CLSI – A One Health Perspective on Susceptibility Testing

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Zoetis, Inc

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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.

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One Health Drivers

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

- 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!

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Making the One Health Connection

- 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

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Classification of Antibacterials by Importance in Human Health is the Basis for Microbiological Risk Assessments in Animal Health[1]

Human Use Only	Critically Important[2]	Highly Important	Important	Not Important
Carbapenems	3rd Gen Cephs	Penicillin	1st Gen Cephs	Bacitracin
Linezolid	4th Gen Cephs	Oxacillin	2nd Gen Cephs	Tiamulin
Vancomycin	Fluorquinolones	Carbenicillin	Cephamycins	Avilamycin
Oritivancin	Macrolides	Ampicillin	Quinolones	Ionophores
Dalbavancin	Trim/Sulfa	Amoxicillin		
Daptomycin		Amoxi-Clavulanate		
5th Gen Cephs		Amp-Sulbactam		
		Aminoglycosides		
		Lincosamides		
		Tetracylines		
		Streptogramins		
		Rifamycins		
		Chloramphenicol		

[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.

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AST and V-AST: A History of Collaboration

- 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

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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

Human Origin Bacteria	Veterinary AMR
EARs-NET	NARMS
NARMS	MARAN
CIPARS	DANMAP
ResistVet	GERM-VET
WHONET-Argentina	CIPARS
	ITAVARM

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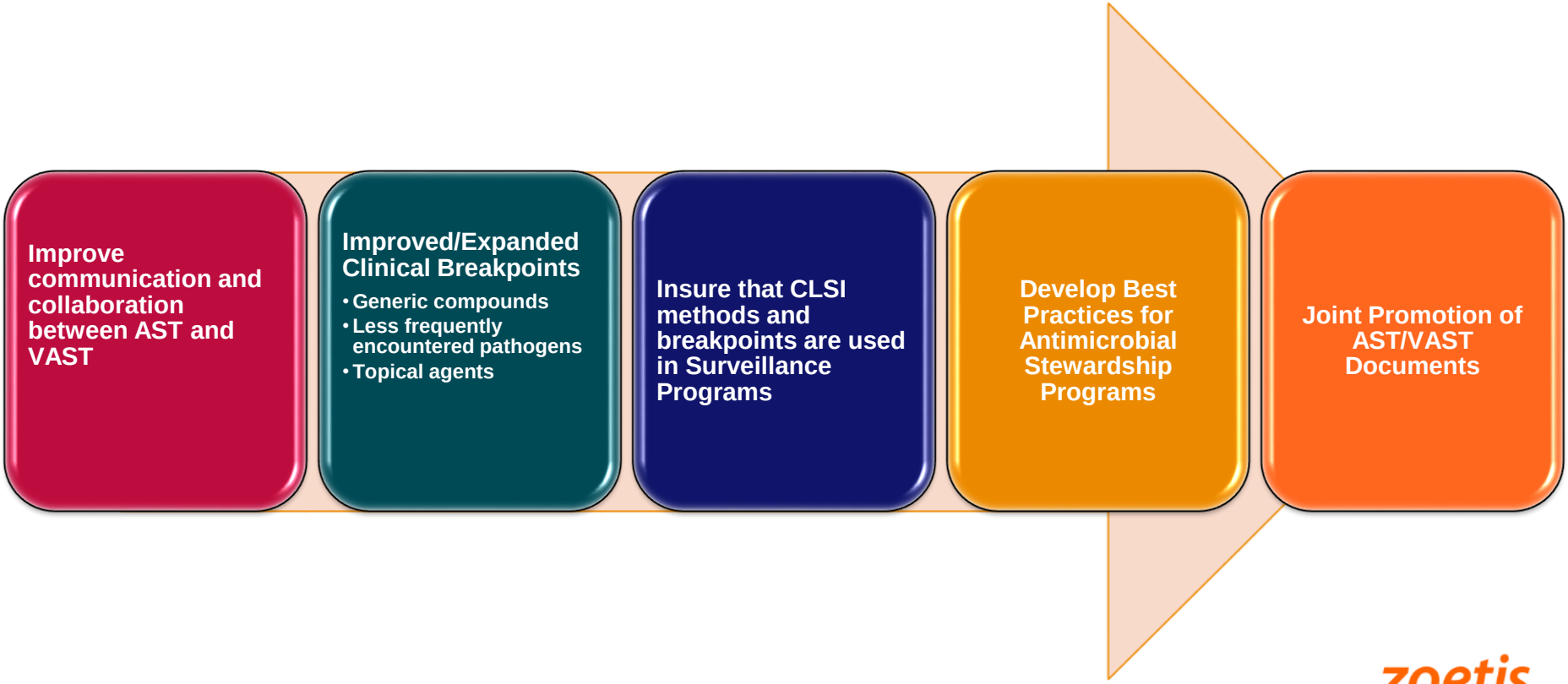
Future CLSI ONE HEALTH INITIATIVES



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CLINICAL AND LABORATORY STANDARDS INSTITUTE®

CLSI Educational Workshop January 14, 2017

One Health - One Medicine

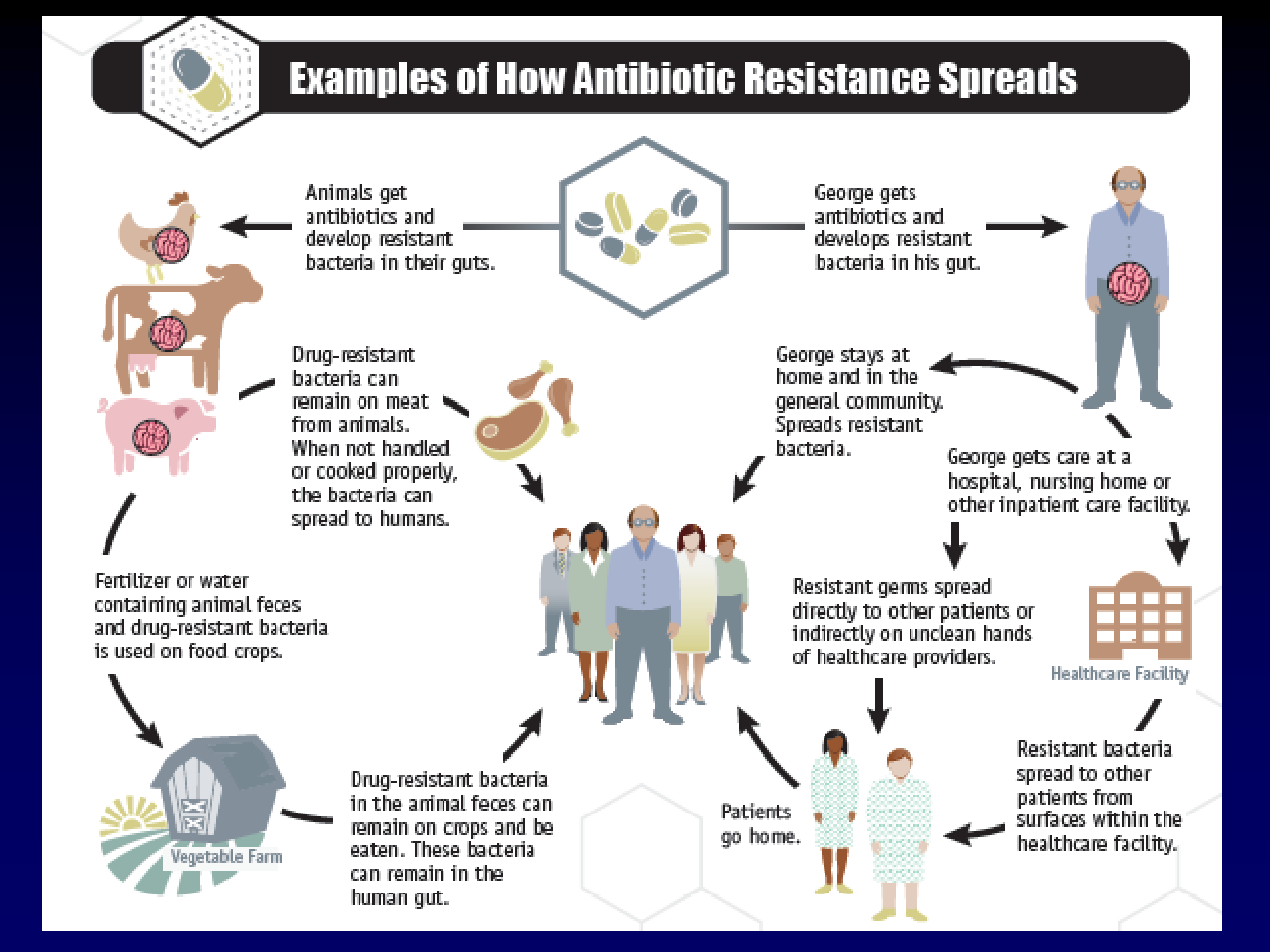
CLSI Veterinary Antimicrobial Susceptibility Testing Subcommittee (VAST)

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

CLSI Educational Workshop

How VAST Develops Breakpoints for Generic Drugs (and how/why they differ from M100 breakpoints)

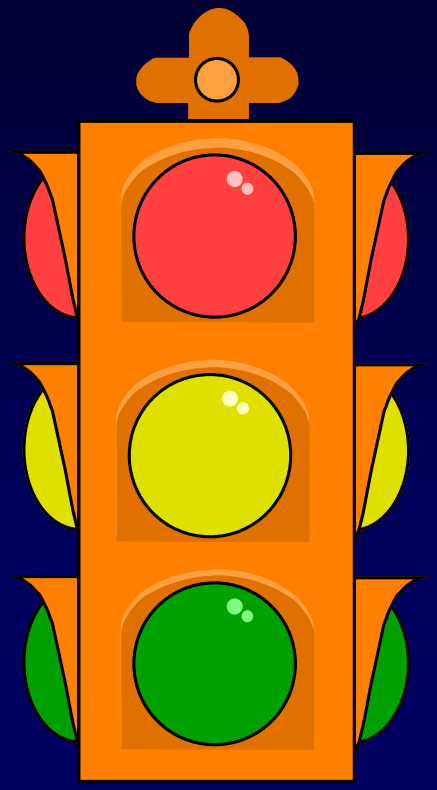
Examples of How Antibiotic Resistance Spreads

