



Understanding Antimicrobial Sensitivity Testing

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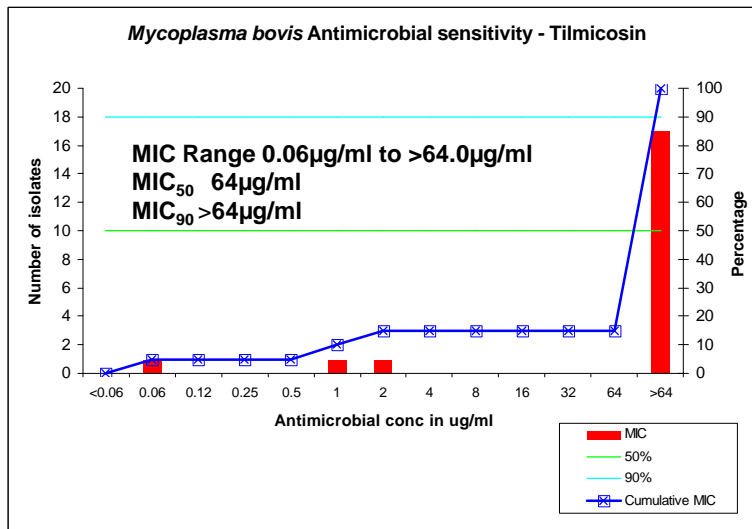
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Understanding Antimicrobial Sensitivity Testing

- Minimum inhibition concentrations (MIC)
- Mycoplasmacidal concentrations (MMC)
- Pharmacokinetics and Pharmacodynamics
- Breakpoints
- Antimicrobial Resistance

Minimum Inhibition Concentration (MIC)

- *In vitro* test – that measures the effectiveness of the antimicrobial at inhibiting growth



MIC Testing

- Broth Dilution, Agar Dilution, Inhibition Discs, E test.

Standardised Testing

- Antimicrobials, dissolved at known concentrations.
- Media that supports the growth of the organism being tested. No antimicrobials in media.
- Growing organism – at known concentration.
- Method of assessing End point - growth/inhibition.
- Controls

MIC Testing - Antimicrobials

- Purity of antimicrobial – the active ingredient.

STABILITY

- Does the diluent effect antimicrobial effectiveness?

Antimicrobial	Diluent
Chloramphenicol	alcohol and water
Erythromycin	alcohol and water
Sulphamethoxazole	0.1N NaOH and water
Trimethoprim	Lactic acid and water

- Quinolones and Chloramphenicol are light sensitive
- Incubation conditions – temperature, CO₂ - time
is the active antimicrobial ingredient breaking down?

MIC Testing - Media

- The selected media must support optimum growth of the organism being tested.
- It must not contain antimicrobials – as they may interact with the antimicrobial being tested (antagonistic or synergistic effect).
- Most media that supports mycoplasma growth contains serum, which may also affect results

MIC Testing - Organism

- The organism must be pure and its identification confirmed.
- Ideally the organism should be in the logarithmic phase of growth.
- It should be inoculated at between 10^3 and 10^5 colour changing units (ccu) or colony forming units (cfu).


MIC Testing – End Point

- Growth – colonies on agar, buttons of cells in microbroth dilutions, swirls of growth in broths.
- pH indicator – not useful for non-acid producers
- Other indicators – Alamar blue, tetrazolium.

MIC Testing – Controls

- Controls without antimicrobials to compare growth/pH/ indicator change.
- Known standards! (None for Veterinary *Mycoplasma species*.)

MIC Testing – Microbroth dilution

		Oxytetracycline	Spectinomycin	
Concentration in $\mu\text{g/ml}$				
128	0.5			
64	0.25			
32	0.125			
16	0.06			
8	0.03			Oxytetracycline MIC 0.03 $\mu\text{g/ml}$
4	0.015			
2	0.008			Spectinomycin MIC 8.00 $\mu\text{g/ml}$
1	0.0 Control			

Other MIC testing methods

Agar dilution – Similar to broth dilution, however antimicrobial concentration and organism added to molten agar and then poured.

Disc sensitivity – Measures zones of inhibition – does not give an MIC value – dependent on antimicrobial diffusion through agar.

