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CLSI Veterinary Antimicrobial Susceptibility Testing Subcommittee (VAST)

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CLSI-VAST Generic Drug Working Group

June 14-15, 2019

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Generic Drug Working Group

Proposal

- To update susceptibility testing breakpoints for ampicillin in horses

Current breakpoint

VET 08, Table 2D

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	MIC Breakpoints and Interpretive Categories (µg/mL)			Comments
				S	I	R	
Penicillins							
Horses							
A	Respiratory	Ampicillin	*S. equi* subsp. *equi* and subsp. *zooepidemicus*	≤0.25	–	–	(13) For strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed.

No dose listed, but 22 mg/kg q12h was used for analysis.

Proposed action: VET 08, Table 2

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	MIC Breakpoints and Interpretive Categories (µg/mL)			Comments
				S	I	R	
Penicillins							
Horses							
A	Respiratory	Ampicillin	*S. equi* subsp. *equi* and subsp. *zooepidemicus*	≤0.25	–	–	(13) For strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed. (X) Ampicillin breakpoints were determined from an examination of MIC distributions of isolates and PK-PD analysis of ampicillin in horses after administration at a dose of 22 mg/kg IM or IV every 12 hours.

Proposed action: VET 08, Table 2

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	MIC Breakpoints and Interpretive Categories (µg/mL)			Comments
				S	I	R	
Penicillins							
Horses							
A	Respiratory Skin, Soft Tissue	Ampicillin	*Staphylococcus aureus*	≤ 0.25	0.5	≥ 1	Ampicillin breakpoints were determined from an examination of MIC distributions of isolates and PK-PD analysis of ampicillin in horses after administration at a dose of 22 mg/kg IM or IV every 12 hours.
A	Respiratory Skin, Soft Tissue	Ampicillin	Enterobacteriaceae	≤ 0.25	0.5	≥1	

Ampicillin: Dosage Regimens (Published Doses)

Injectable (IV, or IM)

Ampicillin solution

- Dogs: 10-20 mg/kg, q6-8h.
- Cats: 10-20 mg/kg, q6-8h.
- Horses: 10-22 mg/kg, q8h IV
- Horses: 10-22 mg/kg, q12h IM

Ampicillin sodium: Label & Indications

Amp-Equine (Ampicillin Sodium) Zoetis

INDICATIONS FOR USE

Horses

Treatment of respiratory tract infections (pneumonia and strangles) due to *Staphylococcus* species, *E. coli*, and *Proteus mirabilis* and skin and soft tissue infections (abscesses and wounds) due to *Staphylococcus* species, *Streptococcus* species, *E. coli* and *Proteus mirabilis*, when caused by susceptible organisms.

Ampicillin sodium: Label & Indications

Amp-Equine (Ampicillin Sodium) Zoetis

DOSAGE AND ADMINISTRATION

Horses

3 mg per pound of body weight twice daily by intravenous or intramuscular injection. (6.7 mg/kg every 12 hours)

Ampicillin Sodium Solution

Ampicillin Protein Binding in Horses

- Variable; depending on study
- Ampicillin ranged from 6.8-8% up to 37-38%
- For this analysis, a value of 20% was used *(fu 0.80)*
- 20% is consistent with other species

Pharmacokinetic Data for Ampicilin in Horses

Ampicillin Data for Horses:
IV administration

Half-life	(SD)	VD	(SD)	CL	(SD)	AUC	(SD)	Reference
hour		L/kg		ml/kg/hr		ug hr/mL		
0.62		0.18	0.01	210		32.82	0.93	Sarasola & McKellar, 1993
0.725		0.3	0.04	268	30	38.4	4.3	Sarasola et al,1992
1.55								Durr et al, 1976
1.41		0.17						Bowman et al 1986
0.75		0.21						Horspool et al 1992
1.72	0.16	0.71	0.1	285	37			Ensink et al 1992
0.657	0.06	0.33	0.07	340.8	48			Wilson et al, 1988
0.7		0.26	0.02	365.4	34.7	28.8	2.6	Sarasola & McKellar, 1995
1.07	0.16	0.34	0.09	267.64	29.7	33.3	4.18	**Mean**

Ampicillin Data for Horses:
IM administration

T-half	(SD)	VD	(SD)	CL	(SD)	AUC	(SD)	Cmax	(SD)	K01	(SD)	Tmax	(SD)	Reference
Hr		L/kg		ml/kg/hr		ug hr/mL		ug/mL		/hr		hr		
2.3	0.61	0.71	0.21	209.8	17.2	61.1	4.8	31.1	8.7	15.2	17.4	0.32	0.2	Van Den Hoven et al 2003

Microbiology

Distribution of Minimum Inhibitory Concentration Values for Ampicillin on Gram Positive Organisms: Feb. 2001-Jan. 2002										
Species	**Bacteria**	**<=0.12**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**> 8**
Equine	*Staph. aureus (n=71)*	25	0	4	1	5	9	6	5	16
	Strep. equi or zooepidemicus (n=313)	264	0	40	0	3	2	0	0	4

Distribution of Minimum Inhibitory Concentration Values for Ampicillin on Gram Positive Organisms: Feb. 2002-Jan. 2003									
Species	**Bacteria**	**<=0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**> 8**
Equine	*Staph. aureus (n=81)*	35	2	5	2	7	8	1	21
	Strep. equi or zooepidemicus (n=241)	238	2	0	1	0	0	0	0

Distribution of Minimum Inhibitory Concentration Values for Ampicillin on Gram Positive Organisms: Feb. 2003-Jan. 2004									
Species	**Bacteria**	**<=0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**>8**
Equine	*Staph. aureus (n=64)*	27	5	2	5	6	4	2	13
	Strep. equi or zooepidemicus (n=213)	200	13	0	0	0	0	0	0