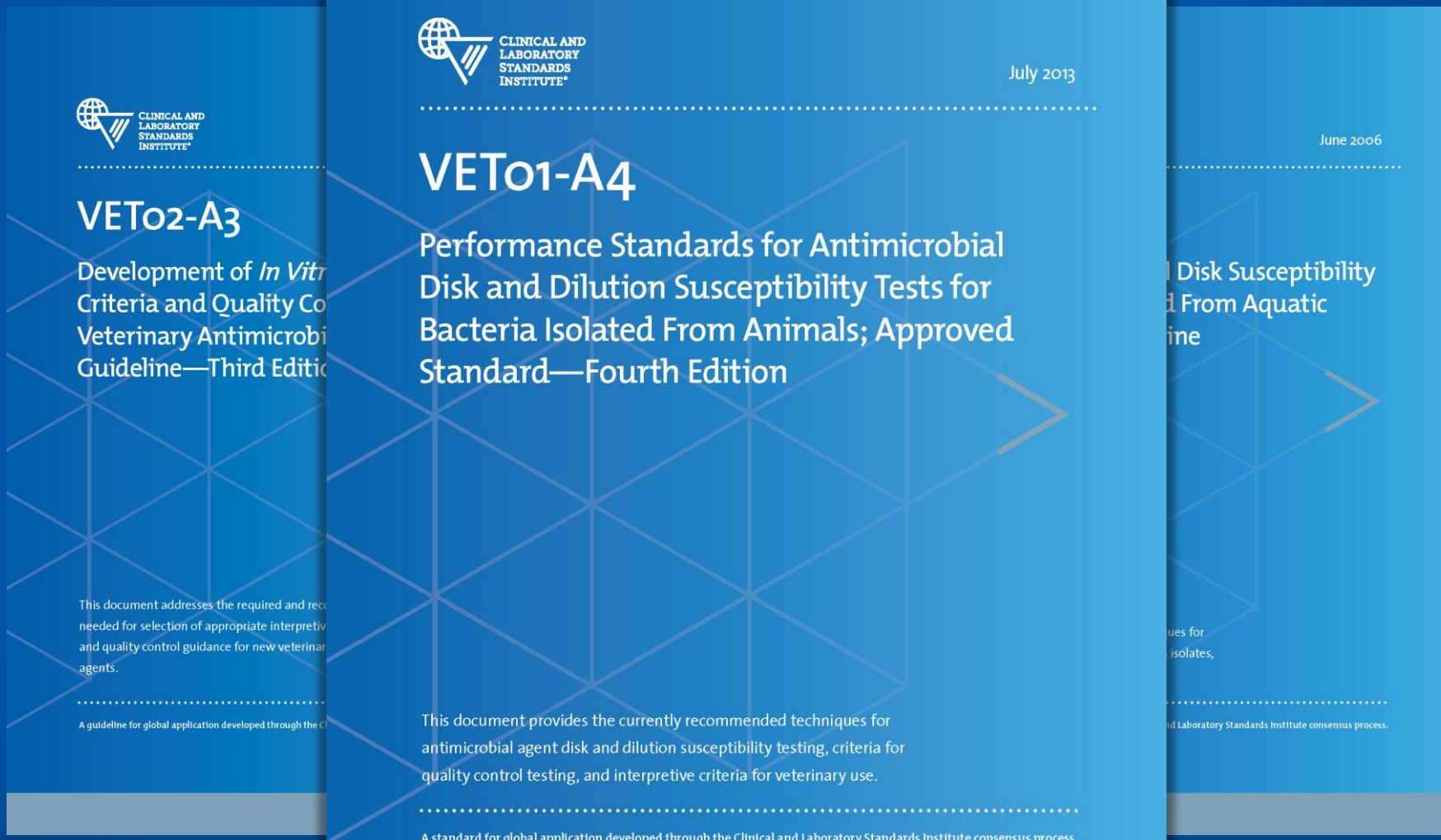


We are “One Health”

CLSI Interpretive Categories

- Resistant
- Intermediate
- Susceptible




CLINICAL AND LABORATORY STANDARDS INSTITUTE
VET02-A3
Development of *In Vitr*
Criteria and Quality Co
Veterinary Antimicrobi
Guideline—Third Editi
This document addresses the required and rec
needed for selection of appropriate interpretiv
and quality control guidance for new veterinar
agents.
A guideline for global application developed through the Cl
CLINICAL AND LABORATORY STANDARDS INSTITUTE
July 2013
VET01-A4
Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Fourth Edition
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.
June 2006
l Disk Susceptibility
d From Aquatic
ine
ues for
isolates,
d Laboratory Standards Institute consensus process.

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CLSI VET 02

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

CLINICAL AND LABORATORY STANDARDS INSTITUTE™

(Formerly NCCLS)
Providing NCCLS standards and guidelines,
ISO/TC 212 standards, and ISO/TC 76 standards

Veterinary Antimicrobial Susceptibility Testing subcommittee (VAST)

- Role of the Generic Drug Working Group (GWG)

CLSI VET 02

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)

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- 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)

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✓ Ampicillin (horses & pigs)

✓ Tetracycline (cattle & pigs)

- Enrofloxacin (pigs)

✓ Penicillin G (horses, cattle, pigs)

Clinical Laboratory and Standards Institute (CLSI)

CLSI-VAST (VET01-S2, 2013) has updated breakpoints for susceptibility testing:

- Cephalosporins (1st gen): ≤ 8 µg/mL → ≤ 2 µg/mL
- Amoxicillin-Clavulanate: ≤ 8 µg/mL → ≤ 0.25 µg/mL
- Ampicillin: ≤ 8 µg/mL → ≤ 0.25 µg/mL
- Gentamicin: ≤ 4 µg/mL → ≤ 2 µg/mL
- Chloramphenicol: No change (≤ 8 µg/mL)
- Oxacillin (Resistant *Staph pseudintermedius*): ≥ 4 µg/mL → ≥ 0.5 µg/mL

CLSI-VAST (VET01-S3, 2014)
New breakpoints for susceptibility testing:

- ✓ Doxycycline: ≤ 4 µg/mL → ≤ 0.125 µg/mL (dogs and horses)

- ✓ Amikacin: ≤ 16 µg/mL →
  - Dogs ≤ 4 µg/mL
  - Horses ≤ 4 µg/mL
  - Foals ≤ 2 µg/mL

CLSI-VAST (VET01-S4)
New breakpoints for susceptibility testing (not yet published)

- ✓ Minocycline: ≤ 4 µg/mL → ≤ 0.5 µg/mL
- ✓ Piperacillin and Tazobactam: ≤ 16 µg/mL →
  - Dogs ≤ 8 µg/mL
- ✓ Ciprofloxacin (dogs): ≤ 0.06 µg/mL (Human breakpoint is ≤ 1 µg/mL; therefore, recommended no listing.)

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How Do We Create Standards?

3.7 Development of Interpretive Criteria for Generic or Older Compounds (VET 02)

Where does the dose come from?

- Established consensus documents.
  - United States Pharmacopeia Drug Information (USP-DI) Expert Panel (www.USP.org; J Vet Pharm Ther 2003)
  - ACVIM Consensus Statements
  - ISCAID (International Society of Companion Animal Infectious Diseases) guidelines

3.7 Development of Interpretive Criteria for Generic or Older Compounds (VET 02)

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.

3.7 Development of Interpretive Criteria for Generic or Older Compounds (VET 02)

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 CO_{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)

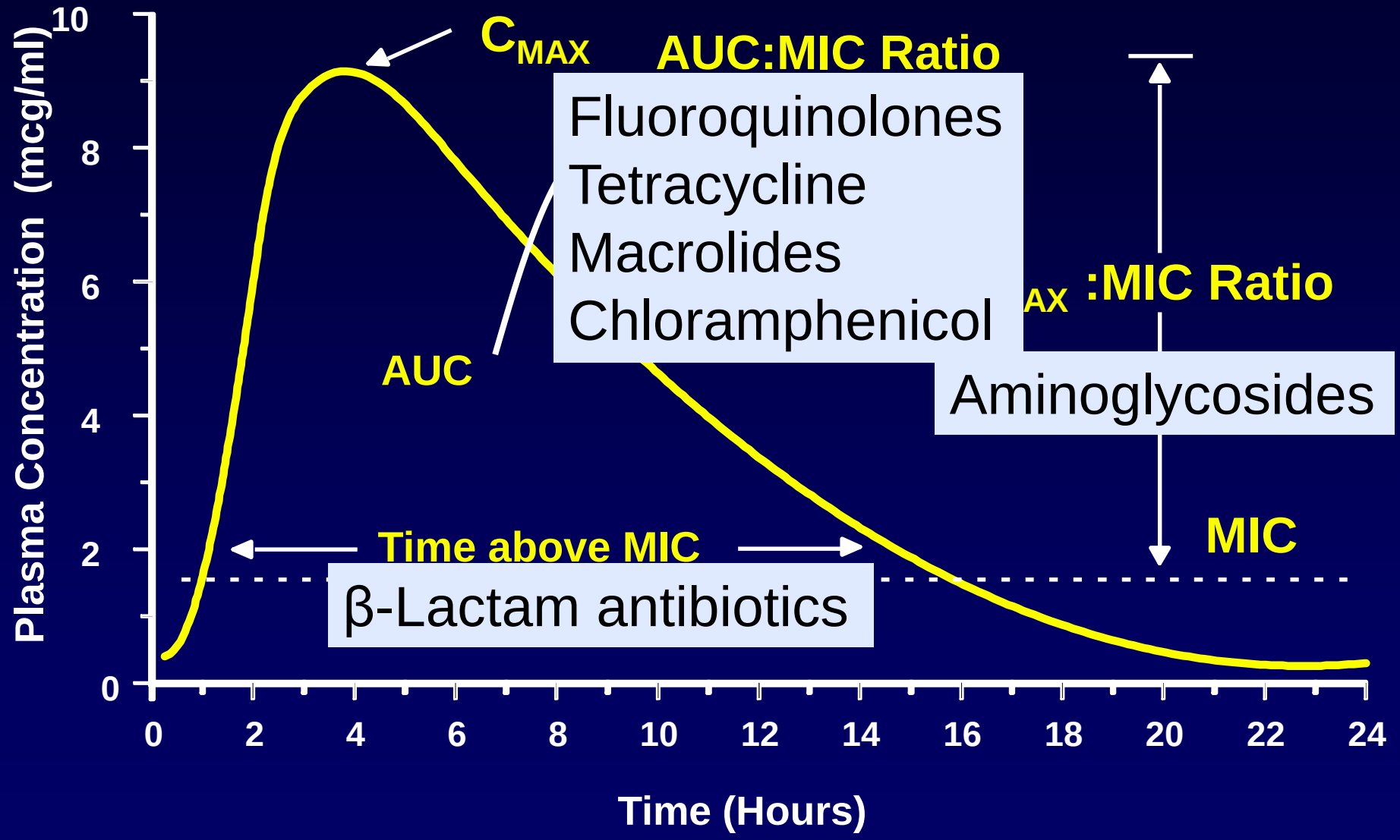
Pharmacokinetic Data

- 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



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 > MIC =

  ln (Dose/[VD x MIC]) x (T ½ / ln2) x (100 / DI)

