



**Summary Minutes
Subcommittee on Veterinary Antimicrobial Susceptibility Testing
Buena Vista Palace
Lake Buena Vista, Florida
6-7 January 2011**

A meeting of the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) was held on 6-7 January 2011 in Lake Buena Vista, Florida. The following were in attendance:

**Jeffrey L. Watts, PhD, RM (NCRM)
Chairholder**

Pfizer Animal Health

Mark G. Papich, DVM, MS

North Carolina State University

Members Present

Donald Bade

Steven D. Brown, PhD

Viginia R. Fajt, DVM, PhD, DACVCP

Thomas R. Fritsche, MD, PhD

Henry S. Heine, PhD

Robert P. Hunter, MS, PhD

Stefan Schwarz, DVM

Peter Silley, PhD

Ching Ching Wu, DVM, PhD

Gary E. Zurenko, MS

Microbial Research, Inc.

The Clinical Microbiology Institute

Texas A & M University

Marshfield Clinic

Ordway Research Institute, Inc.

Elanco Animal Health

Friedrich-Loeffler-Institute (FLI)

MB Consult Limited

Purdue University School of Veterinary
Medicine

Micromyx, LLC

Advisors Present

Cindy Lindeman, BS

Jennifer Lorbach, BS, MBA

Marilyn N. Martinez, PhD

Thomas R. Shryock, PhD

Shabbir Simjee, PhD

Clyde Thornsberry, PhD

John Turnidge, MD

Pfizer Animal Health

Trek Diagnostic Systems

FDA Center for Veterinary Medicine

Elanco Animal Health

Elanco Animal Health

Eurofins Medinet

Women's and Children's Hospital

Reviewers Present

Tara Bidgood, DVM, PhD, DACVCP

Timothy S. Frana, DVM, MPH, PhD

Charles Giesecker, MS

Daniel J. Keil, DVM, PhD, DACVM

Pfizer Animal Health

Iowa State University

FDA Center for Veterinary Medicine

Bayer Healthcare – Animal Health

Scott B. Killian
Cynthia C. Knapp, MS
Maureen Mansfield
Lori T. Moon, MT(ASCP)

Markus Rose, DVM, PhD.
Maria M. Traczewski, BS, MT(ASCP)
Cornelia Wilhelm

Trek Diagnostaic Systems
Trek Diagnostic Systems
Trek Diagnostic Systems
MSU Diagnostic Ctr. for Population & Animal
Health
Intervet Innovation GmbH
The Clinical Microbiology Institute
Intervet Innovation GmbH

Guests Present

Tina Crosby
Maureen K. Davidson, PhD
Anno De Jong
Luca Guardabassi, DVM, PhD
Brian Lubbers
Ian Morrissey
Chris Pillar
Michael T. Sweeney
Pierre-louis Toutain

FDA Center for Veterinary Medicine
FDA Center for Veterinary Medicine
Bayer Animal Health GmbH
University of Copenhagen
Kansas State Veterinary Diagnostic Laboratory
Quotient Bioresearch Ltd.
Eurofins Medinet
Pfizer Animal Health
Inra and National Veterinary School-Toulouse

CLSI Staff Present

Tracy Dooley, BS, MT(ASCP)
Marcy Hackenbrack, MCM, M(ASCP), BA
Clair A. Evans

Opening Remarks

Dr. Watts began the meeting Thursday, 6 January at 8:30 a.m. He stated that the purpose of Thursday's session was to provide an opportunity for the working groups to address their agenda topics and obtain input from the subcommittee. Sponsor presentations and final working group reports would be presented to the full subcommittee during Friday's session.

Minutes of Prior Meeting

The minutes of the 16-17 June 2010 meeting held in Atlanta had been approved by electronic comment and vote by the subcommittee prior to the meeting. The final version was included in the meeting materials and will be posted to the CLSI website on a page specific for the VAST subcommittee that is being created at this time.