EUCAST Data

Ampicillin / *E. coli*

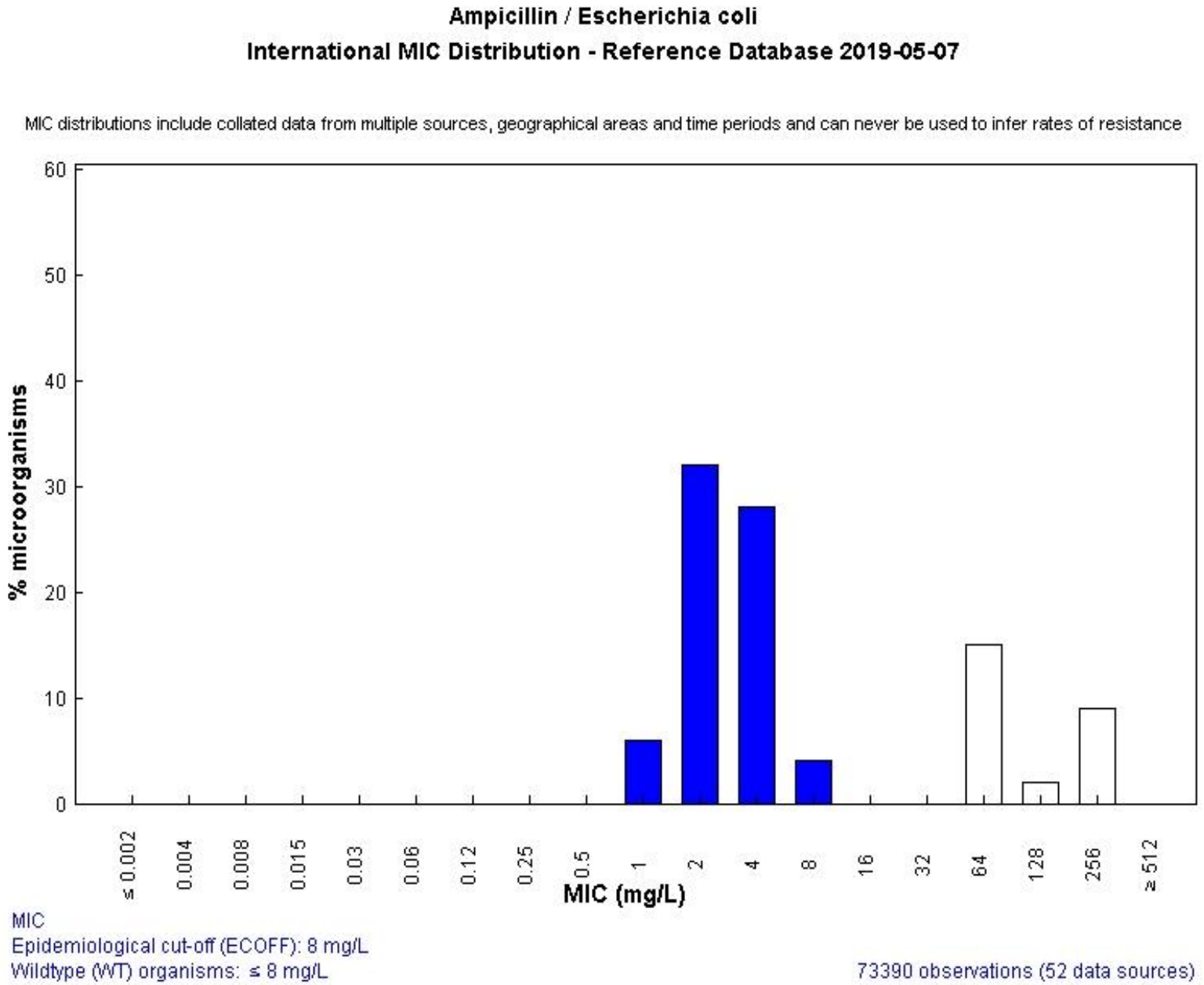


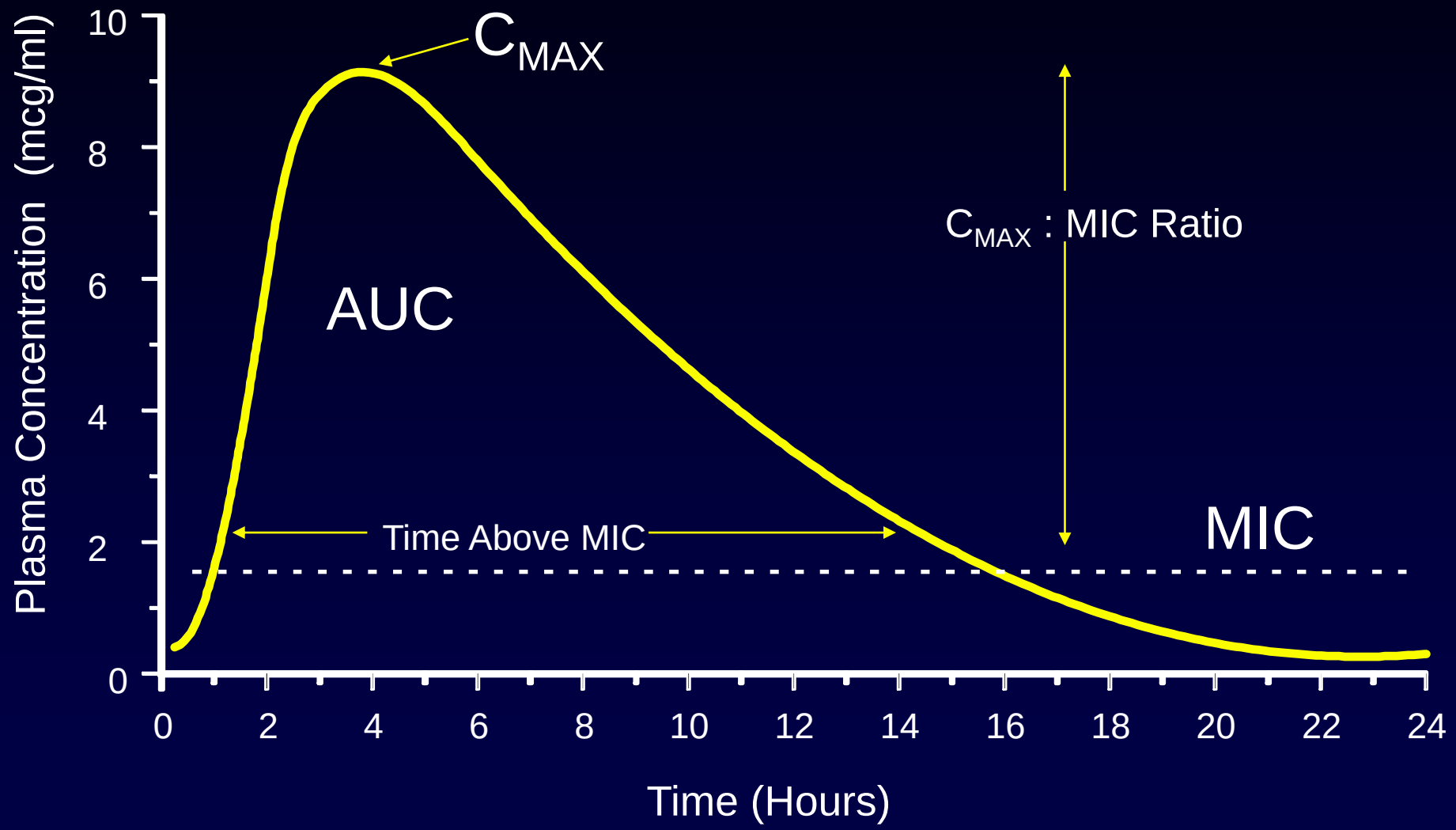
Figure 1: MIC distribution and ECOFF for Ampicillin and E. coli (available from EUCAST)