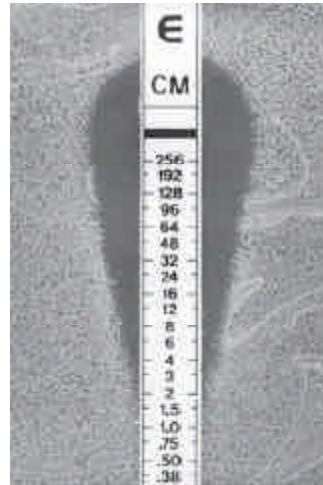
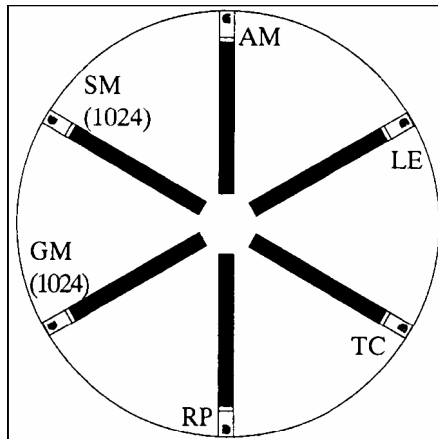
ATP - dependent luminometry.

Flow cytometry.

E test – strips with gradient of antimicrobial, used to measure inhibition of growth on agar plates.

Other MIC testing methods

E test – strips with gradient of antimicrobial, used to measure inhibition of growth on agar plates.



MycoplasmaSTATIC or MycoplasmaCIDAL???

At the MIC the antimicrobial is inhibiting growth
Mycoplasma static (not necessary killing the organism). The organism can grow again if the antimicrobials are removed. Allows immune system to catch up.

Mycoplasma static – macrolides, tetracyclines, lincosamides, chloramphenicols.

Mycoplasma cidal – kills the organism – does not require help from the immune system.

Mycoplasma cidal antimicrobials are useful where infections are at sites with reduced immune system contribution – endocarditis, meningitis.

Mycoplasma cidal – aminoglycosides (streptomycin), fluoroquinolones

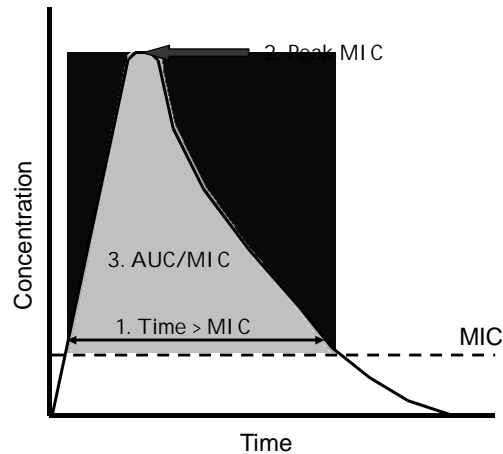
Pharmacodynamics and Pharmacokinetics

1. Time the antimicrobial remains above MIC concentration (time-dependent activity)

Concentration dependent activity or time-independent activity.

2. Ratio of peak concentration of the drug to the MIC

3. Ratio of the area under the concentration-time curve (AUC) to the MIC



Example: Aminoglycosides – concentration-dependent - given as large single dose

Pharmacodynamics and Pharmacokinetics

What happens to the antimicrobials in the animal?

- Inoculation - to site of action (circulating blood).
- Traverse biological membranes & fluid compartments to reach target / receptor site.
- Eliminated (circulating blood).
- Some drugs can't pass all membranes.
- Some drugs accumulate – a result of binding, dissolving in fat, or active transport mechanisms. (serum binding – important measure)
- Some drugs accumulate more in diseased organs (tilmicosin 3X more concentrate in diseased lungs than healthy lungs).
- Some drugs can enhance the immune system (macrolides enhancing phagocytosis).
- Some antimicrobials have anti-inflammatory effect and some now combined with anti-inflammatory drugs - Resflor
- Meat / Milk withdrawal times & COST.

Mycoplasma bovis



Calf pneumonia



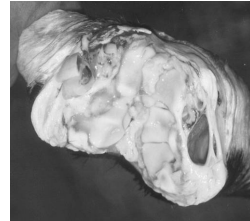
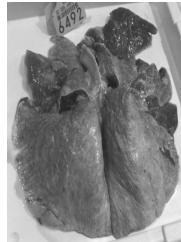
Arthritis



Mastitis



Meningitis/head tilt



Infectious keratoconjunctivitis

Breakpoints

A measure that relates *in vitro* MIC results to *in vivo* effect. i.e. the maximum threshold for predicting successful antimicrobial treatment.

For Veterinary Mycoplasma Infections breakpoints have not been determined.

- Therefore the interpretation of Veterinary Mycoplasma MIC results can not officially be defined into susceptible, intermediate or resistant.