AST Liaison Report

Dr. Heine gave a brief overview of two main items currently being reviewed by the AST Subcommittee:

1. Cephalosporin breakpoint changes – this does not affect the vet committee
2. Plans to change salmonella breakpoints for the fluoroquinolones – not sure if this will affect the vet group and he will keep the committee informed of the changes made.

CLSI Update

Ms. Dooley provided a brief overview of the new CLSI streamlined document development process to improve the timeliness and quality of new and revised CLSI standards and guidelines.

Additional information is available on the CLSI website and free informational webinars are being planned that will explain the changes in greater detail.

Recently Published

M45-A2, *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria* – August 2010

M53-P, *Laboratory Testing and Diagnosis of HIV Infections* - October 2010

M100-S21, *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement* – January 2011

Upcoming Publications

M24-A to A2, *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes* – estimated for publication March 2011

X08-R, *Generation, Presentation and Application of AST Data for Bacteria of Animal Origin; A Report* – estimated for publication June 2011

Working Group Reports

Generic Working Group

Working Group Participants – Co-Chairholders Mark Papich and Ching Ching Wu; Members – Shabbir Simjee, Cindy Lindeman, Bruce Craig, John Turnidge, Stefan Schwarz, Marilyn Martinez, Tara Bidgood.

1) Penicillin G Update

Dr. Papich gave an overview of the data for penicillin G that the working group is currently reviewing for cattle, equine, and swine. The current breakpoint in Table 2B is 0.12 and based on the data it looks like a veterinary-specific breakpoint can also be set at 0.12. The organisms that breakpoints can mostly be set for include *Mannheimia*, *Histophilus*, *Pasteurella*, Streps, and gram negatives (eg, *E.coli*).

The working group will look at PK data from previous research as well as published data and conduct PK/PD analysis and bring this information back in June. **The working group requests anyone with data, especially for swine to send this to Mark, as well as Ms. Lindeman and Dr. Wu.**

2) Revisiting Interpretive Criteria for Enrofloxacin in Pigs

Dr. Papich provided an informational review of the interpretive criteria previously set by the subcommittee in 2009 for enrofloxacin in pigs (≤ 0.25 , 0.5, ≥ 1 for gram negatives; ≤ 0.5 , 1, ≥ 2 for *S. suis*) and a study he conducted at the request of the drug sponsor since other published studies have used different routes of administration (eg, IM, IV or oral), rather than that approved in the U.S. as well as different dose than that approved in the U.S.

The study conducted was to show the distribution of enrofloxacin using an *in-vivo* ultrafiltration sampling technique after injection of enrofloxacin to pigs. The objectives of the study include:

- Determine plasma and interstitial fluid drug concentrations and pharmacokinetic parameters;
- Determine the influence of drug properties (protein binding and lipophilicity) on tissue distribution;
- and

- To evaluate an *in vivo* ultrafiltration device as an alternative to current models used to measure drug concentrations at target sites.

With enrofloxacin injected at the approved dose - Pigs: 7.5 mg/kg

- Determine plasma drug concentrations and pharmacokinetic parameters in pigs for enrofloxacin and its active metabolite ciprofloxacin (if present).
- Determine *in vitro* plasma protein binding for enrofloxacin and ciprofloxacin.

The study conclusions showed:

- The three tissues measured represented clinically-relevant sites;
- The unbound antimicrobial tissue concentrations can be used to predict efficacy and reduction of resistance based on PK-PD principles;
- The metabolite ciprofloxacin contributes substantially to the total drug concentration after administration of enrofloxacin to cattle, but not in pigs;
- Unbound concentrations of enrofloxacin/ciprofloxacin in tissues can be shown to be above C_{MAX}/MIC or AUC/MIC needed for MIC values of susceptible bacteria in pigs and cattle;
- Protein binding is in the moderate range for enrofloxacin and ciprofloxacin in calves, and enrofloxacin in pigs;
- Protein binding did not impair drug diffusion to tissues; and
- Drug concentrations in tissues exceeded the levels predicted by the unbound plasma concentrations alone.