Beta-Lactam Antibiotics
Aminopenicillins: Ampicillin

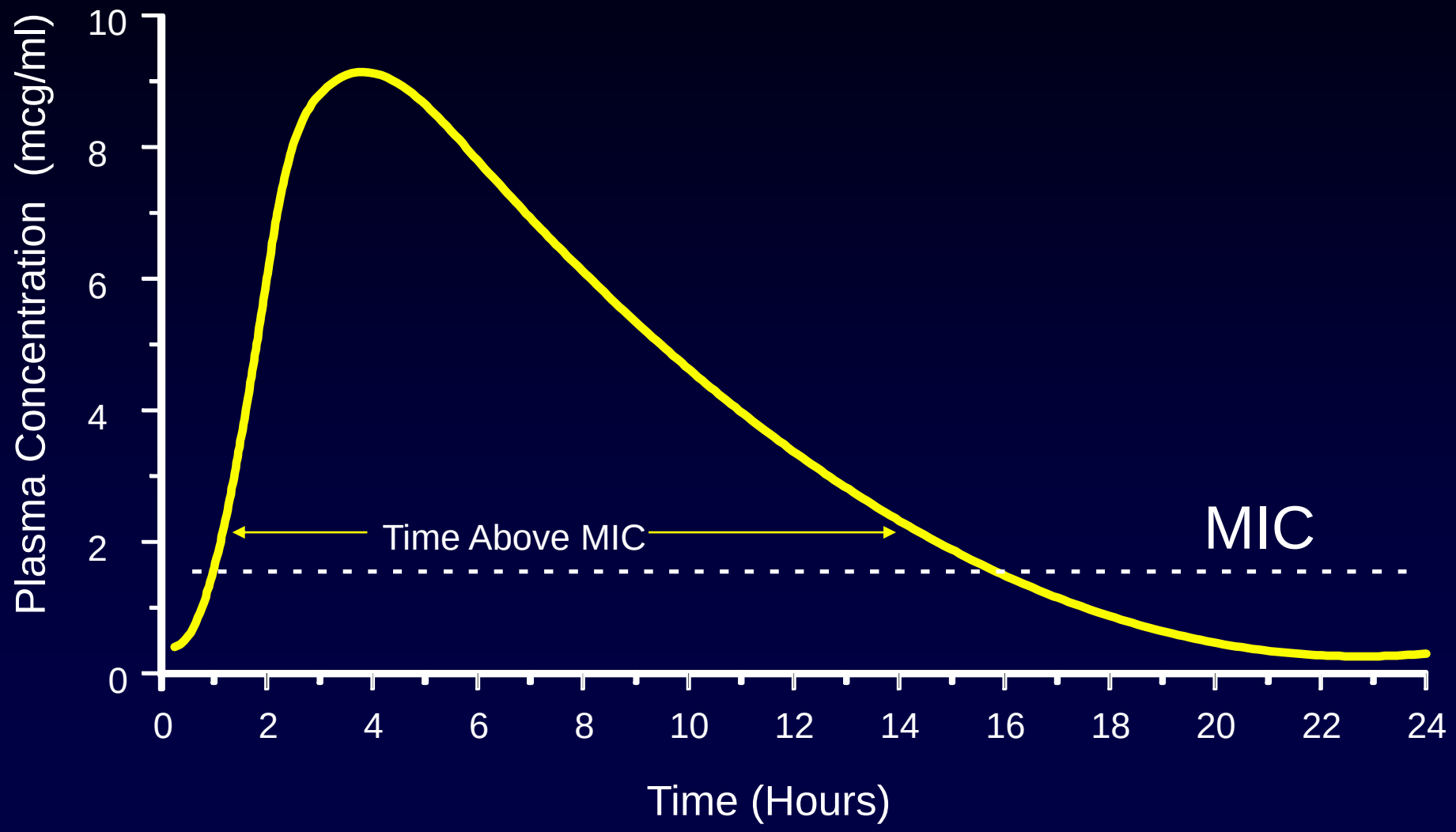
Antibacterial Features

- Bactericidal
- Time-dependent activity

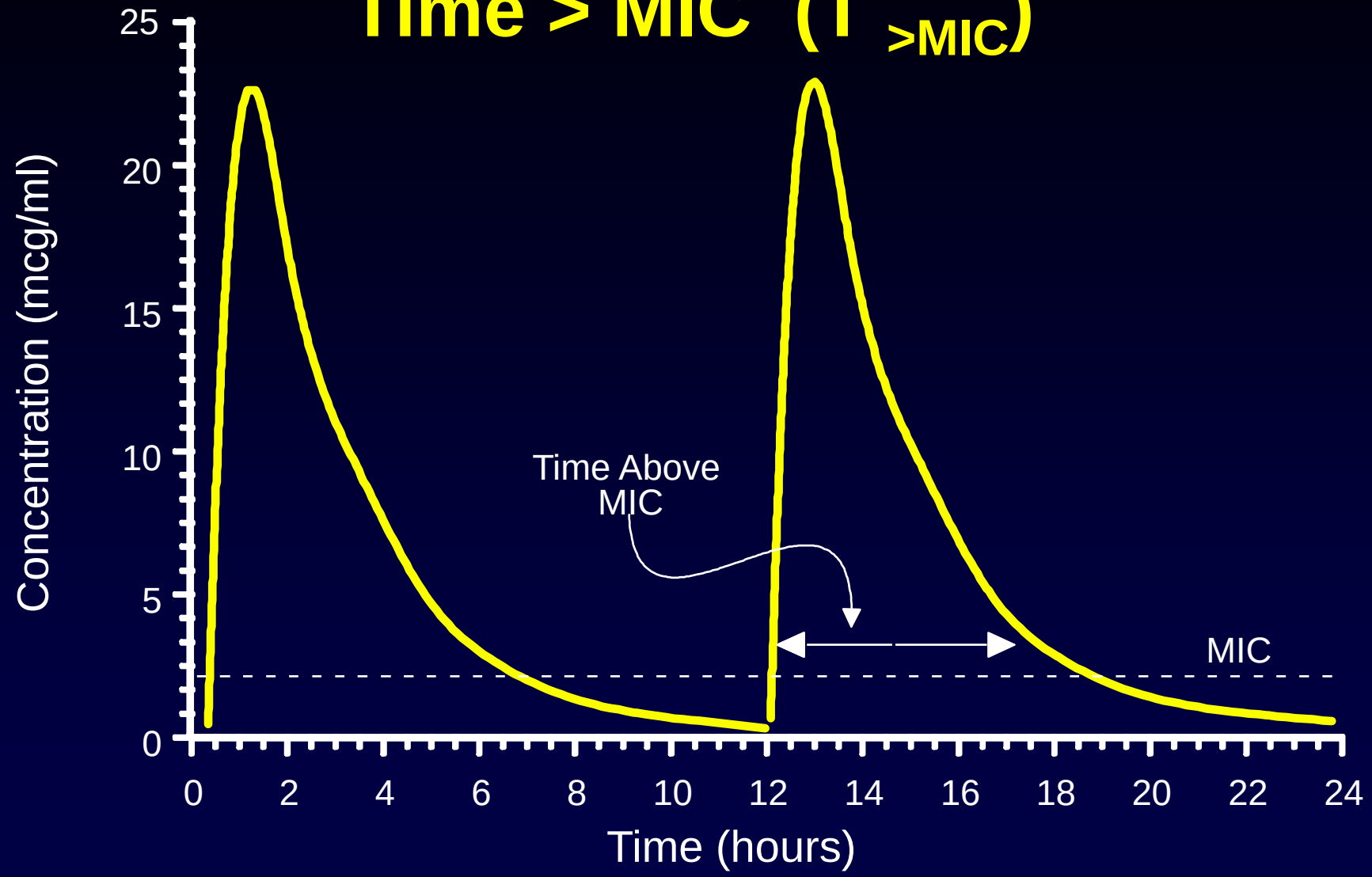
Pharmacokinetic-Pharmacodynamic (PK-PD) Analysis



Time > MIC ($T_{>MIC}$)



Time > MIC ($T_{>MIC}$)



PK-PD Target for T > MIC for Penicillins

CLSI Vet02-A4 Section 4.4.3 "Establishing a Pharmacokinetic-Pharmacodynamic Target"

Table C2:

- *Gram-negative & Streptococci*
  - *f* T>MIC = 30-40% (stasis)
  - *f* T>MIC = 60-70% (max kill)
- *Staphylococcus* spp.
  - *f* T>MIC = 20-30% (stasis)
  - *f* T>MIC = 40-50% (max kill)

PK-PD Targets for ß-lactam Antibiotics

(Turnidge Clin Infect Dis 27: 10, 1998)

- T > MIC is best predictor of outcome.
- Neutropenic animals
  - T > MIC 90-100% of dosing interval
  - T > MIC 50-60% of dosing interval when there is a PAE
- Non-Neutropenic animals
  - T > MIC 20% for carbapenems
  - T > MIC 25-30% for penicillins
  - T > MIC 25-40% for cephalosporins

PK-PD Determination of Dosages for ß-lactam Antibiotics

- **T > MIC 50% of dosing interval.**

(Toutain et al. The pharmacokinetic-pharmacodynamic approach to a rational dosage regimen for antibiotics. Research in Veterinary Science 73: 105-114, 2002)

