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Antimicrobial Susceptibility Testing

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In the past, the WVDL used the Kirby Bauer disc diffusion testing method for antimicrobial susceptibility testing. In January 2006, the WVDL switched to a broth dilution method that provides not only susceptibility information but also minimum inhibitory concentration (MIC) information that is not available with the Kirby Bauer method. In this document, we attempt to answer many of the frequently asked questions regarding antimicrobial susceptibility testing. Of particular importance is the concept of efficacy ratios. Efficacy ratios can be used to calculate which antimicrobial drug has the highest predicted efficacy or activity against a given bacterial isolate. It is our hope that this document will be useful to our clients, the practicing veterinarians.

- **What is the Kirby Bauer disk diffusion test?** The Kirby Bauer test is a qualitative assay whereby discs of paper are impregnated with a single concentration of different antibiotics. The discs are placed on the surface of an agar plate that has been inoculated with test bacteria. During incubation, the antibiotics diffuse outward from the discs creating a concentration gradient. After 18-24 hours, the zone diameter (zone of inhibition) is measured and reference tables are used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antimicrobial drugs.

- **What is the broth dilution method?** The broth microdilution method is a liquid culture method whereby a standard amount of bacteria are inoculated into the wells of a 96 well micro-titer plate that contain different dilutions of antimicrobial drugs. For example in the standard bovine/porcine panel, five wells contain the antibiotic ceftiofur with dilutions of 8, 4, 2, 1, 0.5 and 0.25 ug/ml and four wells contain spectinomycin with dilutions of 64, 32, 16 and 8 ug/ml, respectively. After 18-24 hours, the plates are examined either visually or with an analytical instrument for evidence of bacterial growth. Results are recorded as minimum inhibitory concentrations (MIC).

- **What is the MIC?** The MIC is the highest dilution (lowest concentration) of antimicrobial drug that completely inhibits bacterial growth. The MIC value is reported with interpretation guidelines (S, I, R) that have been established by the Clinical and Laboratory Standards Institute (CLSI). Occasionally, no interpretation (NI) is reported with the MIC result because no interpretative guidelines have been established for the bacteria/drug combination being tested.

- **What are break-points?** Break-points are the bacteria/drug combination that correlates with the interpretation guidelines established by CLSI. For example, the break-points for florfenicol in cases of bovine pneumonia are ≤ 2 (S), 4 (I) and ≥ 8 (R) ug/ml when *Pasteurella multocida* is tested.

- **What are efficacy ratios?** Efficacy ratios are calculated by taking the resistant break-point MIC and dividing it by the MIC obtained by the broth dilution method. It is a tool that can be used to evaluate the relative efficacy of different antimicrobial drugs. For example, recently the WVDL obtained the following MIC results from an isolate of *Mannheimia haemolytica*.

Drug	**MIC ug/ml**	**Interpretation**	**Resistant Break-point MIC ug/ml**	**Efficacy Ratio**
Ceftiofur	0.5	S	≥ 8	16
Florfenicol	0.25	S	≥ 8	32
Oxytetracycline	0.5	S	≥ 16	32
Spectinomycin	16	S	≥ 128	8
Tilmicosin	8	S	≥ 32	4
Tulathromycin	4	S	≥ 64	16

In this case, the efficacy ratio for ceftiofur is 16. The efficacy ratio was calculated by taking the resistant break-point MIC for ceftiofur (8 ug/ml) and dividing it by the measured MIC of 0.5 ug/ml.

Therefore, the two antimicrobial drugs with the greatest predicted efficacy are florfenicol and oxytetracycline. It is important to point out that the final decision of which antimicrobial drug should be used lies with the practicing veterinarian.

The table below can be used for calculation of efficacy ratios.

Penicillin	*Staphylococci* *Enterococci* *Streptococci* *Listeria monocytogenes*	≥ 0.25 ≥ 16 ≥ 4 ≥ 4
Penicillin/novobiocin	Bovine mastitis	≥ 4
Pirlimycin	Bovine mastitis	≥ 4
Rifampin	Small animal/equine	≥ 4
Spectinomycin	Poultry: respiratory disease Porcine: colibacillosis Cattle: respiratory disease	≥ 128
Sulfonamides including Sulfadimethoxine, Sulphathiazole, Sulfachlorpyridazine		≥ 512
Tetracyclines (oxytetracylcine & chlortetracycline)	Non-streptococcal spp *Streptococci*	≥ 16 ≥ 8
Tiamulin	Porcine respiratory disease	≥ 32

Ticarcillin	Small animal/equine	≥ 128
Ticarcillin-clavulanic acid	Small animal/equine	≥ 128
Tilmicosin	Bovine respiratory disease Porcine respiratory disease	≥ 32
Trimethoprim/sulfamethoxazole	Urinary tract infection Other bacteria	≥ 2 ≥ 4
Tulathromycin	Bovine respiratory disease Porcine respiratory disease	≥ 64

*Data from CLSI (formally NCCLS) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard –Second Edition M31-A2 and Informational Supplement M31-S1.