3) A Proposal of Clinical Breakpoints for Cefoperazone for Bovine Mastitis Pathogens

Dr. Schwarz provided an overview of a study conducted to assess cefoperazone susceptibility/resistance among bovine mastitis pathogens to see if interpretive criteria from CLSI document M100 could be applicable to bovine mastitis pathogens.

Testing was done using a comparative analysis of MICs (broth microdilution) and zone diameters of bovine mastitis pathogens following recommendations in M31-A3 using:

- 75 µg disks + MIC range (0.06 – 32 µg/mL)
- QC strains: *E. coli* ATCC®25922, *S. aureus* ATCC®25923, *S. aureus* ATCC®29213
- Bacteria tested (independent clinical isolates from the last 3 years):
 - *S. aureus*: 114 (Germany) + 74 (USA) = 188
 - CoNS: 121 (Germany) + 75 (USA) = 196
 - *E. coli*: 103 (Germany) + 74 (USA) = 177
 - *S. agalactiae*: 101 (Germany) + 73 (USA) = 174
 - *S. dysgalactiae*: 102 (Germany) + 74 (USA) = 176
 - *S. uberis*: 100 (Germany) + 75 (USA) = 175

Based on the results he proposed the following breakpoints:

Antimicrobial Agent	Disk Content	Zone Diameter (mm)			MIC Breakpoint (µg/mL)			Comments
		S	I	R	S	I	R	

Cefoperazone

<i>Escherichia coli</i>	75 µg	≥ 27	22-26	≤ 21	≤ 2	4	≥ 8	Cefoperazone breakpoints were determined from an examination of MIC distribution of isolates and pharmacological data of cefoperazone. The dosage regimen used was 100 mg/affected quarter administered twice in 24 h intervals.
<i>S. aureus</i> CoNS	75 µg	≥ 27	22-26	≤ 21	≤ 2	4	≥ 8	
<i>S. agalactiae</i> <i>S. dysgalactiae</i> <i>S. uberis</i>	75 µg	≥ 21	16-20	≤ 15	≤ 2	4	≥ 8	

Based on the data reviewed the subcommittee recommended that Dr. Schwarz come back in June to try to establish epidemiological cutoff values for disk and MIC.

Editorial Working Group

Working Group Participants – Chairholder Gary Zurenko; Members – Jo Abraham, Steve Yan, Jeff Watts, Mark Papich, Henry Heine, Stefan Schwarz, Maria Traczewski, Ching Ching Wu.

The subcommittee reviewed the current drafts of the M31 text and tables and agreed to the additional changes listed below:

M31 text:

- Review text in the 4th paragraph of the Foreword regarding the M45-like document to be developed and modify if necessary.
- Added text to last paragraph of the Scope and first paragraph of Section 5.6.1 referring readers to the M42-M49 supplement where aquatic animal-specific interpretive criteria can be found.
- Definitions Section
 - Deleted metaphylaxis from definition Control as follows: Control/~~metaphylaxis~~
 - Modified the definition for growth promotion.
- Section 5
 - Deleted the reference to FDA/CVM and replaced with regulatory agency.
 - Modified text in 5th paragraph defining extra label use.
- Added new Section 6.6 on disk diffusion testing of *Campylobacter jejuni*.
- Mr. Zuenko along with Drs. Papich, Schwarz, and Heine will work on edits to Sections 6.8 and 12 for Staphylococci. The revised text will then be incorporated into M31 and circulated to the committee for review.
- Added new Sections 9.2 and 11.3 on agar dilution and broth microdilution testing of *Campylobacter jejuni*.

- Section 11.2 – Since Supplement C is so hard to obtain, Mr. Bade, Ms. Lindeman, and Ms. Traczewski will re-look at the Supplement C data and see if an alternate can be used (eg, IsoVitaleX).
- Dr. Heine will check with the M45 Working Group to see if LHB can be used for *P. multocida* isolates that fail to grow on CAMHB.