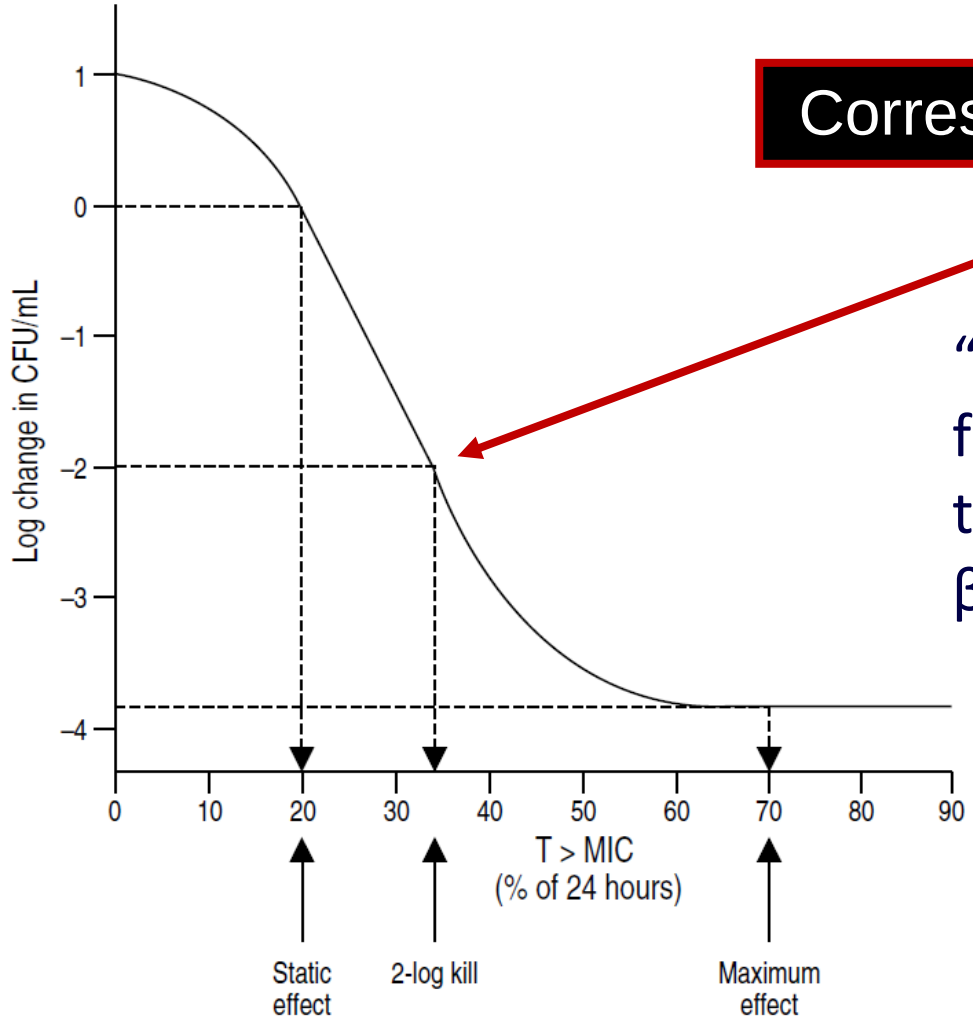
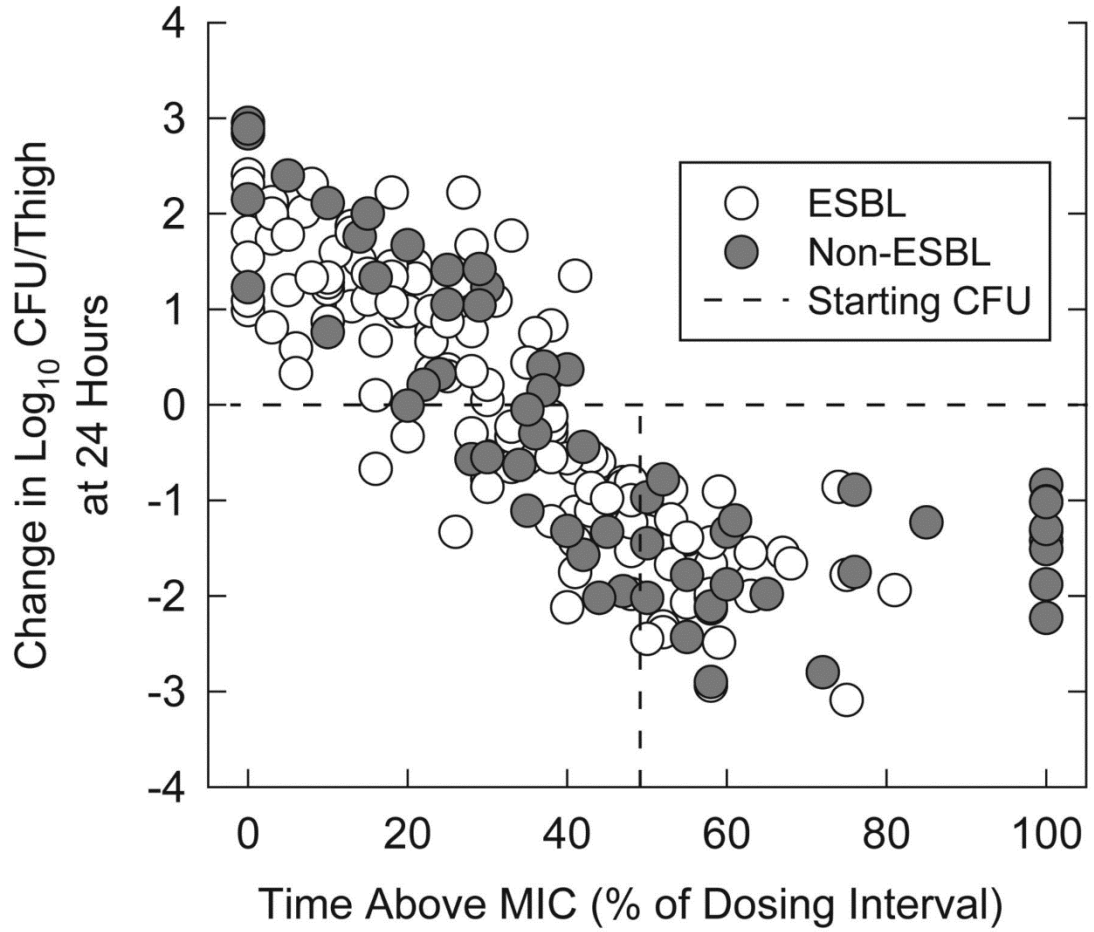


Fig. 2. Theoretical plot of relationship between the β-lactam T > MIC and eradication of a potential pathogen.