Breakpoints - Calculations

Original calculation

$$\text{Breakpoint concentration} = \frac{C_{\max} f X s}{e t}$$

C_{\max} = maximum serum concentration (usually 1 h post dose)

e = factor by which the C_{\max} should exceed MIC (4)

f = factor to allow for protein binding

t = factor to allow for serum elimination – half life

s = reproducibility factor.

Now the following points are also considered:

- concentrations attained at specific sites,
- presence of microbiologically active metabolites
- interaction between parent compounds and their metabolites
- high tissue concentrations v low serum levels (macrolides)

Resistance is something that should also be considered

Breakpoints - Examples

	Susceptible	Resistant
<u>Oxytetracycline:</u>		
<i>Staphylococci & Streptococci</i>	= 1	= 2
<i>Enterobacteriaceae</i>	= 1	= 2
<u>Chloramphenicol</u>		
<i>Staphylococci & Streptococci</i>	= 2	= 4
<i>Enterobacteriaceae</i>	= 8	= 16
<u>Ciprofloxacin</u>		
<i>Enterobacteriaceae</i>	= 0.5	= 1
<i>E. coli, Proteus</i> (from urinary tract)	= 4	= 8
<i>N. gonorrhoeae</i>	= 0.03	= 0.06

Antimicrobials may not be effective

• ANTIMICROBIAL RESISTANCE

- But AR is not the only explanation
- Site (s) of the infection
- Mycoplasmastatic effect
- Animal infection too severe
- Organism protected by biofilm
- Individual animal treated, but infection spread to others, allowing re-infection.

Antimicrobial Resistance

• Antimicrobial resistance can be induced *in vitro* in a few steps by subculturing at sub-MIC levels.

• Resistance is often the result of single point mutations in a gene.

Fluoroquinolone: Mutation in *gyrA*, similar to that described for *E. coli*

		2		2																	
		5		6																	
	- - - - -	0	- - - - -	0 - - - -																	
<i>M. bovis</i>	S	G	A	T	T	C	T	T	C	G	G	T	T	A	T	G	A	A	G	C	A
NCTC	R	G	A	T	T	C	T	T	C	G	G	T	T	A	T	A	A	A	G	C	A
400B07	R	G	A	T	T	C	T	T	C	G	G	T	T	A	T	A	A	A	G	C	A
420B07	R	G	A	T	T	T	T	C	G	G	T	T	A	T	G	A	A	G	C	A	

Macrolides: Mutation in 23S rRNA, similar to that described for *E. coli*

Tetracyclines: As yet the mechanism for our *M. bovis* resistant isolates has not been described.

Mutation Prevention Concentration

Used more for the fluoroquinolones

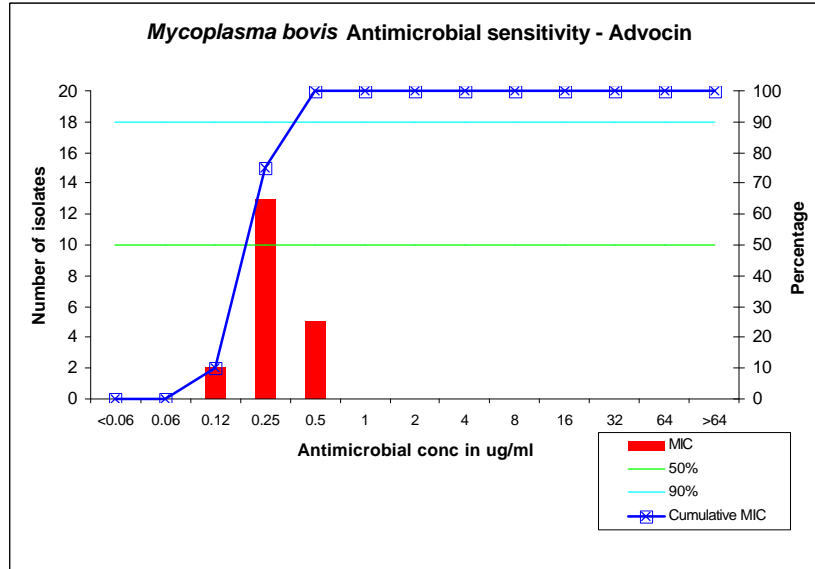
- **Limit the selection of resistant mutants**
- **It is the MIC of the least susceptible mutant**
- **Tests 10^{10} cells**

- **10^{10} cells is large enough for mutant sub-populations to be present**
- **Clinical infections rarely contain more than 10^{10} cells**
- **Testing more than 10^{10} cells is logistically difficult.**