M31 tables:

- Table 1
 - Delete imipenem from Group D under Horses
 - Add nitrofurantoin (dogs only) in Group D for Dogs and Cats
 - Dr. Papich will get together a list of commonly used drugs used to add under Dogs and Cats in Group D. Some of the drugs mentioned that Dr. Papich will verify include: imipenem, rifampin, ticarcillin, and ticarcillin-clavulanate.
- Table 2A
 - Under β -lactam/ β -lactamase Inhibitor Combinations change coagulase-positive staphylococci to *Staphylococcus* spp.
 - Edit list of organisms for Cephalothin for Dogs (skin and soft tissue) as per January 2009 minutes
- Table 2B
 - Add nitrofurantoin for UTI only

All changes will be incorporated into the documents and circulated to the Editorial Working Group and the Subcommittee to review in preparation to finalize the documents and submit them for vote by April 2011.

Veterinary Mycoplasma Working Group

Working Group Participants –Chairholder Ching Ching Wu; Members – Joann Kinyon, Cecile Bebear, Mary Brown, Don Bade, Lynn Duffy, Roger Ayling, Ken Waites

Dr. Wu gave an overview of a preliminary study conducted to determine the MIC of florfenicol against 50 clinical isolates of *M. bovis*. Testing was conducted:

- using frozen MIC plates
- three labs (PU, CMI, CO)
- each lab tested 51 strains in triplicate
- one replicate in each of the three lots of media

Four sources of media were evaluated (Accumedia, BD, Remel, Difco PPLO), using the 2 methods presented at the last meeting: HBAN (Hayflick's medium with alamar blue)–detects oxidation reaction in the electron transport chain during respiration and the standard method using phenol red.

Preliminary testing showed that the choice of medium and growth indicator will be a critical component of developing the AST methods for *Mycoplasma* spp. The next steps of the working group will be to:

- determine medium to be used for the organisms to be tested
- determine the concentration ranges of each antimicrobial agents
- determine when and how the test should be read
- identify QC strains and establish QC ranges

Dr. Wu will provide further updates of the working groups progress at the next meeting of the subcommittee.

International Harmonization Working Group

Working Group Participants – Chairholder Tom Shryock; Members – Peter Silley, Bob Walker, Stefan Schwarz, Jeff Watts, Ruby Singh, Bernd Stephan.

1). Dr. Shryock discussed the proposal previously reviewed by the subcommittee for developing a new guideline that would address additional pathogens and antibiotics not currently addressed in M31. This document would be similar to the M45 document - *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently-Isolated or Fastidious Bacteria* developed by the human AST subcommittee.

The proposal was sent to the Consensus Committee on Microbiology (previously designated as the Area Committee on Microbiology) for approval and some concerns were raised with regards to the limited data that may be available on infrequently recovered veterinary isolates. In an effort to try and move forward with this proposal, the consensus committee recommended that since this project will require additional time to develop beyond the current CLSI timeline (22 months), to form a small informal working group that will work under the guidance of the VAST Subcommittee. The working group would then begin to initiate work for the document (literature searches, gathering data, etc) and plan to meet when VAST meets as well as conduct conference calls in the interim to get the work done and possibly have an initial draft. The proposal for the document can then be submitted for approval of the CLSI Chairholders Council. Once approved, this then allows 4-6 months to finalize the document and initiate the voting process.

Dr. Shryock then outlined a proposed path forward:

1. Appoint a Working Group
2. Determine the pathogens to address and determine the appropriate drugs (a proposed list of organisms includes: *Brachyspira* spp., *Clostridium perfringens*, *H. somni*, *S. suis*, *B. bronchiseptica*, *H. parasuis*, *Paenibacillus larvae* [Foul brood], *Rhodococcus equi*, and *Fusobacterium necrophorum*)
3. Assign categories for reprint or data collection and evaluation
4. Compile in M45 format
 - derive scattergram or MIC histogram
 - provide supporting data as available
 - propose ECV or breakpoint(s)

The subcommittee agreed to form a working group to be lead by Ms. Tracezewski. Working Group members include Mr. Bade, Dr. Fritsche, and Mr. Sweeny. The working group will initiate gathering information on the organisms to be addressed and provide an update in June on whether there is sufficient data to proceed.