Corresponds to 2 log kill for 35% T>MIC

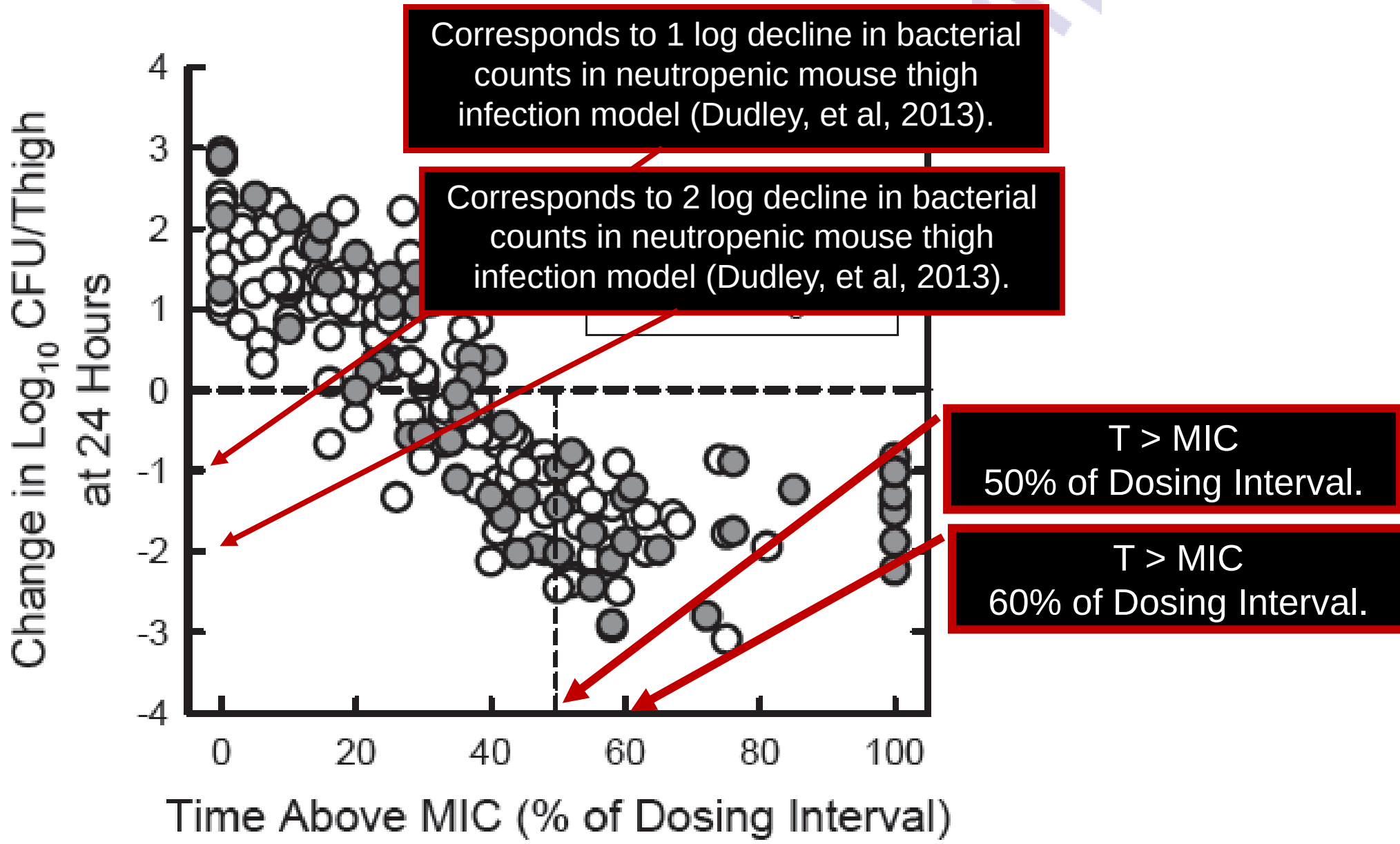
"An unbound drug serum concentration present for > 40–50%of the dosing interval has been taken as predictive of bacteriological efficacy for β-lactams."

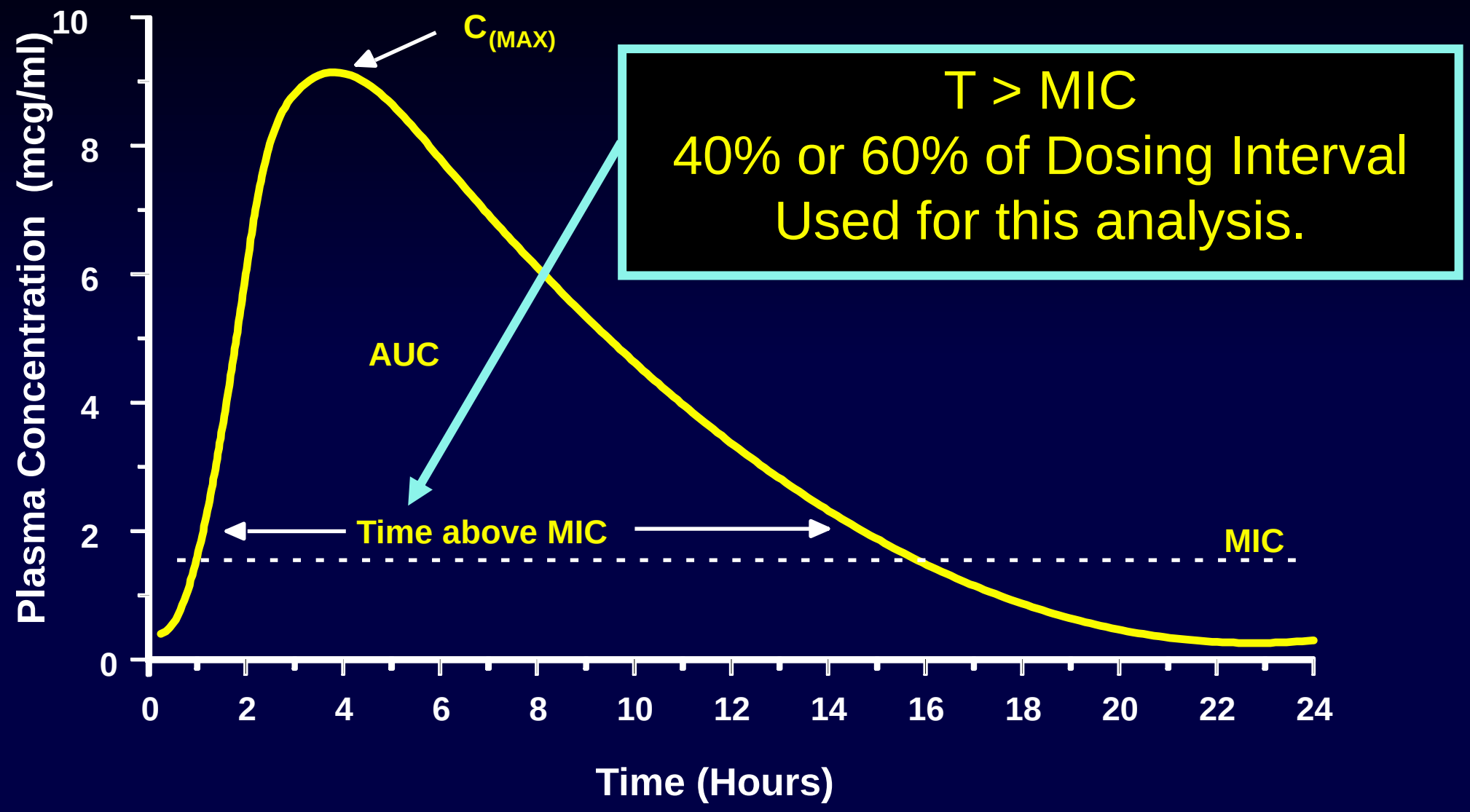
MacGowan. Clin Microbiol Infect 2004; 10 (Suppl. 2): 6–11.