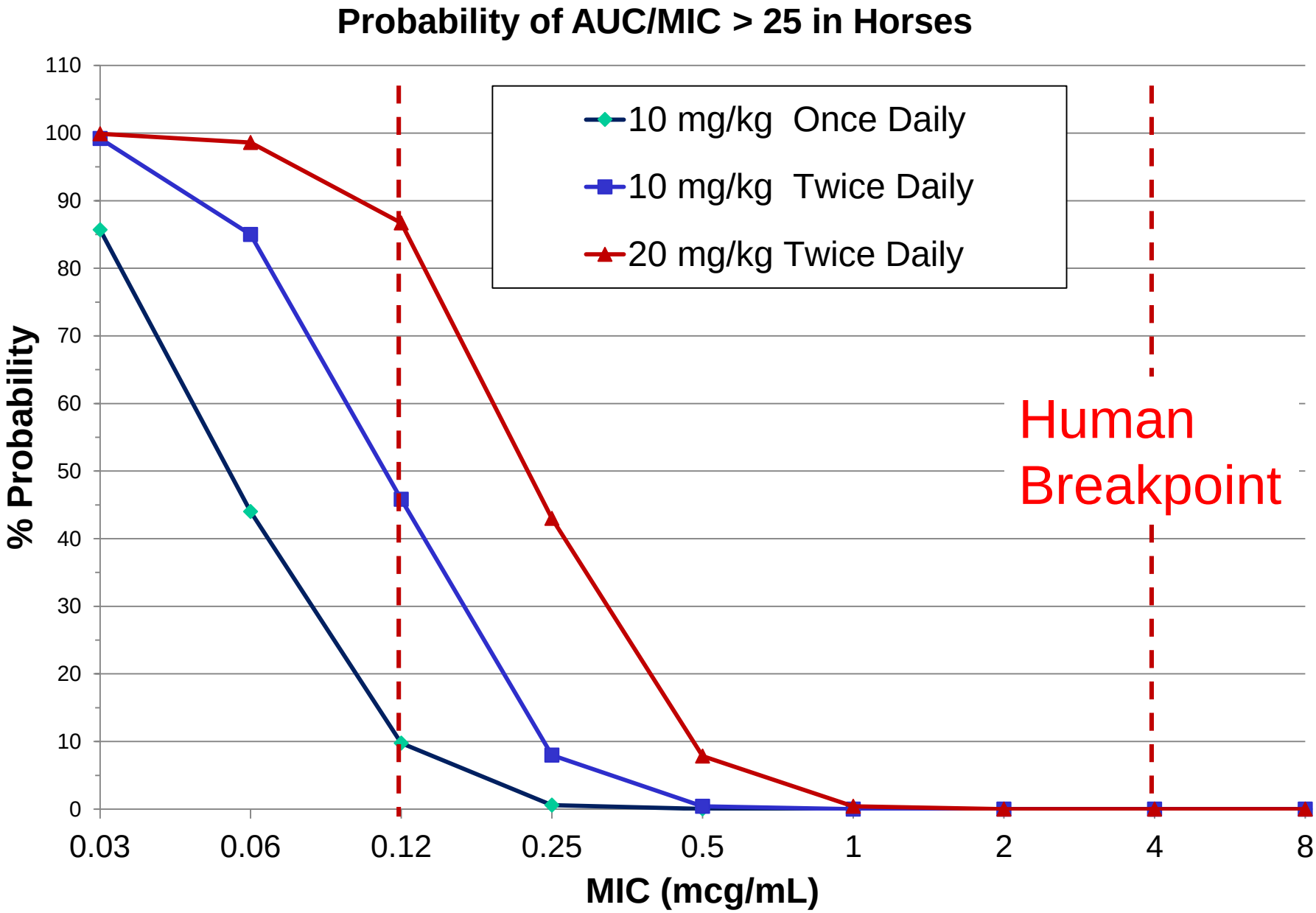
- VD = volume of distribution
- ln2 = natural logarithim 2
- T ½ = half-life
- Dose
- DI = dose interval

Determination of AUC / MIC

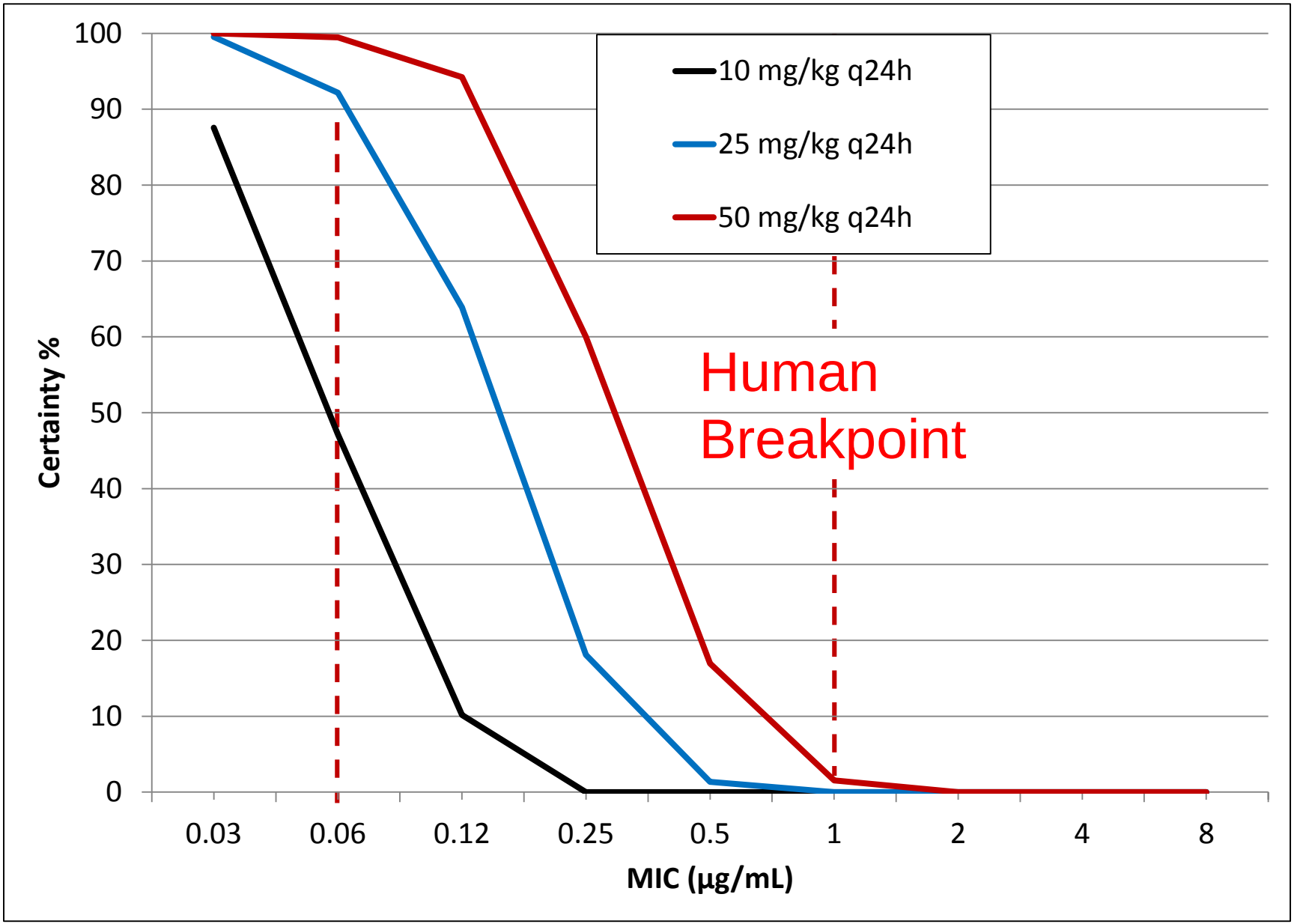
$$\text{AUC/MIC} = \frac{\text{fu} \cdot \text{F} \cdot 24\ \text{hr} \cdot \text{Dose}}{\text{CL} \cdot \text{MIC}}$$

- Clearance (CL)
- Fraction absorbed (F)
- Protein binding (fraction unbound, *fu)*
- Dose
- MIC

Probability of Target Attainment (PTA) for doxycycline administered to horses



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



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?

NC STATE UNIVERSITY

COLLEGE OF VETERINARY MEDICINE

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‘One Health’ in a Clinical Microbiology Laboratory Practice

Thomas R. Fritsche
Division of Laboratory Medicine
Marshfield Clinic

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

100 YEARS

ENRICHING LIVES **TOGETHER**
FOR GENERATIONS

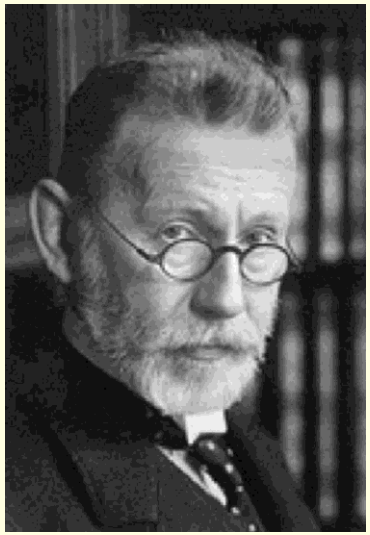
Laboratory Operations

- 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

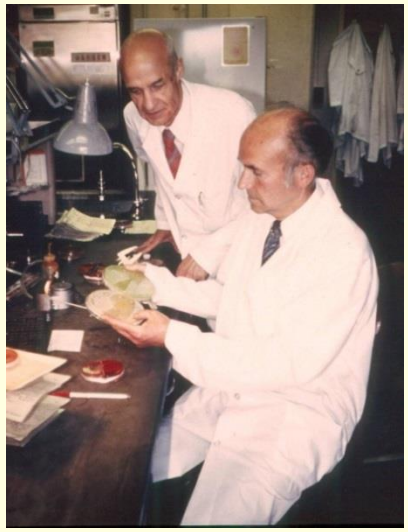
The Paradigm of Clinical Bacteriology: 106 Years in the Making (1860-1966)

