuni/coli</td>
<td>Erythromycin</td>
<td>EUCAST ECOFF</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Campylobacter jejuni/coli</td>
<td>Florfenicol</td>
<td>EUCAST ECOFF</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Campylobacter jejuni/coli</td>
<td>Ciprofloxacin</td>
<td>EUCAST ECOFF</td>
<td>Yes*</td>
<td>Yes*</td>
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<td>Nalidixic acid</td>
<td>EUCAST ECOFF</td>
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<td>Yes*</td>
</tr>
<tr>
<td>Campylobacter jejuni/coli</td>
<td>Tetracycline</td>
<td>EUCAST ECOFF</td>
<td>Yes*</td>
<td>Yes*</td>
</tr>
<tr>
<td>Enterococcus faecium/faecalis</td>
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<td>Yes</td>
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<td>CLSI bp</td>
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* VAST also proposes ECV
Ex: VAST’s Use of WGS Data to Propose an ECV

Step 1. Population Data

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<th>Log₂MIC</th>
<th>Raw Count</th>
<th>Cum. Count</th>
<th>Modal MIC</th>
<th>Log₂MIC Mode</th>
<th>Max Log₂MIC</th>
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<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>No mechanisms</th>
<th>All mechanisms</th>
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<tr>
<td>&gt;32</td>
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</table>

ECOFFinder calculated an ECV ≤ 8

WGS data validates an ECV ≤ 8

EUCAST approved an ECOFF ≤ 16
## VAST ECOFF Conclusions

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<tr>
<th>Pathogen</th>
<th>Drug</th>
<th>Use EUCAST ECOFF</th>
<th>EUCAST ECOFF Change Needed</th>
<th>EUCAST ECOFF Change Possible</th>
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<td></td>
</tr>
<tr>
<td></td>
<td>gentamicin</td>
<td>no?</td>
<td></td>
<td>yes, from 2 to 1 µg/mL</td>
</tr>
<tr>
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<td>sulfisoxazole</td>
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<td></td>
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<tr>
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<td>ciprofloxacin</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>yes, from 16 to 8 µg/mL</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>azithromycin</td>
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<tr>
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<td>sulfisoxazole</td>
<td>no data</td>
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</tr>
<tr>
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<td>ciprofloxacin</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>nalidixic acid</td>
<td>no?</td>
<td></td>
<td>yes, from 16 to 8 µg/mL</td>
</tr>
<tr>
<td></td>
<td>amoxicillin/clav acid</td>
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<tr>
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</tr>
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<td>ceftiofur</td>
<td>yes</td>
<td></td>
<td></td>
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<tr>
<td><em>C. coli</em></td>
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<td>erythromycin</td>
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<td>gentamicin</td>
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<tr>
<td></td>
<td>nalidixic acid</td>
<td>yes</td>
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<tr>
<td></td>
<td>tetracycline</td>
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<td><em>C. jejuni</em></td>
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<td>erythromycin</td>
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<td>gentamicin</td>
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<td>azithromycin</td>
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<tr>
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<td>florfenicol</td>
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</tr>
</tbody>
</table>
Sequencing and resistance gene ID

- Whole-genome sequencing performed on MiSeq platform
  - Assembly by CLC Genomics Workbench
  - Resistance genes identified by in-house scripts, with 85% identity cutoff to genes in ResFinder database
- Presence of resistance determinants correlated to previously determined MICs
Use of WGS Data to Propose ECVs

Establishing Genotypic Cutoff Values to Measure Antimicrobial Resistance in *Salmonella* and *Escherichia coli*

Gregory H. Tyson*, Cong Li, Sherry Ayers, Patrick F. McDermott and Shaohua Zhao

*FEMS Micro Letters 2016. 363:1-5*

- accepted, AAC 2017
Previous work

• Correlated presence of resistance genes/resistance-associated mutations with NWT or R phenotype
  – For *Salmonella*, *E. coli*, *Campylobacter*
  – Correlations agreed approximately 99% of the time
• For some drugs, correlations much lower
Genotypic Cutoff Value (GCV)

- Term coined to denote: the highest MIC of the population of bacteria lacking resistance determinants to a given drug. A vast majority of isolates above this MIC must possess resistance mechanisms.
- Determined using Visual Inspection
- Previously used this technique (but didn’t call it GCV) to change NARMS cutoffs (E. coli and Salmonella) for streptomycin