2). Dr. Schwarz presented information on a project that is parallel to the veterinary M45-like document. The Antibiotic Resistance Working Group of the German Veterinary Medical Society (DVG) was formed to develop antimicrobial susceptibility testing procedures for veterinary pathogens focusing on:

- *Haemophilus parasuis* (swine),
- *Bordetella bronchiseptica* (swine, dog),
- *Arcanobacterium pyogenes* (cattle, swine),
- *Rhodococcus equi* (horse),
- *Ornithobacterium rhinotracheale* (poultry)
- *Riemerella anatipestifer* (poultry)

The working group will begin trying to determine suitable medium and test conditions. For QC, strains from CLSI documents M31-A3 and M100-S20 will be tested for their MICs using the test media and test conditions agreed upon. Testing will then be conducted using 50-100 recent isolates (last 5 years) of the respective pathogen group. Also, ring trials will be conducted to find out whether routine diagnostic laboratories are able to adopt the new AST methodology.

The goal of the working group is to obtain agreement with VAST-CLSI on how to do the project so that they know the conditions for an eventual approval of the AST procedures (before the project starts). Dr. Schwarz will present the results at upcoming VAST-CLSI meetings as soon as results are available for a specific pathogen group.

Education Working Group

Working Group Participants – Chairholder Virginia Fajt; Members – Mike Apley, Bob Badel, Jennifer Lorbach, Tom Shryock, Ching Ching Wu.

Dr. Fajt provided an overview of the efforts of the working group. The 2 articles drafted to be sent to well read journals (eg, JVDI) are completed and will be sent to editors as discussed previously. The articles are:

Article 1 – “Recommendations for Researchers” detailing the use of CLSI Veterinary standards.

Article 2 – “Guidelines for Clinical Use” detailing how to use and implement the VAST documents.

M37 Revision Working Group

Working Group Participants – Chairholder Marilyn Martinez; Members - Josh Hayes, Rob Hunter, Cindy Lindeman, Mark Papich, Peter Silley, Shabbir Simjee, Steve Yan.

Dr. Martinez discussed the aspects of the existing M37 document and the needed revisions and clarifications. As discussed previously, the three-pronged approach has demonstrated weakness and the working group wants to improve clarity regarding how CO_{CL} is established and describe conditions under which setting a CO_{CL} estimate is not feasible.

In an effort to try and define criteria to be met in order to have a valid estimation of the CO_{CL} , the working group proposed that if the available data do not meet the set criteria, then a CO_{CL} cannot be determined. In this situation, “S” will be based solely on CO_{PD} and CO_{WT} (i.e., based upon the same criteria that we currently use for in the “generics working group”).

Criteria for establishing a CO_{CL} :

- The isolates should be derived from pre-treatment cultures
- The MICs of the isolated should be linked to the clinical outcomes of the individual animals.
- Currently, within the FDA, individual animal information is available for most companion animal antimicrobial products. The following table put together by Dr. Hayes at CVM/FDA is the information they see for food animals:

	Pretreatment Culture?
Cattle	
Respiratory Disease	Yes
Pink Eye	Yes
Mastitis	Yes
Foot Rot	Yes
Swine	
Respiratory Disease	No (generally)
PPE	No
Chickens	
Necrotic Enteritis	No

In post-treatment cultures, the remaining pathogens may not have been susceptible to the drug. Therefore, the resulting MIC values may reflect pathogens that failed to be killed at the labeled dose. Because of this post-treatment cultures should not be used to set CO_{CL} but the information may be helpful when setting “S”.