From: Background and Rationale for Revised Clinical and Laboratory Standards Institute Interpretive Criteria (Breakpoints) for Enterobacteriaceae and Pseudomonas aeruginosa: I. Cephalosporins and Aztreonam

Clin Infect Dis. 2013;56(9):1301-1309. doi:10.1093/cid/cit017


Corresponds to 1 log decline in bacterial counts in neutropenic mouse thigh infection model (Dudley, et al, 2013).
Corresponds to 2 log decline in bacterial counts in neutropenic mouse thigh infection model (Dudley, et al, 2013).
T > MIC
50% of Dosing Interval.
T > MIC
60% of Dosing Interval.
Change in Log10 CFU/Thigh at 24 Hours
4
3
2
1
0
-1
-2
-3
-4
0
20
40
60
80
100
Time Above MIC (% of Dosing Interval)


T > MIC
40% or 60% of Dosing Interval
Used for this analysis.
C(MAX)
AUC
Time above MIC
MIC
Plasma Concentration (mcg/ml)
0
2
4
6
8
10
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (Hours)

PK-PD Data

Determination of T > MIC

- % T > MIC =
  ln (Dose/[VD x MIC]) x (T ½ / ln2) x (100 / DI)

- VD = volume of distribution
- T ½ = half-life
- DI = dose interval

Monte Carlo Simulations

- % T > MIC
- Crystal Ball software (Oracle)
- 1,000 random trials simulated
- Input
  - MIC (range from 0.03 µg/mL– 8 µg/mL)
  - Dose: 6.6 mg/kg every 12 hours
  - VD (mean & variance)
  - T ½ (mean & variance)
  - Protein binding: 20 % *(fu 0.80*)

Results

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

f T> MIC 40% PTA Ampicillin Horses
6.6 or 22 mg/kg IV or IM 12h

	MIC (mcg/mL)								
Dose Regimen	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
6.6 mg/kg IV q12h	100	99.9	99.6	96.01	70.5	22.05	1.144	0	0
6.6 mg/kg IM q12h	100	100	99.83	99.89	97.85	84.59	38.94	3.06	0
22 mg/kg IV q12h	100	100	100	100	99.3	92.05	59.35	13.5	0.05
22 mg/kg IM q12h	100	100	100	100	100	99.54	97.39	77.71	23.84

** value in each cell represents the probability of target attainment to reach a fT>MIC target of 40% for each dose listed.*