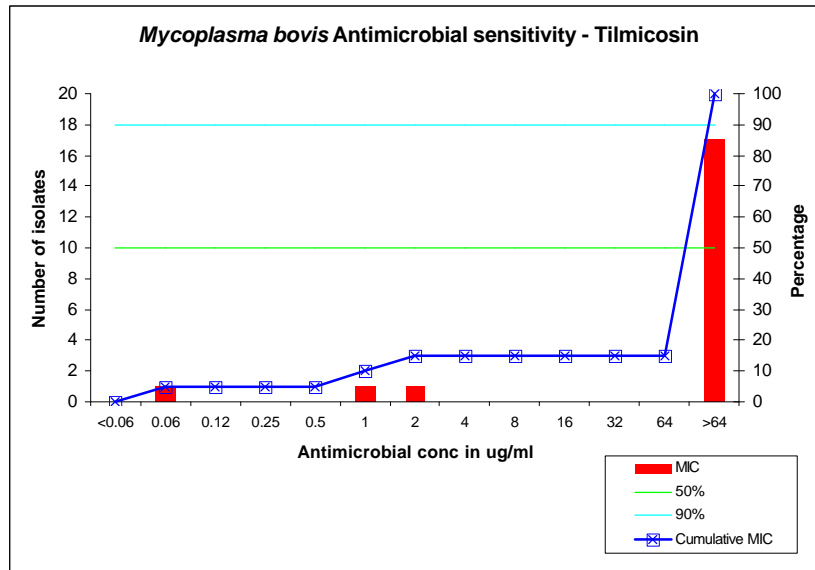
Treatment Decision - Information

- **Treat early**
- **Ensure correct disease diagnosis**
- **Select antimicrobial that is likely to be most effective – PK/PD information**
- **Obtain MIC information – check for antimicrobial resistance**
- **Use the antimicrobial at the correct concentration**
- **Decide - Treat one animal – in contract animals or all animals – quarantine?**
- **Monitor for antimicrobial resistance developing**