Dr. Martinez then provided some discussion points for criteria for establishing a CO_{CL} for pretreatment cultures:

- For any given pathogen, we need to have at least 30 isolates from the treated group in order to differentiate between clinical failures and successes with the proposed drug/dosage regimen.
- To be used to set CO_{CL}, one of the following conditions must be met:
 - At the proposed CO_{CL}, less than 10% of those isolates were associated with therapeutic failure.
 - At the proposed CO_{CL}, there is a definable difference in the number of clinical successes versus failures (statistics?).
- If all of the treated animals were deemed to be clinical successes, then the CO_{CL} will be defined as the MIC associated with the highest MIC value observed in 4 or more clinical cases in the treated group.
- If there were therapeutic failures and if the MIC of the failures could not be distinguished from the clinical successes, then a CO_{CL} cannot be defined and “S” will be based solely upon CO_{PD} and CO_{WT}.

Path Forward – Some additional points mentioned below as well as the points discussed for establishing CO_{CL} will be worked on by a small group to include Drs. Martinez, Turnidge, Papich Watts, and Toutain and bring back to the committee.

- Methods for establishing CO_{PD} have been described in M37 – review to see if the general discussion is adequate.
- Possibly expand the appendix to include a more detailed discussion of drugs whose tissue concentrations differ from blood (e.g., macrolides, tetracyclines and pleuromutilins)?
- What “benchmark” PK-PD targets should be used when such studies are not available?

Aquaculture Working Group

Mr. Giesecker provided an overview of the current roster changes and activities of the Aquaculture Working Group. The original Chairholders of the working group, Drs. Reimschuessel and Hawke, who oversaw the development and publication of two approved level guidelines for performing susceptibility testing on bacteria isolated from aquatic animals: *Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated From Aquatic Animals; Approved Guideline (M42-A)* and *Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated From Aquatic Animals; Approved Guideline (M49-A)* as well as a recently published supplement – M42/M49-S1 that includes updated tables with clinical breakpoints for *Aeromonas salmonicida* and epidemiological cutoff values for *Aeromonas salmonicida* have recently

stepped down to now serve as members. Dr. Ron Miller from CVM/FDA has assumed the role as Chairholder.

Current efforts of the working group are focused on trying to develop standardized susceptibility testing methods for some of the more fastidious bacterial pathogens of fish, as well as address clinical breakpoints and epidemiological cutoff values for other important bacterial pathogens of fish.

He then provided an overview of a study conducted to determine MIC QC ranges in broth for tests of Flavobacteria. This study was initiated due to recent FDA/CVM approvals of proprietary formulations of Florfenicol and Oxytetracycline to control infections in fish caused by *Flavobacterium columnare* and *F. psychrophilum* and a need for standardized antimicrobial susceptibility testing methods.

The proposed QC ranges for *Escherichia coli* ATCC® 25922 and *Aeromonas salmonicida* subsp. *salmonicida* ATCC® 33658 were approved by the VAST Subcommittee as follows (**Approved 10-0**):

Antimicrobial Agent	MIC (µg/mL)	
	18 °C/ 92-96 hrs	28 °C/ 44-48 hours
Ampicillin	0.06 – 0.25	0.06 – 0.25
Enrofloxacin	0.004 – 0.03	0.004 – 0.015
Erythromycin	4 – 16	4 – 16
Florfenicol	0.25 – 1	0.25 – 1
Flumequine	0.015 – 0.06	0.015 – 0.06
Ormetoprim/sulfadimethoxine	0.03/0.59 – 0.25/4.75	0.06/1.19 – 0.25/4.75
Oxolinic Acid	0.008 – 0.03	0.008 – 0.03
Oxytetracycline	0.06 – 0.25	0.06 – 0.25
Trimethoprim/sulfamethoxazole	0.015/0.3 – 0.06/1.19	0.03/0.59 – 0.12/2.38