Salmonella WGS – MIC data correlations

Chloramphenicol

- No mechanisms
- All mechanisms

Amoxicillin-clavulanate

- R not predicted
- No mechanisms
- R predicted

Ampicillin

- No mechanisms
- All mechanisms

Ciprofloxacin

- No mechanisms
- All mechanisms
# Salmonella Ciprofloxacin MICs by Mechanism

## Graph

The graph shows the distribution of MIC (mg/L) values for Salmonella isolates. The x-axis represents MIC values ranging from 0 to >4 mg/L, while the y-axis represents the number of isolates. The graph is color-coded to show different mechanisms:
- **GCV ECOFF ECV BP**
- **No mechanisms**
- **All mechanisms**

## Table

### Table: MIC (mg/L) by Mechanism

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<th>MIC (mg/L)</th>
<th>No mechanisms</th>
<th>qnr genes</th>
<th>One gyrA mutation</th>
<th>Two gyrA mutations</th>
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<tr>
<td>0.03</td>
<td>344</td>
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### Summary of GCVs for *Salmonella*

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<th>CLSI susceptible (S): treatment success likely</th>
<th>EUCAST ECV: wild-type (WT)</th>
<th>GCV: no resistance mechanism*</th>
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<td>Amoxicillin-clavulanate</td>
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<td>≤ 8</td>
<td>≤ 8</td>
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<tr>
<td>Ceftiofur</td>
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<td>≤ 2</td>
<td>≤ 2</td>
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<td>Gentamicin</td>
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<td>≤ 2</td>
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<tr>
<td>Tetracycline</td>
<td>≤ 4</td>
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<td>≤ 4</td>
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<td>≤ 16</td>
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<td>Ciprofloxacin</td>
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<td>Trimethoprim-sulfamethoxazole</td>
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<td>≤ 1</td>
<td>≤ 0.5</td>
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</table>

* Determined by authors using visual inspection method
Results

• Only 81 of 22,486 isolates had MICs that did not correlate to their GCV definitions, many due to overlap of population with and without acquired resistance mechanisms
  – **99.6% total correlation**

• WGS will provide a more accurate measure to report %NWT (not %R.....yet)

• Demonstrates ability to predict MIC based on genotypic information alone
  – Some resistance mechanisms differ markedly by level of resistance conferred
# NARMS Now: Interactive Data Displays

## Antimicrobial resistance genes in *Salmonella*, 2014

Whole genome sequencing (WGS) has entered a new age in infectious disease science, with the power to greatly enhance diagnosis, surveillance and treatment. WGS can be used to predict antimicrobial resistance for a number of bacteria, including the foodborne pathogen, *Salmonella*. In addition, WGS data reveal the range of gene causing resistance to a particular antimicrobial.

Please note: Minor differences may be encountered when comparing results from the static data tables and the Interactive data dashboards. The data dashboards are limited to those isolates that were subjected to WGS analysis. A few isolates were not available for testing and therefore excluded from the displays presented here.

This dashboard allows users to explore how resistance varies in the most common serotypes of *Salmonella*. To get started, select an antimicrobial.

**Select an Antimicrobial agent**

- Ampicillin

**Select from the most common serotypes found in human and animal *Salmonella* infections:**

- [Select serotype]

### Number of Isolates resistant to Ampicillin

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### Number of Ampicillin resistance genes found

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Note: The table below lists the number of *Salmonella* isolates tested from each source sample. When a specific serotype is selected, the numbers in the table change to reflect total samples of that serotype.

For humans, only isolates that were resistant to at least one antimicrobial agent via phenotypic testing were sequenced (n=1376). Nineteen isolates that lost resistance between phenotypic testing and whole genome sequencing (continued by repeated phenotypic testing) were excluded from the analysis, resulting in a final N of 1157.

### Total number of isolates tested

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<tr>
<th>Source</th>
<th>2,127</th>
<th>143</th>
<th>101</th>
<th>86</th>
<th>44</th>
<th>13</th>
<th>103</th>
<th>215</th>
<th>20</th>
<th>275</th>
<th>325</th>
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Source: [FDA](http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm416741.htm)
New WGS Resources

• NCBI has released a comprehensive, centralized resistance gene database (4000+), including translated gene sequences (3500+)

• Associated analytic tools will be released
Acknowledgements

US FDA – CVM’s Office of Research

• Greg Tyson
• Patrick McDermott
• Shaohua Zhao
• Cong Li
• Sherry Ayers
• Jonathan Sabo
• Claudia Lam
My Recommendations

1. Joint AST/VAST WG to develop an official CLSI position on:
   - How ECVs should and should not be used
   - When is it appropriate to use CBPs for surveillance when ECVs are available?
   - How surveillance data should be reported

   - Others?

Example

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Antimicrobial</th>
<th>%NWT</th>
<th>%R</th>
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<tbody>
<tr>
<td>Salmonella</td>
<td>Streptomycin</td>
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<td></td>
<td>Gentamicin</td>
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<td></td>
<td>Ampicillin</td>
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