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

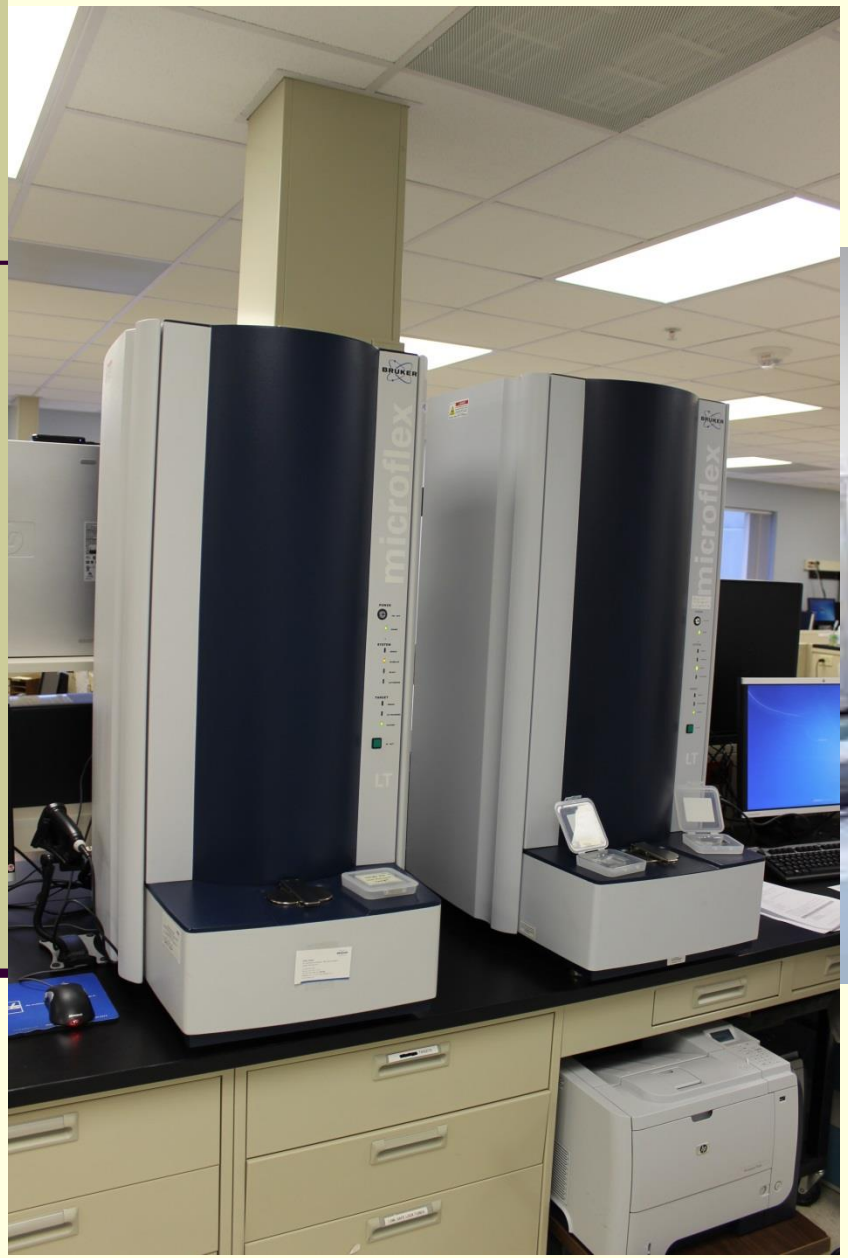
CLSI M58 Guidance Document in Development

- *"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



Bruker MicroFlex Biotyper™

bioMerieux Vitek® MS

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

MALDI Biotyper Screen Shot

ID	Name	Position	Chip	Detected Species	Score
psae 27853	A1	A1	0	Pseudomonas aeruginosa	2.427
896841	A2	A2	0	Escherichia coli	2.308
896841-2	A3	A3	0	Escherichia coli	2.471
889887	A4	A4	0	Providencia stuartii	2.468
889887-2	A5	A5	0	Providencia stuartii	2.462
899983	A6	A6	0	Enterobacter aerogenes	2.467
100095	A7	A7	0	Staphylococcus felis	2.199
106249	A8	A8	0	Staphylococcus intermedius	1.831
894373-2	B1	B1	0	Enterococcus faecalis	2.259
114233	B2	B2	0	Klebsiella pneumoniae	2.435
113051	B3	B3	0	Klebsiella pneumoniae	2.475
113051-2	B4	B4	0	Mannheimia granulomatis	2.143
896380	B5	B5	0	Staphylococcus intermedius	1.946
107872	B6	B6	0	Escherichia coli	2.314
107872-2	B7	B7	0	Pasteurella canis	2.329
100855	B8	B8	0	Pasteurella canis	2.277
107346	C1	C1	0	Pseudomonas aeruginosa	2.372
107346-2	C2	C2	0	Pseudomonas aeruginosa	2.294
101629	C3	C3	0	Escherichia coli	2.575
101629-2	C4	C4	0	Enterococcus faecium	2.407
108164	C5	C5	0	Staphylococcus pseudintermedius	2.145
899622	C6	C6	0	Bordetella bronchiseptica	2.582
899622	C7	C7	0	Bordetella bronchiseptica	2.470
898715	C8	C8	0	Staphylococcus aureus	2.358
898715-2	D1	D1	0	Staphylococcus aureus	2.452
114139	D2	D2	0	Staphylococcus pseudintermedius	.962
107512	D3	D3	0	Pseudomonas putida	1.876
114364	D4	D4	0	Pasteurella multocida	2.285

Costs: Johns Hopkins Experience for 952 Isolates Annualized to 47,845 Isolates (279 spp.)*

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

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

Tan et al. J. Clin. Microbiol. 50:3301, 2012

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

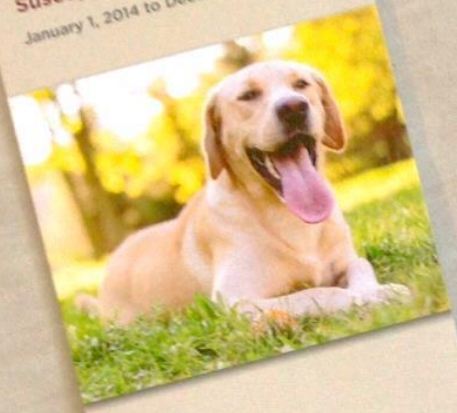
ThermoFisher ARIS™ System using Broth Microdilution MIC Panels

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

Canine Prevalent Pathogens & Antimicrobial Susceptibility Patterns

January 1, 2014 to December 31, 2015



Marshfield Labs
A division of Marshfield Clinic
marshfieldlabs.org

Feline Prevalent Pathogens & Antimicrobial Susceptibility Patterns

January 1, 2014 to December 31, 2015

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Equine, Bovine, and Avian Prevalent Pathogens and Antimicrobial Susceptibility Patterns

January 1, 2014 to December 31, 2015

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Marshfield Labs
A division of Marshfield Clinic

2015 Outpatient Cumulative Antibiogram

Microbiology Section
Division of Laboratory Medicine,
Marshfield Clinic
Marshfield Wisconsin

Contact Dr. Thomas Novicki or Dr. Thomas Fritsche at ext. 16300 (715-221-6300) for additional information regardijng this report. Contact the Marshfield Clinic Pharmacy Drug Information Service at ext. 19800 (715-221-9800) for dosing and other drug information.

MINISTRY
Saint Joseph's Hospital

2015 Inpatient Cumulative Antibiogram

Microbiology Section
Division of Laboratory Medicine,
Marshfield Clinic
Marshfield Wisconsin

Contact Dr. Thomas Novicki or Dr. Thomas Fritsche at ext. 16300 (715-221-6300) for additional information regarding this report. Contact the Saint Joseph's Hospital Pharmacy at ext. 77687 (715-387-7687) for dosing and other drug information.

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 Antibiograms
% Susceptible

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

Human isolates 2015; Canine isolates 2014-2015

Human/Canine Antibiograms % Susceptible

	S. aureus	
	H 621	**C 231**
OX	76	75
PEN	21	20
ENO	-	78
LVX	76	-
MAR	-	79
TET (DOX)	94	97
SXT	99	98

Human isolates 2015; Canine isolates 2014-2015

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

	Oxacillin % Resistant (n)			
Year	SIG*	*S. schleiferi*	*S. aureus*	Total
2012	19.5 (1688)	38.5 (135)	36.5 (96)	21.7 (1919)
2013	19.7 (2432)	41.4 (239)	18.9 (127)	21.4 (2798)
2014	19.2 (3140)	37.5 (392)	25.5 (145)	21.3 (3677)
2015	20.6 (3341)	32.2 (391)	26.6 (137)	21.9 (3869)
Totals	19.8 (10601)	37.4 (1157)	26.9 (505)	21.6 (12263)

**S. intermedius* group

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

	Mupirocin % Resistant (number tested); 200 ug disk			
Year	SIG*	*S. schleiferi*	*S. aureus***	Total
2012	0 (0/261)	0 (0/35)	0 (0/5)	0.0 (0/301)
2013	0.7 (2/289)	7.7 (3/39)	14.3 (1/7)	1.8 (6/335)
2014	0 (0/200)	5.6 (1/18)	0 (0/5)	0.4 (1/223)
2015	0.7 (2/271)	4.3 (1/23)	0 (0/2)	1.0 (3/296)
Totals	0.4 (4/1021)	4.3 (5/115)	5.2 (1/19)	0.9 (10/1155)

**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!

MARSHFIELD CLINIC®

U.S. FOOD & DRUG ADMINISTRATION

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

CLSI Educational Workshop, January 14, 2017

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

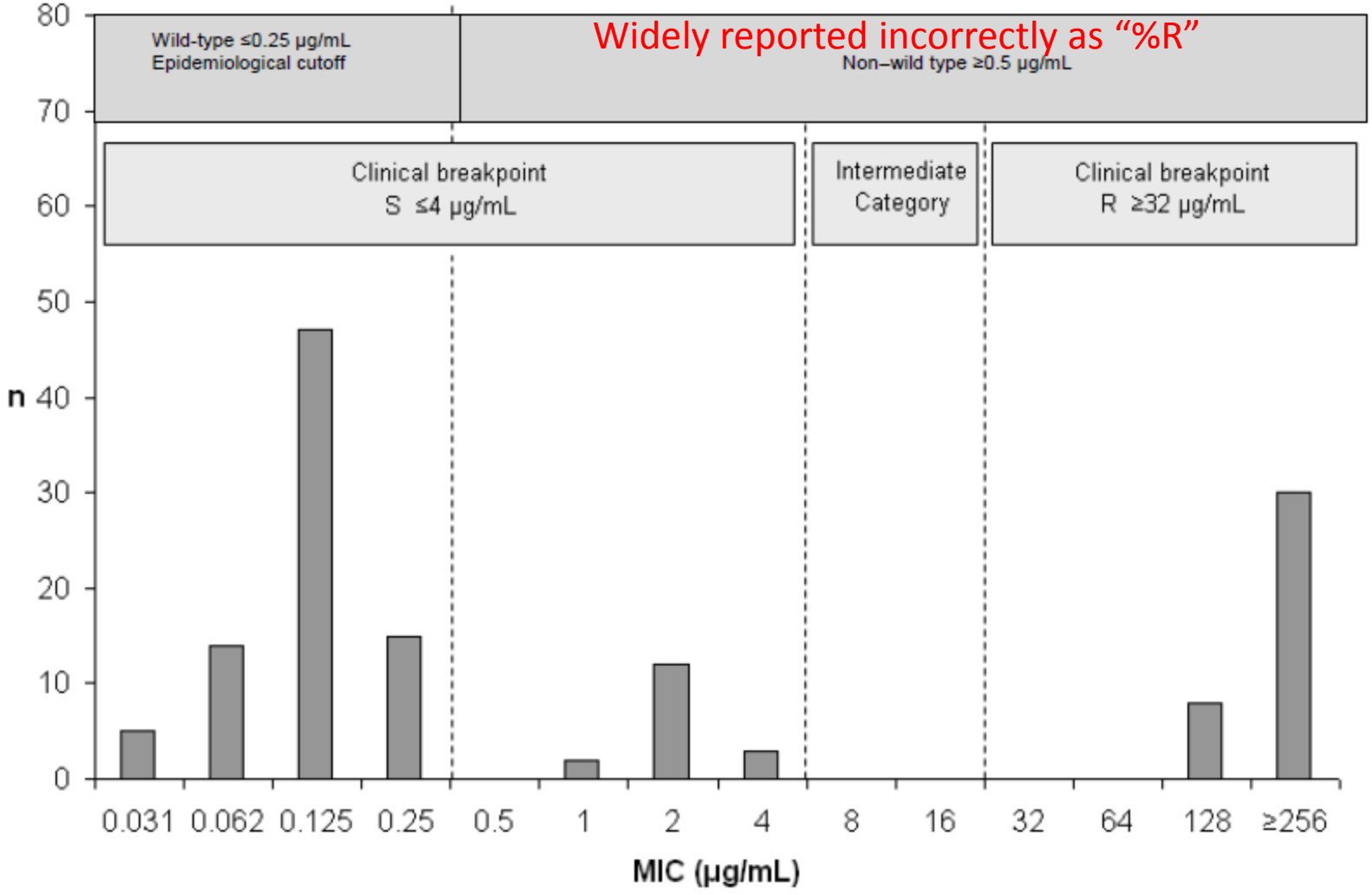


Figure 1. Distribution of MICs and Categorization by Clinical Breakpoints Contrasted to ECVs

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

Rev. sci. tech. Off. int. Epiz., 2012, **31** (1), 33-41

Susceptibility testing methods, resistance and breakpoints: what do these terms really mean?