***Escherichia coli*, ATCC® 25922**

Antimicrobial Agent	MIC (µg/mL)	
	18 °C/ 92-96 hrs	28 °C/ 44-48 hours
Ampicillin	2 – 8	1 – 4
Enrofloxacin	–	0.002 – 0.015
Erythromycin	4 – 16	16 – 64
Florfenicol	4 – 32	2 – 8
Flumequine	0.06 – 0.25	0.06 – 0.5
Ormetoprim/sulfadimethoxine	No Range Approved	0.12/2.38 – 0.5/9.5
Oxolinic Acid	0.03 – 0.12	0.03 – 0.12
Oxytetracycline	0.12 - 1	0.12 – 1
Trimethoprim/sulfamethoxazole	0.015/0.3 – 0.12/2.38	0.015/0.3 – 0.12/2.38

Presentations

Nitrofurantoin Susceptibility in Small Animal Urinary Tract Pathogens

Dr. Guardabassi presented information on a study conducted to evaluate the *in vitro* antimicrobial activity of nitrofurantoin against *E. coli* and *S. pseudintermedius* isolated from dogs and cats. Currently there are no veterinary specific breakpoints or MIC distribution data for treating UTI infections in small animals. In conducting the study, they wanted to see if nitrofurantoin was active against multidrug-resistant bacteria that are emerging in small animals (eg, ESBL’s and MRSP) and what concentrations should be achieved in dog urine to kill the pathogen and to prevent selection of resistant mutants?

Testing was conducted using:

- 269 bacterial isolates (88% isolated between 2005 and 2010)
 - 106 *E. coli* and 163 *S. pseudintermedius*
 - 240 canine and 29 feline
- MDR bacteria of known genetic background
 - 13 MRSP belonging to 7 sequence types
 - 5 ESBL-producing *E. coli* (CTX-M-1 and SHV-12)
 - 9 CMY-producing *E. coli*
- Methods
 - MIC testing by agar dilution according to CLSI (M31-A3)
 - Range 2-128 ug/ml
 - Dimethylformamide as solvent
 - MPC testing according to Blondeau 2009
 - Heavy inoculum density ($\geq 10^{10}$ CFU/ml)
 - Large inoculum size (100 μ l)
 - MIC of presumptive mutants was tested according to CLSI
 - Time-killing curves in 2 strains for each species
 - Initial bacterial concentration 5×10^5 CFU/ml
 - Drug concentrations corresponding to MIC and MPC of the strain
 - Bacterial counts at 5 time points (0, 1, 3, 5 and 24 h)

The results from the study showed that all strains were killed at concentrations achievable in dog urine ($\leq 32 \mu\text{g/mL}$) and the drug has potential in small animal medicine with multi-resistant strains as an alternative to carbapenems.

Dr. Guardabassi's lab is willing to help generate the necessary data to determine the clinical breakpoint for treatment of small animals (dogs and cats) and what the optimal dosage should be. He will provide further updates at a later time.

He then requested that **nitrofurantoin be added to Table 1, Group D in M31 as well as Table 2B. Approved 10-0.**

He also asked if the current solvent listed in Table 8 for nitrofurantoin (phosphate buffer pH 8) is appropriate. The subcommittee recommended that this remain as is until further testing is conducted. To change the recommended solvent a comparison study would need to be conducted with the appropriate QC organisms.

In Vitro Antimicrobial Activity of Cephalexin Against *S. pseudintermedius* and *E. coli* Isolated from Small Animals

Dr. Guardabassi presented information on a study conducted to determine if there may be diagnostic implications in light of the new CLSI clinical breakpoint ($R > 8 \mu\text{g/mL}$) for cephalothin (traditionally been used to predict susceptibility to first generation cephalosporins) and the potential risk that ~20% of *S. pseudintermedius* isolates would be erroneously categorized as resistant by the agar dilution method.

The study was conducted using:

- The agar dilution method according to CLSI M31-A3 (range 0.5-128 ug/ml)
- Mueller-Hinton agar plates stored at 4°C (max 3 days)
- 225 strains from 3 countries (2001-2010)
 - 107 *S. pseudintermedius*
 - 118 *E. coli*

- Resistant strains with characterized background
 - 8 MRSP
 - 18 CMY/ESBL-producers

Study results showed the MIC distribution is clearly bimodal and reflects the presence/absence of *mecA* as well as a two-fold shift to higher MICs was observed for both species in comparison with the study by Stegmann et al.