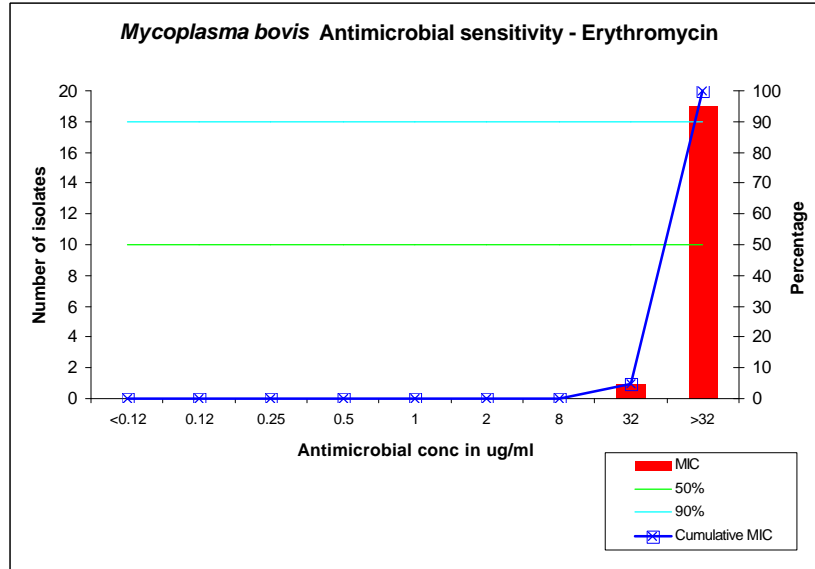
Mycoplasma bovis MIC Results



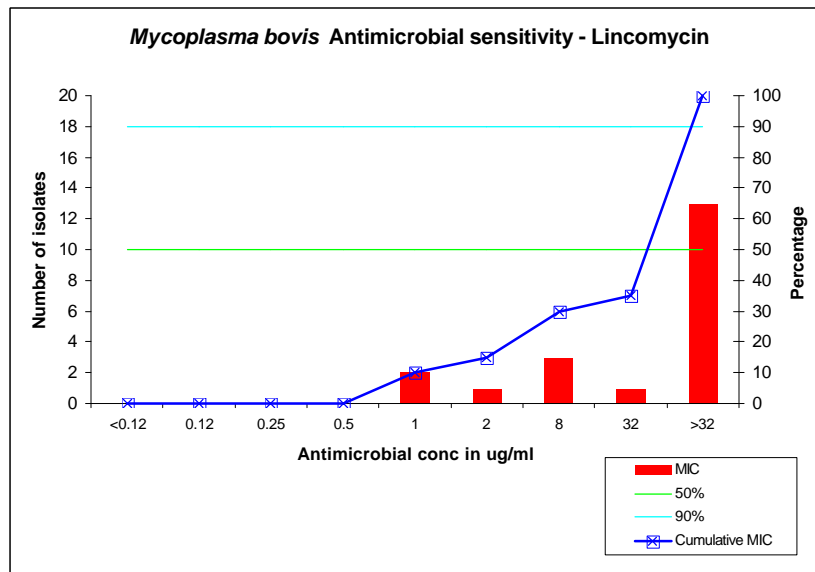
Mycoplasma bovis MIC Results



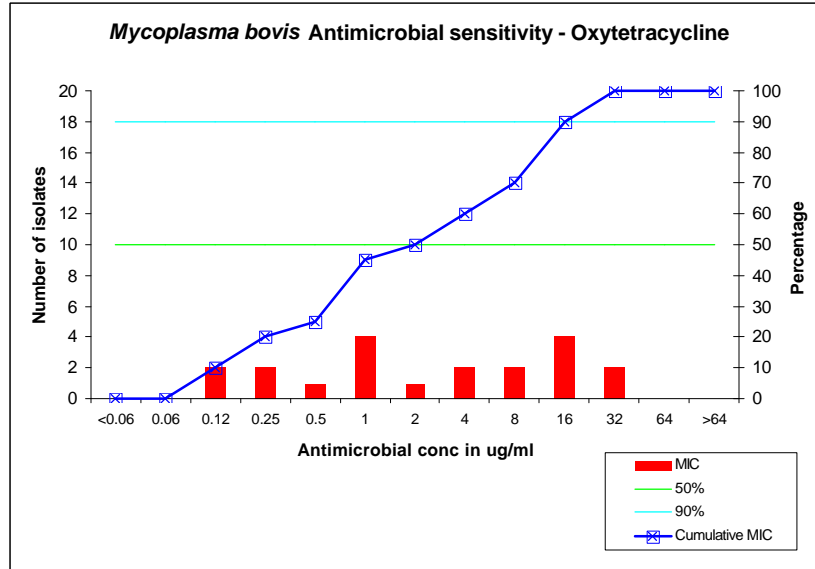
Mycoplasma bovis MIC Results



Mycoplasma bovis MIC Results



Mycoplasma bovis MIC Results



Antimicrobial effectiveness against Mycoplasma bovis

	<i>M. bovis</i> (20 UK strains)		<i>M. bovis</i> (20 Turkish strains)	
	MIC Range	MIC 50	MIC Range	MIC 50
Tilmicosin	<math><0.06</math>- >64.00	>64.00	>32.00- >32.00	>32.00
Oxytetracycline	0.12- 32	2	8 >32.00	32.00
Spectinomycin	2.00- >64.00	8	2.00- 8.00	8.00
Erythromycin	32.00- >32.00	>32.00	0.12- >32.00	>32.00
Ciprofloxacin	<math><0.25</math>- 2	1	2.00- 32.00	32.00
Clindamycin	0.25- >32.00	>32.00	0.12- 8.00	0.25
Lincomycin	1.00- >32.00	>32.00	0.12- 2.00	1.00
Enrofloxacin	0.12- 1	0.25	1.00- 32.00	32.00
Danofloxacin	0.12- 0.5	0.25	0.50- 8.00	8.00
Tylosin	ND		1.00- 32.00	32.00
Florfenicol	2.00- 16	4	2.00- 32.00	32.00
Chloramphenicol	0.25- 32	8	8.00- 32.00	32.00
Marbofloxacin	0.50- 1.00*	1.00	0.25- >8.00	8.00
Draxxin	ND		0.25- >8.00	0.25

* Isolate recently with MIC 64.00

Requirements

- International Standardised methods for MIC testing for Veterinary *Mycoplasma species* need to be established.
- International standard control strains need to be established, initially for the main Veterinary *Mycoplasma species*.
- Breakpoints need to be determined, for the different *Mycoplasma species* in the different hosts and with different clinical signs. i.e. *Mycoplasma bovis* mastitis, does that have different breakpoints to *Mycoplasma bovis* respiratory disease? - *In-vivo* experiments.
- New methods need developing for rapid diagnosis and antimicrobial sensitivity testing (molecular detection of antimicrobial resistance – PCR's - micro-array?)

Conclusion

- Described how the laboratory obtains MIC
- Explained "static" and "cidal"
- PK/PD
- Breakpoints
- MIC data
- Limitations and need for standardisation
- Antimicrobial resistance
- Need for *in-vivo* data on antimicrobial effectiveness.