P. Silley

MB Consult Limited, Enterprise House, Ocean Village, Southampton SO14 3XB, United Kingdom
Department of Biomedical Sciences, University of Bradford, West Yorkshire, BD7 1DP, United Kingdom

Summary
The Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing can be considered the major international contributors to antimicrobial susceptibility testing. In this review, the author considers the differences between the respective organisations, examines the terminology used in antimicrobial susceptibility testing and argues for an urgent need to harmonise these definitions. While this may seem somewhat surprising, the terminology used to define resistance does differ. In this context, attention is given to the trend for 'resistance' to be defined by the epidemiological cut-off value, rather than by the long-established clinical breakpoint. The author goes on to discuss susceptibility testing methodologies and present an approach to setting clinical breakpoints.

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

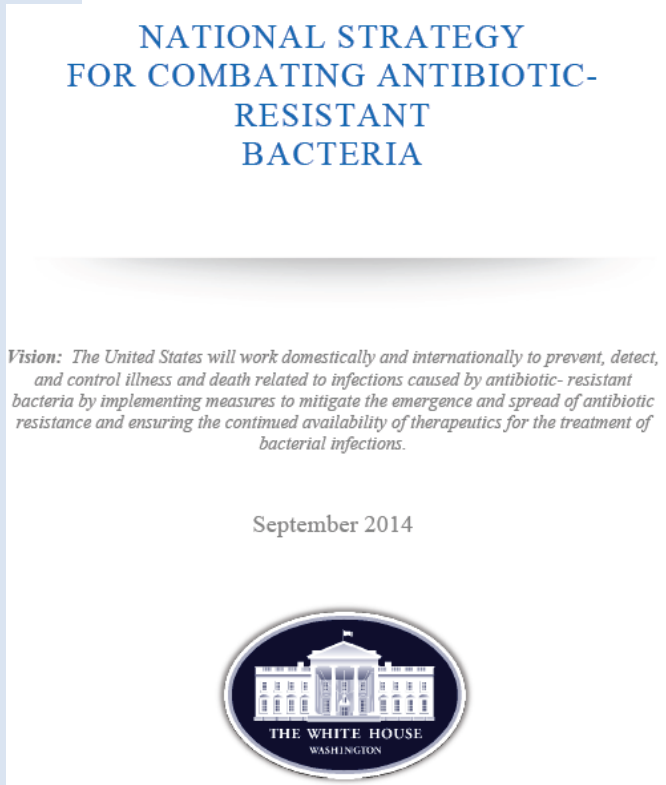
U.S. Presidential CARB Initiative

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.



AMR Harmonization

OIE Efforts

White et al. (2001)

- Introduced the term *'microbiological breakpoints'*

WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future

OIE Ad hoc Group

CHAPTER 6.7. (2012)

HARMONISATION OF
NATIONAL ANTIMICROBIAL RESISTANCE
SURVEILLANCE AND MONITORING PROGRAMMES

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.

Rev. sci. tech. Off. int. Epiz., 2001, 20 (3), 849-858

Antimicrobial resistance: standardisation and harmonisation of laboratory methodologies for the detection and quantification of antimicrobial resistance

D.G. White [1], J. Acar [2], F. Anthony [3], A. Franklin [4], R. Gupta [5], †T. Nicholls [6], Y. Tamura [7], S. Thompson [8], E.J. Threlfall [9], D. Vose [10], M. van Vuuren [11], H.C. Wegener [12] & M.L. Costarrica [13]

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(4) The National Veterinary Institute (SVA), Department of Antibiotics, SE 751 89 Uppsala, Sweden
(5) College of Veterinary Sciences, Veterinary Bacteriology, Department of Microbiology, G.B. Pant University of Agriculture and Technology, Pantnagar 263 145 Uttar Pradesh, India
(6) National Offices of Animal and Plant Health and Food Safety, Animal Health Science and Emergency Management Branch, Department of Agriculture, Fisheries and Forestry, P.O. Box 858, Canberra ACT 2601, Australia
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(9) Public Health Laboratory Service, Central Public Health Laboratory, Laboratory of Enteric Pathogens, 61 Collindale Avenue, London NW9 5HT, United Kingdom
(10) David Vose Consulting, Le Bourg, 24400 Les Lèches, France
(11) University of Pretoria, Faculty of Veterinary Science, Department of Veterinary Tropical Diseases, Private Bag X04, Onderstepoort 0110, South Africa
(12) World Health Organization, Detached National Expert, Division of Emerging and Transmissible Diseases, Animal and Food-related Public Health Risks, 20 avenue Appia, 1211 Geneva, Switzerland
(13) Food and Agriculture Organization, Food Quality and Standards Service, Senior Officer, via delle Terme di Caracalla, 00100 Rome, Italy

This report, prepared by the OIE Ad hoc Group of experts on antimicrobial resistance, has not yet received the approval of the International Committee of the OIE

Summary

The Ad hoc Group of experts on antimicrobial resistance of the Office International des Epizooties has developed a guideline on the standardisation and harmonisation of laboratory methodologies used for the detection and quantification of antimicrobial resistance. The existing methods (disk diffusion [including concentration gradient strips], agar dilution and broth dilution) are reviewed, including a comparison of their advantages and disadvantages. The definitions of resistance characteristics of bacteria (susceptible, intermediate and resistant) are addressed and the criteria for the establishment of breakpoints are discussed. Due consideration has to be given to these aspects in the interpretation and comparison of resistance monitoring or surveillance data. The use of validated laboratory methods and the establishment of quality assurance (internal and external) for microbiological laboratory work and the reporting of quantitative test results is recommended. Equivalence of different methods and laboratory test results is also recommended to be established by external proficiency testing, which should be achieved by the means of a reference laboratory system. This approach allows the comparison of test results obtained using different methods generated by laboratories in different countries.

AMR Monitoring and Harmonization

WHO GLASS, 2016

- *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 GL

- 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

AMR Monitoring and Harmonization

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*

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

FDA

www.eucast.org

MIC distributions and ECOFFs

MIC distributions and ECOFFs

Link to the website with 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*

VET05-R to VET05-A

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.

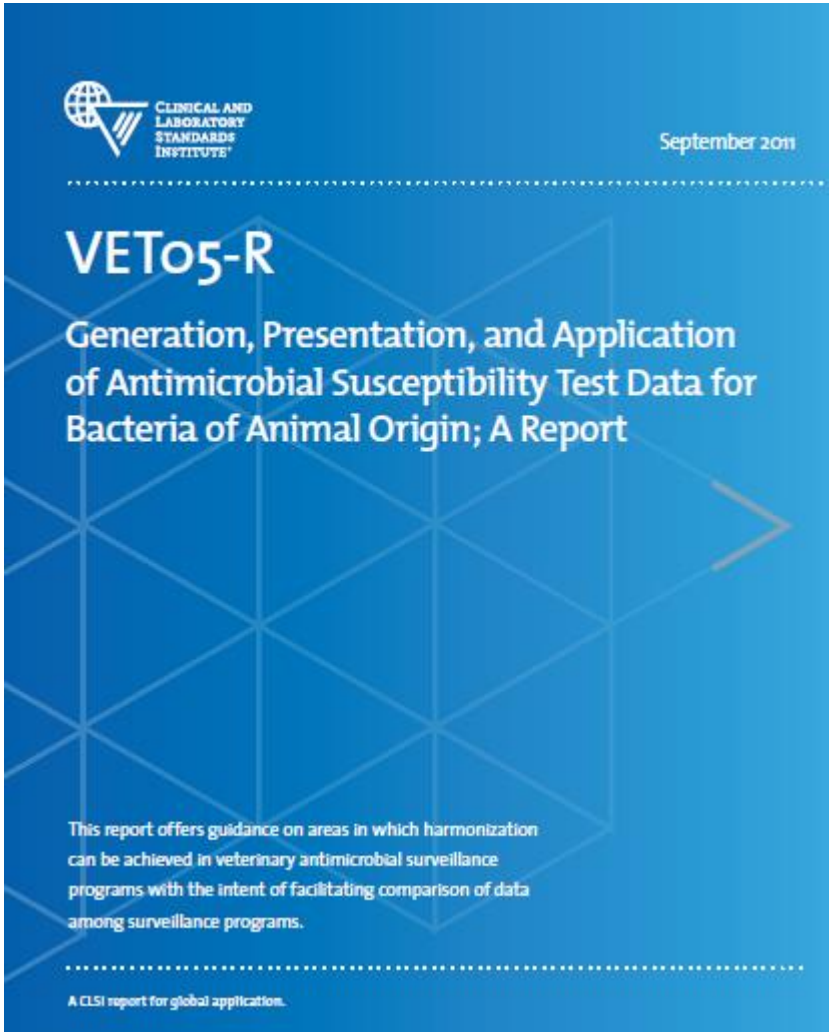
Emphasize ECOFFs for surveillance and not CBPs

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

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Consultation on Report from the EUCAST Subcommittee on the Role of Whole Genome Sequencing (WGS) in Antimicrobial Susceptibility Testing of Bacteria

The report is open for comment by 24 June 2016. Please send comments, with supporting data or references where appropriate, to the EUCAST Scientific Secretary (derek.brown222@btinternet.com). Please use the accompanying form for your comments.

Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers

Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

This draft guidance document is being distributed for comment purposes only.