In discussing the need for a method-specific cephalixin breakpoint, Dr. Papich offered to try and pull the necessary data. The subcommittee also discussed possibly pulling the first generation cephalosporins in the next edition of M31. Dr. Turnidge agreed to bring a proposal to the subcommittee to pull these out of Table 2A.

The subcommittee did agree to **add oxacillin to Table 1, Group D as well as to add comment h regarding the results of oxacillin susceptibility tests used to predict susceptibility to cloxacillin – oxacillin-resistant staph should be reported as resistant to all β -lactams from Table 1 to Table 2A (Approved 10-0).**

Examination of Amoxicillin Breakpoints in Swine

Dr. Toutain discussed the lack of veterinary specific breakpoints for amoxicillin in pigs and the work being done to try and possibly set clinical breakpoints. Data was published by Schwarz et al in Veterinary Microbiology in 2008 to attempt to deduce a clinical breakpoint for amoxicillin applicable to porcine respiratory tract pathogens based on known data of the pharmacokinetics of amoxicillin in swine, results of clinical efficacy studies, and available data on the in vitro susceptibility of pathogens causing porcine respiratory tract infections, such as *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Streptococcus suis*, *Bordetella bronchiseptica*, and *Haemophilus parasuis*. Based on all pharmacological, clinical and microbiological parameters, the suggested clinical breakpoints for amoxicillin to be used for the classification of bacterial pathogens involved in porcine respiratory tract infections: 0.5 $\mu\text{g}/\text{mL}$ for “susceptible”, 1.0 $\mu\text{g}/\text{mL}$ for “intermediate”, and 2 $\mu\text{g}/\text{mL}$ for “resistant”.

He also discussed further work done at Toulouse including population PK in pigs, Monte Carlo simulations, and selection of possible PK/PD breakpoints. Results showed that the investigated PK/PD breakpoint are:

- For oral route (20 mg/kg) : 0.25 $\mu\text{g}/\text{mL}$
- For IM route (30mg/kg) : 0.125 $\mu\text{g}/\text{mL}$

QC Ranges for Disk Diffusion Testing of Gamithromycin for the Treatment of Bovine Respiratory Disease

Dr. Pillar presented Tier 2 quality control study data for disk diffusion testing of gamithromycin (15 μg) against *S. aureus* ATC[®] 25923, *S. pneumoniae* ATCC[®] 49619, and *M. haemolytica* ATCC[®] 33396. Based on the data presented, the following QC ranges were proposed:

Organism	Proposed QC range (mm)	Vote
<i>S. aureus</i> ATCC® 25923	19-26	Approved 10-0
<i>S. pneumoniae</i> ATCC® 49619	20-26	Approved 10-0
<i>M. haemolytica</i> ATCC® 33396	18-26*	Approved 10-0

* This gets added to Footnote c in Table 4 and in Section 6.4.1.

Plans for Next Meeting

The next meeting of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing will be scheduled as a two-day meeting on Wednesday, 15 June and Thursday, 16 June 2011 in Boston, Massachusetts.

The submission deadline for the June meeting will be **Wednesday, 4 May 2011**. Materials for the June meeting will be distributed to the subcommittee on a CD prior to the meeting. The meeting rooms will be equipped with power strips for those who prefer to view the material on their computer instead of printing the material.

Adjournment

Dr. Watts thanked the participants for their attendance and input. The meeting was adjourned at 3:05p.m.

Respectfully submitted,

Tracy Dooley, BS, MT (ASCP)
Standards Administrator

PLEASE NOTE: All slide presentations from the 2-day session can be found on the CLSI website on the Subcommittee for Veterinary Antimicrobial Susceptibility Testing webpage under Microbiology by clicking the link provided below:

[VAST January 2011 Meeting Presentations](#)