Document issued on: May 13, 2016

You should submit comments and suggestions regarding this draft document within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions about this document, contact Heike Sichtig Ph.D., Division of Microbiology Devices at 301-796-4574 or by email at Heike.Sichtig@fda.hhs.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health
Office of *In Vitro* Diagnostics and Radiological Health
Division of Microbiology Devices

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

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- **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

Species	Antimicrobial Agent	Interpretive Category Currently Used by NARMS	EUCAST ECOFF available for… Salmonella	EUCAST ECOFF available for… E. coli
Salmonella/E. coli	Gentamicin	CLSI bp	Yes*	Yes*
Salmonella/E. coli	Streptomycin	NARMS bp (using GCV)	yes	Yes
Salmonella/E. coli	Amoxicillin-Clavulanate	CLSI bp	No*	No*
Salmonella/E. coli	Cefoxitin	CLSI bp	No*	Yes*
Salmonella/E. coli	Ceftiofur	CLSI bp	Yes*	Yes*
Salmonella/E. coli	Ceftriaxone	CLSI bp	No	Yes
Salmonella/E. coli	Sulfisoxazole	CLSI bp	No*	no, sulfameth yes*
Salmonella/E. coli	Trimethoprim-sulfamethoxazole	CLSI bp	Yes	Yes
Salmonella/E. coli	Azithromycin	NARMS bp	No*	No*
Salmonella/E. coli	Ampicillin	CLSI bp	Yes*	Yes*
Salmonella/E. coli	Chloramphenicol	CLSI bp	Yes*	Yes*
Salmonella/E. coli	Ciprofloxacin	CLSI bp	Yes*	Yes*
Salmonella/E. coli	Nalidixic Acid	CLSI bp	Yes*	Yes*
Salmonella/E. coli	Tetracycline	CLSI bp	yes	yes
			Jejuni	coli
Campylobacter jejuni/coli	Gentamicin	EUCAST ECOFF	Yes*	Yes*
Campylobacter jejuni/coli	Telithromycin	EUCAST ECOFF (none set for coli, so jejuni criteria used for both)	Yes*	No*
Campylobacter jejuni/coli	Clindamycin	EUCAST ECOFF	Yes*	Yes*
Campylobacter jejuni/coli	Azithromycin	EUCAST ECOFF	Yes*	Yes*
Campylobacter jejuni/coli	Erythromycin	EUCAST ECOFF	Yes*	Yes*
Campylobacter jejuni/coli	Florfenicol	EUCAST ECOFF	Yes*	Yes*
Campylobacter jejuni/coli	Ciprofloxacin	EUCAST ECOFF	Yes*	Yes*
Campylobacter jejuni/coli	Nalidixic acid	EUCAST ECOFF	Yes*	Yes*
Campylobacter jejuni/coli	Tetracycline	EUCAST ECOFF	Yes*	Yes*
			faecium	faecalis
Enterococcus faecium/faecalis	Gentamicin	CLSI bp	yes	yes
Enterococcus faecium/faecalis	Kanamycin	NARMS bp	no	no
Enterococcus faecium/faecalis	Streptomycin	CLSI bp	yes	yes
Enterococcus faecium/faecalis	Vancomycin	CLSI bp	yes	yes
Enterococcus faecium/faecalis	Tigecycline	NARMS bp	yes	yes
Enterococcus faecium/faecalis	Lincomycin	NARMS bp	no	no
Enterococcus faecium/faecalis	Daptomycin	CLSI bp	yes	yes
Enterococcus faecium/faecalis	Erythromycin	CLSI bp	yes	yes
Enterococcus faecium/faecalis	Tylosin	NARMS bp	no	no
Enterococcus faecium/faecalis	Nitrofurantoin	CLSI bp	yes	yes
Enterococcus faecium/faecalis	Linezolid	CLSI bp	yes	yes
Enterococcus faecium/faecalis	Penicillin	CLSI bp	no	no
Enterococcus faecium/faecalis	Chloramphenicol	CLSI bp	yes	yes
Enterococcus faecium/faecalis	Ciprofloxacin	CLSI bp	yes	yes
Enterococcus faecium/faecalis	Quinupristin/Dalfopristin	CLSI bp (only for faecium)	no	no
Enterococcus faecium/faecalis	Tetracycline	CLSI bp	yes	yes

*** VAST also proposes ECV**

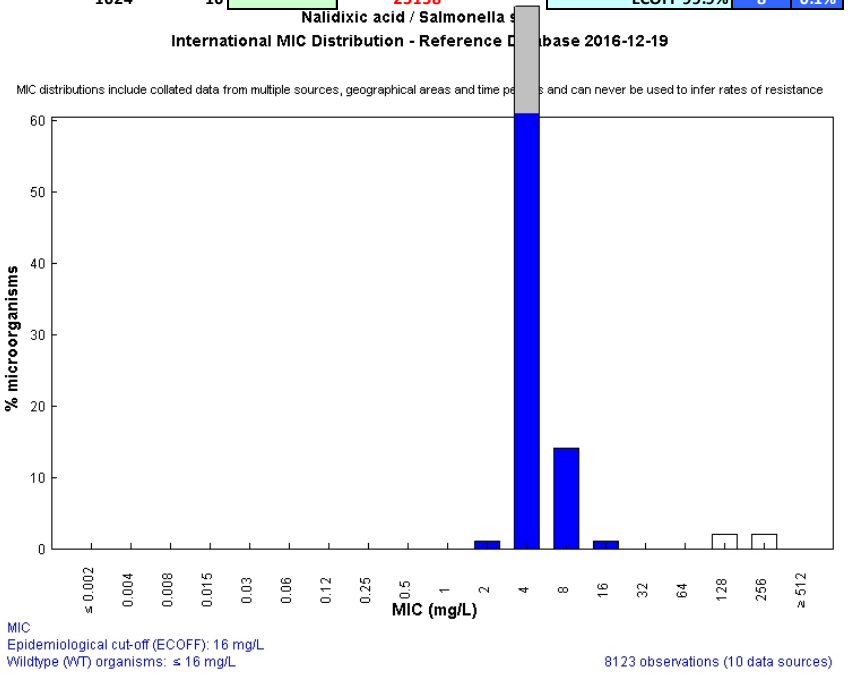
Ex: VAST's Use of WGS Data to Propose an ECV

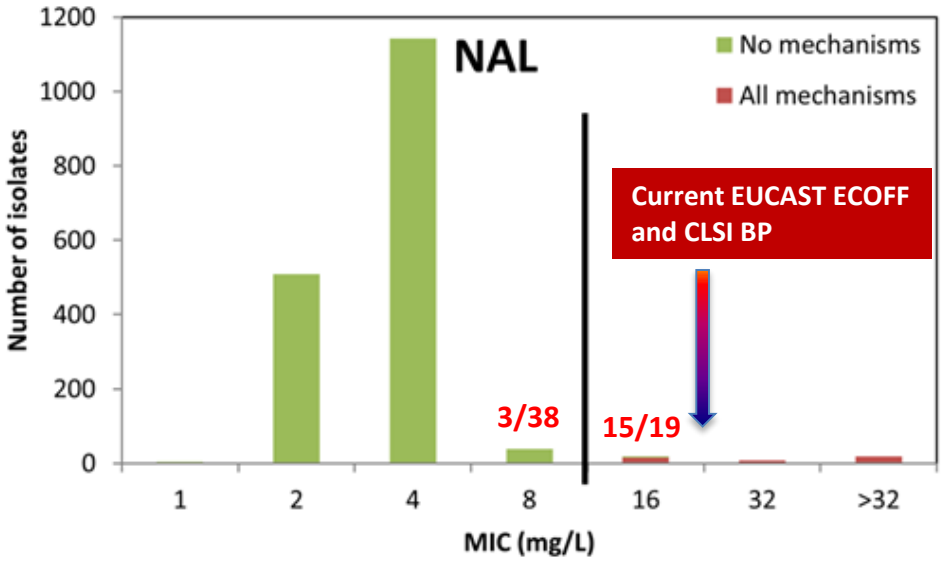
Step 1. Population Data — *Salmonella* | Nalidixic acid | onscale

MIC	Log_2MIC	Raw Count	Cum. Count	Fitted
0.001	-10		0	0.0
0.002	-9		0	0.0
0.004	-8		0	0.0
0.008	-7		0	0.0
0.016	-6		0	0.0
0.03	-5		0	0.0
0.06	-4		0	0.0
0.125	-3		0	0.0
0.25	-2		0	0.0
0.5	-1	11	11	0.1
1	0	154	165	209.0
2	1	8462	8627	8412.4
4	2	15077	23704	15091.4
8	3	1427	25131	1411.1
16	4	26	25157	4.4
32	5	1	25158	0.0
64	6		25158	
128	7		25158	
256	8		25158	
512	9		25158	
1024	10		25158	

Modal MIC	4
Log_2MIC Mode	2
Max Log_2MIC	5
Selected Log2 Mean	1.203 =2.3
Selected Log2 SD	0.502

Selected CO_{WT} Values		%>
ECOFF 95.0%	8	0.1%
ECOFF 97.5%	8	0.1%
ECOFF 99.0%	8	0.1%
ECOFF 99.9%	8	0.1%





ECOFFinder calculated an ECV ≤ 8

WGS data validates an ECV ≤ 8

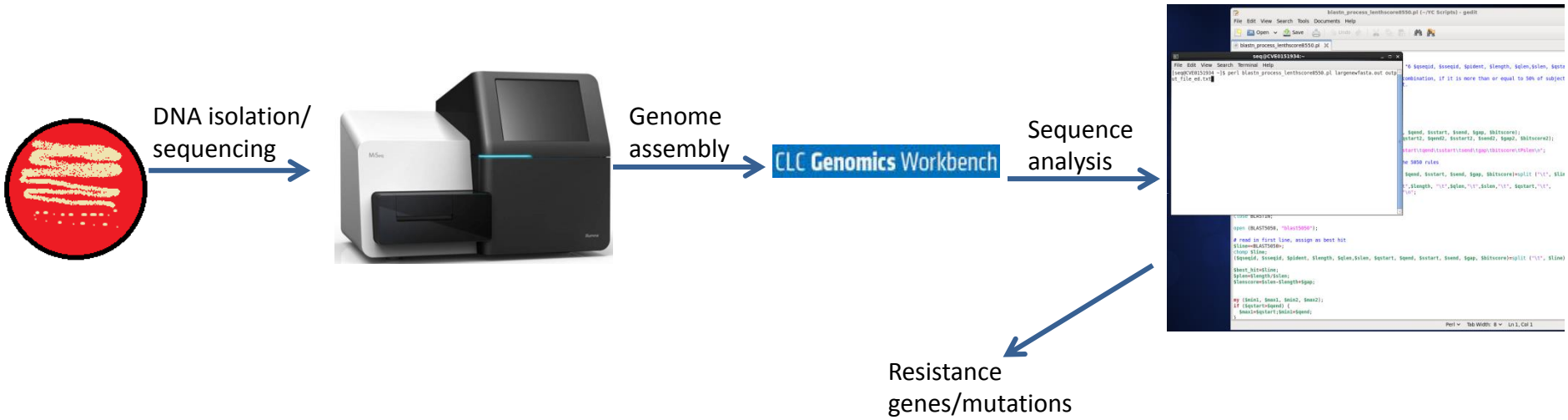
EUCAST approved an ECOFF ≤ 16

VAST ECOFF Conclusions

Pathogen	Drug	Use EUCAST ECOFF	EUCAST ECOFF Change Needed	EUCAST ECOFF Change Possible
Salmonella	ampicillin	yes		
	chloramphenicol	yes		
	gentamicin	no?		yes, from 2 to 1 µg/mL
	sulfisoxazole	no data		
	ciprofloxacin	yes		
	nalidixic acid	no	yes, from 16 to 8 µg/mL	
	amoxicillin/clav acid	no data		
	cefoxitin	yes		
	ceftiofur	yes		
	azithromycin	none set		
E. coli	ampicillin	yes		
	chloramphenicol	yes		
	gentamicin	yes		
	sulfisoxazole	no data		
	ciprofloxacin	yes		
	nalidixic acid	no?		yes, from 16 to 8 µg/mL
	amoxicillin/clav acid	none set		
	cefoxitin	yes		
	ceftiofur	yes		
	azithromycin	no data		
C. coli	ciprofloxacin	yes		
	clindamycin	yes		
	erythromycin	yes		
	gentamicin	yes		
	nalidixic acid	yes		
	tetracycline	yes		
	telithromycin	no data		
	azithromycin	yes		
	florfenicol	yes		
C. jejuni	ciprofloxacin	yes		
	clindamycin	yes		
	erythromycin	yes		
	gentamicin	yes		
	nalidixic acid	yes		
	tetracycline	yes		
	telithromycin	yes		
	azithromycin	yes		
	florfenicol	yes		

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

RESEARCH LETTER – Food Microbiology

Using whole-genome sequencing to determine appropriate streptomycin epidemiological cutoffs for *Salmonella* and *Escherichia coli*

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

FEMS Micro Letters 2016. 363:1-5

Establishing Genotypic Cutoff Values to Measure Antimicrobial Resistance in *Salmonella*

Gregory H. Tyson[1#], Shaohua Zhao[1], Cong Li[1], Sherry Ayers[1], Jonathan L. Sabo[1], Ron A. Miller[2], and Patrick F. McDermott[1]

- 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

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Whole-Genome Sequencing for Detecting Antimicrobial Resistance in Nontyphoidal *Salmonella*

Patrick F. McDermott,[a] Gregory H. Tyson,[a] Claudine Kabera,[a] Yuansha Chen,[a] Cong Li,[a] Jason P. Folster,[b] Sherry L. Ayers,[a] Claudia Lam,[a] Heather P. Tate,[a] Shaohua Zhao[a]

Division of Animal and Food Microbiology, Office of Research, Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, Maryland, USA[a]; Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA[b]

AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology

Whole-Genome Sequencing Analysis Accurately Predicts Antimicrobial Resistance Phenotypes in *Campylobacter* spp.

S. Zhao,[a] G. H. Tyson,[a] Y. Chen,[a] C. Li,[a] S. Mukherjee,[a] S. Young,[a] C. Lam,[a] J. P. Folster,[b] J. M. Whichard,[b] P. F. McDermott[a]

J Antimicrob Chemother 2015; **70**: 2763–2769
doi:10.1093/jac/dkv186 Advance Access publication 3 July 2015

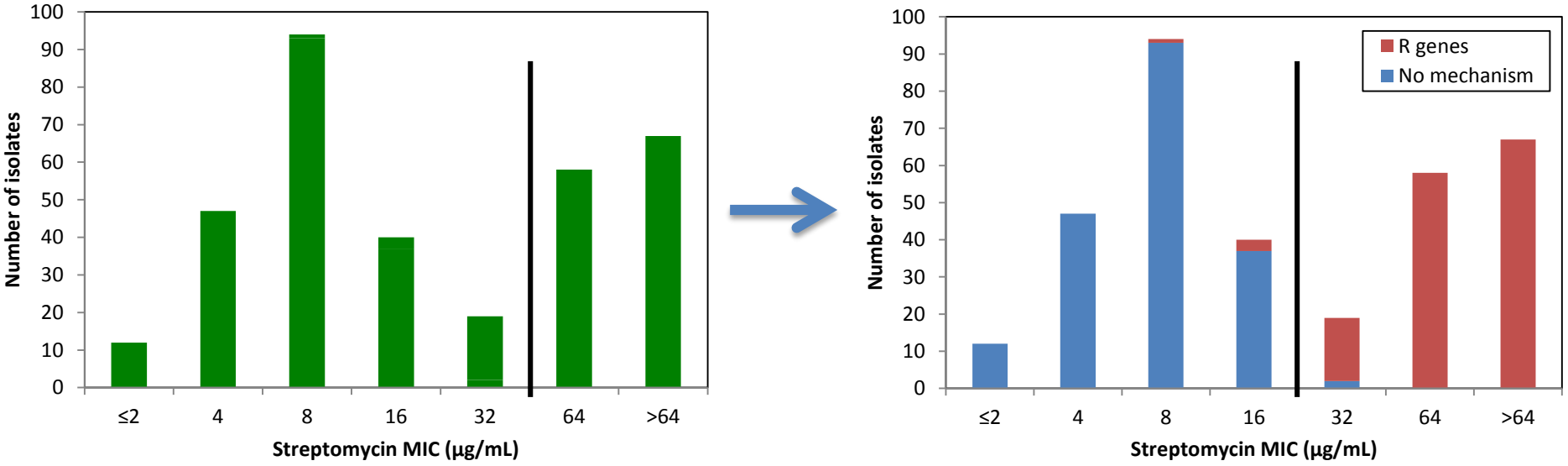
Journal of Antimicrobial Chemotherapy

WGS accurately predicts antimicrobial resistance in *Escherichia coli*

Gregory H. Tyson[1], Patrick F. McDermott[1], Cong Li[1], Yuansha Chen[1], Daniel A. Tadesse[1], Sampa Mukherjee[1], Sonya Bodeis-Jones[1], Claudine Kabera[1], Stuart A. Gaines[1], Guy H. Loneragan[2], Tom S. Edrington[3], Mary Torrence[4], Dayna M. Harhay[5] and Shaohua Zhao[1]*

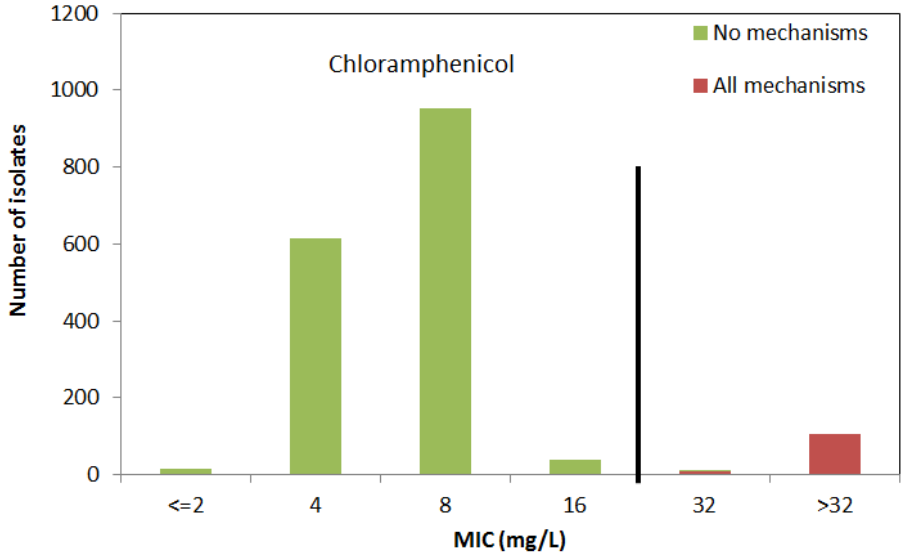
Genotypic Cutoff Value (GCV)

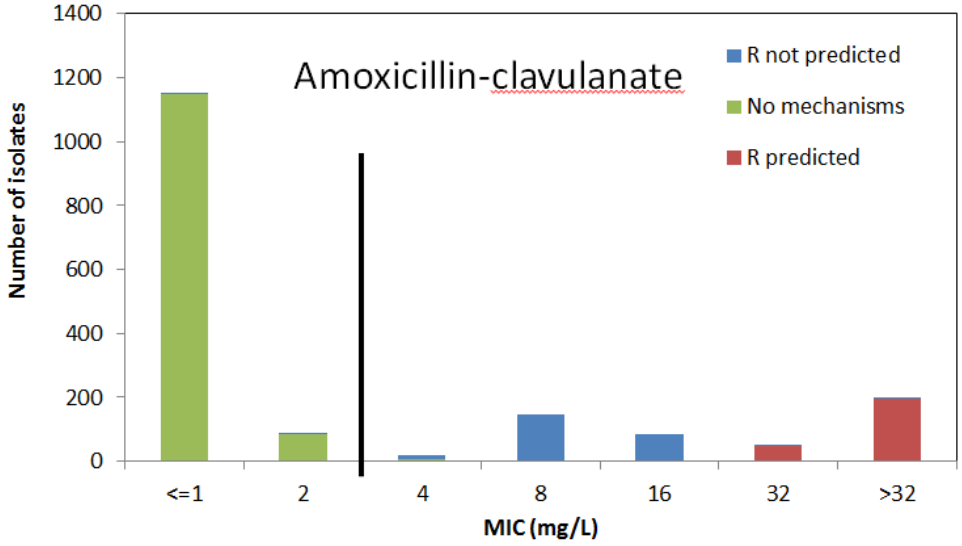
- Term coined to denote: the highest MIC of the population of bacteria lacking resistance determinants to a given drug. A vast majority of isolates above this MIC must possess resistance mechanisms.
- Determined using Visual Inspection
- Previously used this technique (but didn't call it GCV) to change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

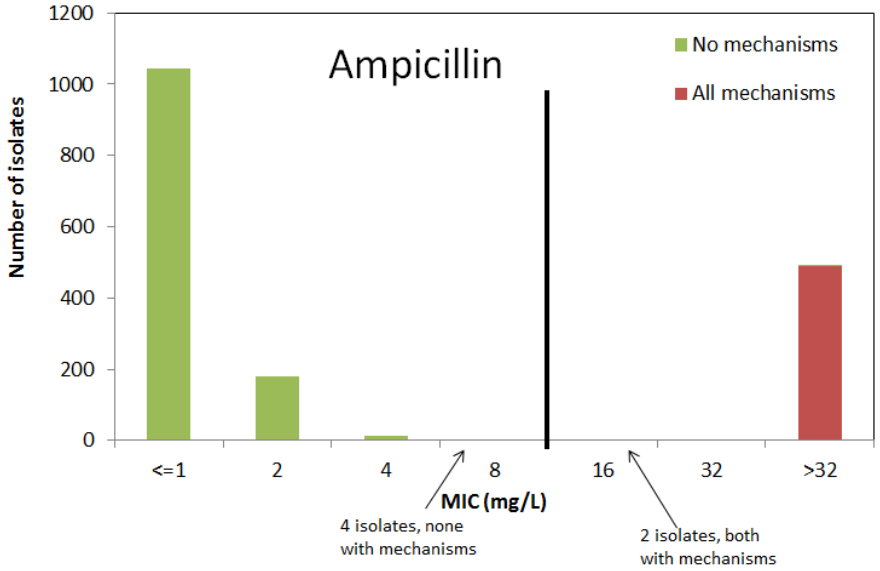


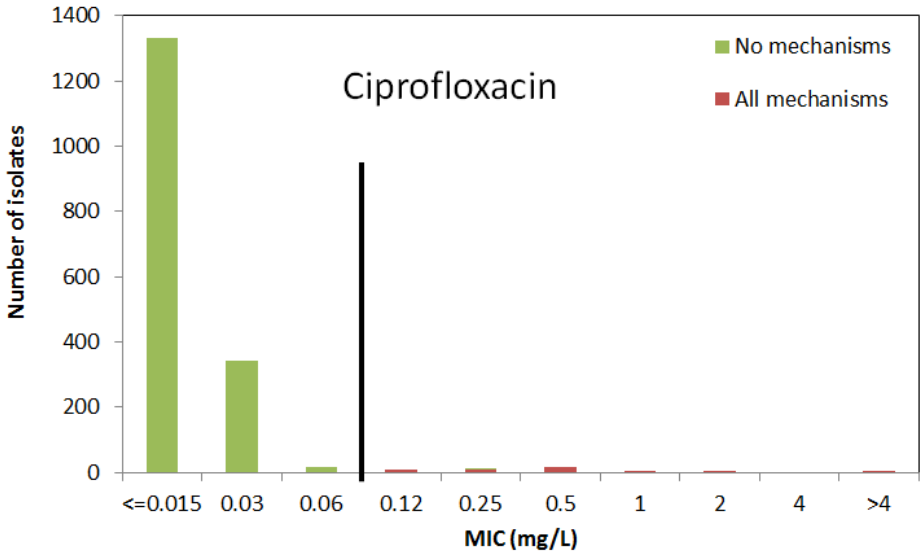
Tyson GH, Li C, Ayers SL, McDermott PF, Zhao S. FEMS Microbiol Lett. 2016 Feb;363(4).

Salmonella WGS – MIC data correlations

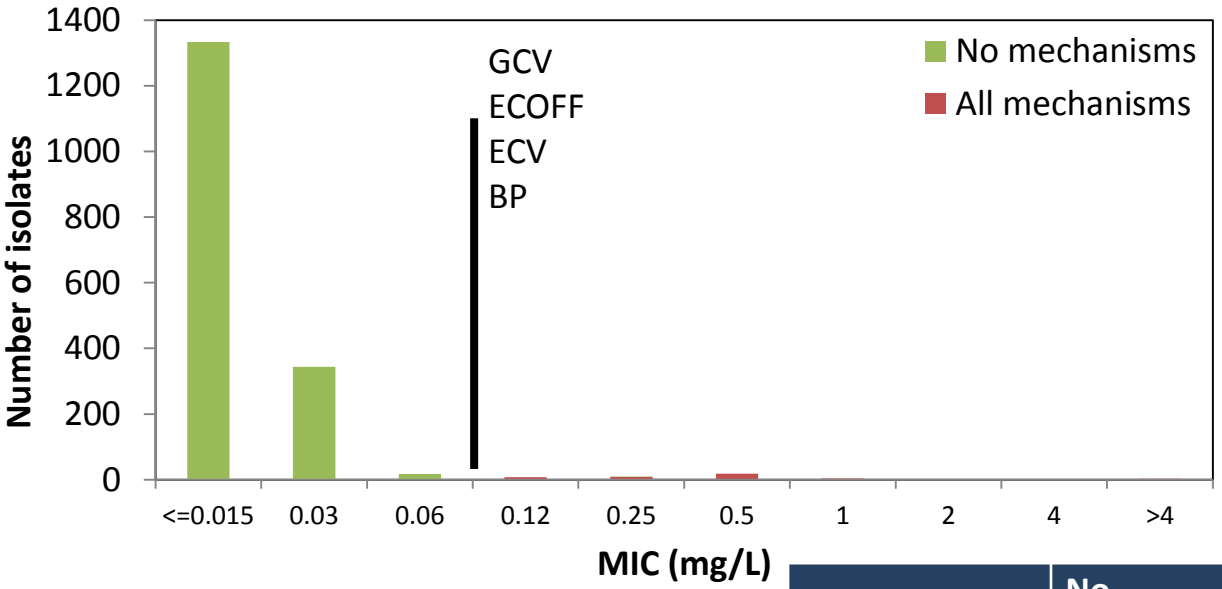








Salmonella Ciprofloxacin MICs by Mechanism



MIC (mg/L)	No mechanisms	*qnr* genes	One *gyrA* mutation	Two *gyrA* mutations
≤ 0.015	1333			
0.03	344			
0.06	17			
0.12		1	7	
0.25	1	4	5	
0.5		17	1	
1		4		
2		1		
4				
> 4				3

Summary of GCVs for *Salmonella*

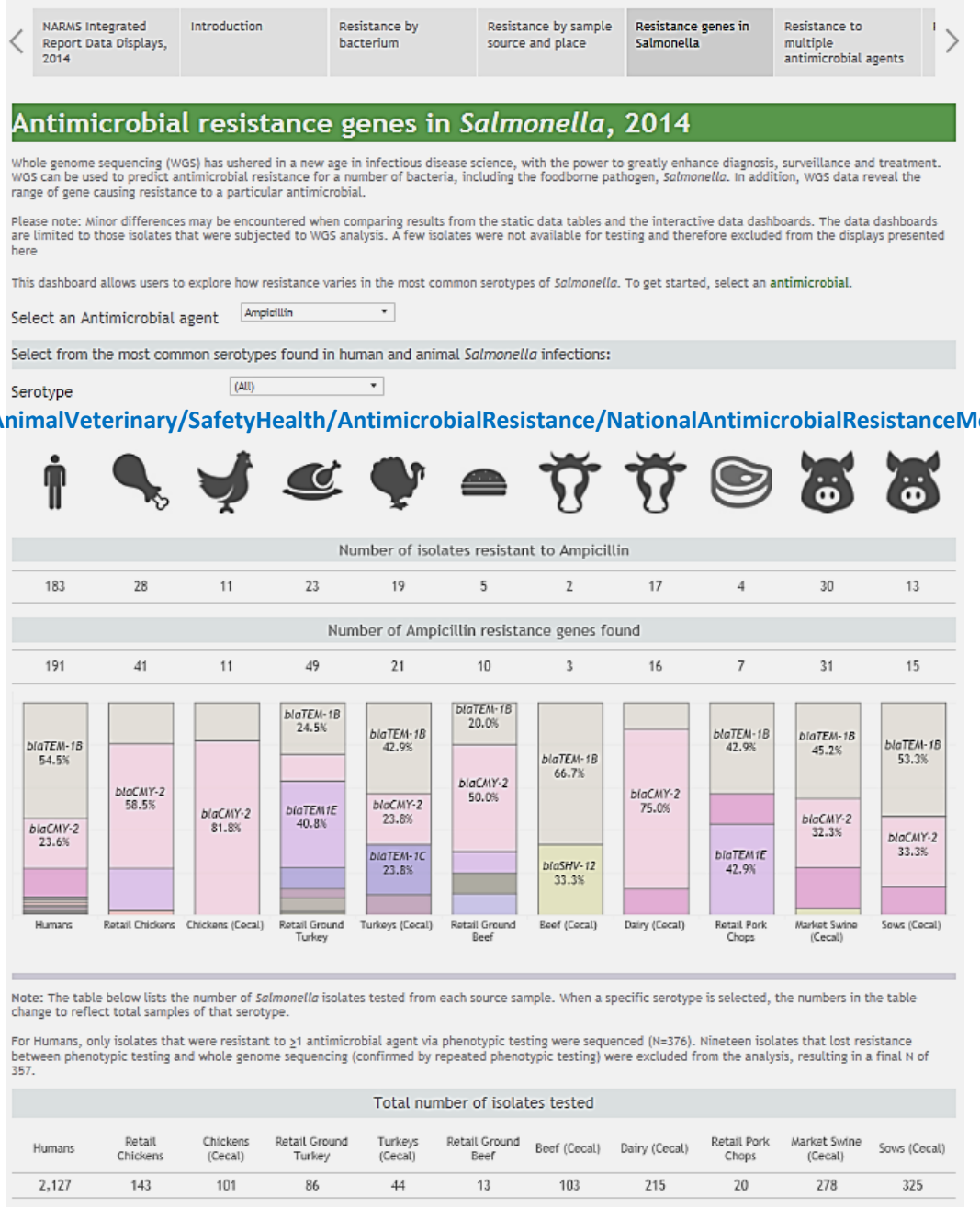
Drug	CLSI susceptible (S): treatment success likely	EUCAST ECV: wild-type (WT)	GCV: no resistance mechanism*
Ampicillin	≤ 8	≤ 4	≤ 8
Amoxicillin-clavulanate	≤ 8	None	≤ 2
Cefoxitin	≤ 8	≤ 8	≤ 8
Ceftriaxone	≤ 1	None	≤ 1
Ceftiofur	≤ 2	≤ 2	≤ 2
Gentamicin	≤ 4	≤ 1	≤ 2
Tetracycline	≤ 4	≤ 4	≤ 4
Chloramphenicol	≤ 8	≤ 16	≤ 16
Ciprofloxacin	≤ 0.06	≤ 0.06	≤ 0.06
Nalidixic acid	≤ 16	≤ 16	≤ 8
Azithromycin	None	None	≤ 16
Sulfisoxazole	≤ 256	None	≤ 256
Trimethoprim-sulfamethoxazole	≤ 1	≤ 1	≤ 0.5

* 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



NARMS Integrated Report Data Displays, 2014 | Introduction | Resistance by bacterium | Resistance by sample source and place | Resistance genes in Salmonella | Resistance to multiple antimicrobial agents

Antimicrobial resistance genes in *Salmonella*, 2014

Whole genome sequencing (WGS) has ushered in 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:

Serotype: (All)

http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm416741.htm

Number of isolates resistant to Ampicillin

Humans	Retail Chickens	Chickens (Cecal)	Retail Ground Turkey	Turkeys (Cecal)	Retail Ground Beef	Beef (Cecal)	Dairy (Cecal)	Retail Pork Chops	Market Swine (Cecal)	Sows (Cecal)
183	28	11	23	19	5	2	17	4	30	13

Number of Ampicillin resistance genes found

Humans	Retail Chickens	Chickens (Cecal)	Retail Ground Turkey	Turkeys (Cecal)	Retail Ground Beef	Beef (Cecal)	Dairy (Cecal)	Retail Pork Chops	Market Swine (Cecal)	Sows (Cecal)
191	41	11	49	21	10	3	16	7	31	15

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 ≥1 antimicrobial agent via phenotypic testing were sequenced (N=376). Nineteen isolates that lost resistance between phenotypic testing and whole genome sequencing (confirmed by repeated phenotypic testing) were excluded from the analysis, resulting in a final N of 357.

Total number of isolates tested

Humans	Retail Chickens	Chickens (Cecal)	Retail Ground Turkey	Turkeys (Cecal)	Retail Ground Beef	Beef (Cecal)	Dairy (Cecal)	Retail Pork Chops	Market Swine (Cecal)	Sows (Cecal)
2,127	143	101	86	44	13	103	215	20	278	325

New WGS Resources

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

https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047

- 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

Pathogen	Antimicrobial	%NWT	%R
Salmonella	Streptomycin	14.6	-
	Gentamicin	18.3	6.0
	Ampicillin	7.5	2.6