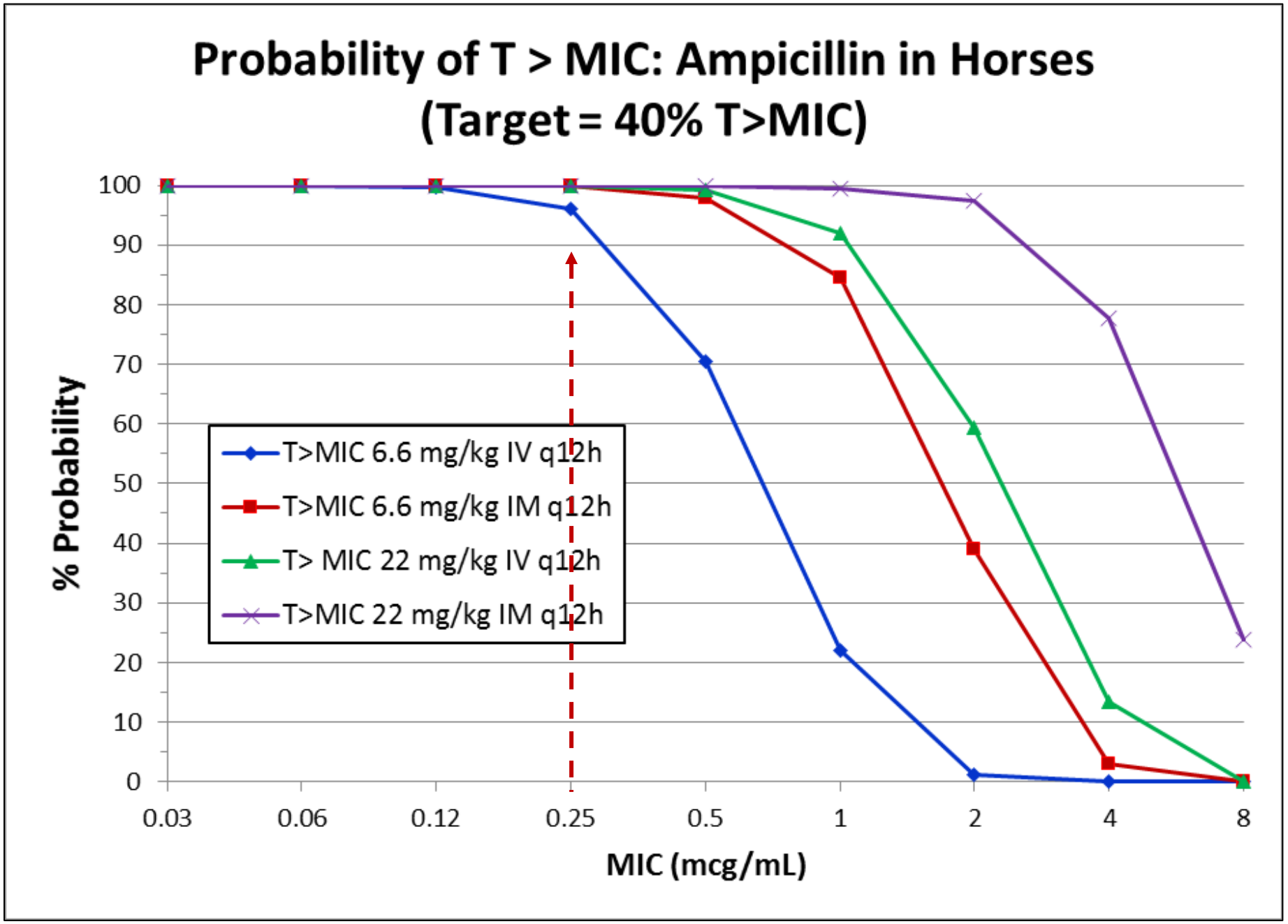
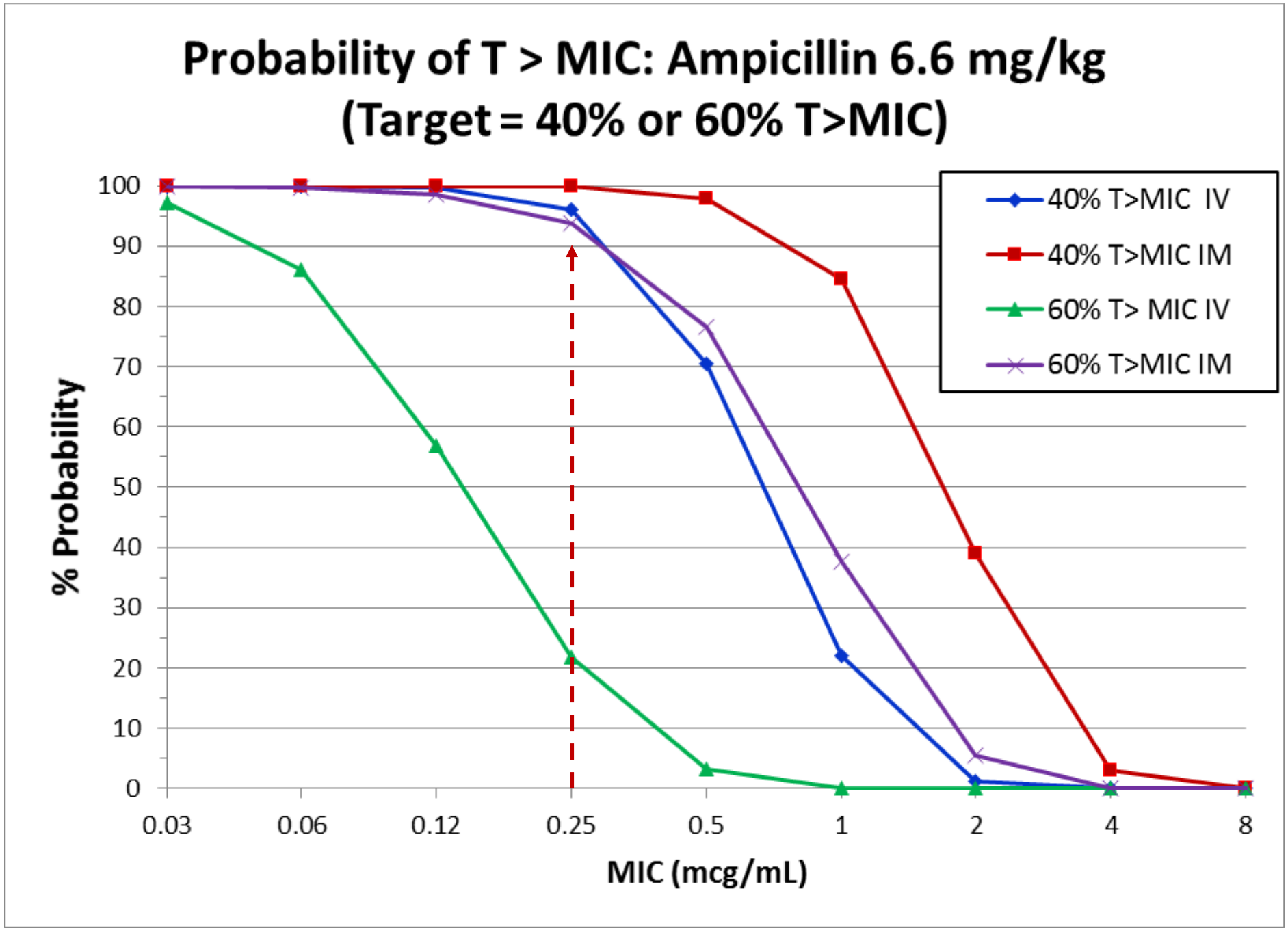


Figure 2: Probability of Target Attainment for Ampicillin in Horses.


Probability of T > MIC: Ampicillin 6.6 mg/kg
(Target = 40% or 60% T>MIC)
% Probability
100
90
80
70
60
50
40
30
20
10
0
0.03
0.06
0.12
0.25
0.5
1
2
4
8
MIC (mcg/mL)
40% T>MIC IV
40% T>MIC IM
60% T> MIC IV
60% T>MIC IM

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

f T> MIC 40 or 60% PTA Ampicillin Horses 6.6 or 22 mg/kg IV or IM 12h

	MIC (mcg/mL)								
Dose Regimen	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
6.6 mg/kg IV q12h 40%	100	99.9	99.6	96.01	70.5	22.05	1.144	0	0
6.6 mg/kg IM q12h 40%	100	100	99.83	99.89	97.85	84.59	38.94	3.06	0
6.6 mg/kg IV q12h 60%	97.08	86.09	56.93	21.76	3.24	0.06	0	0	0
6.6 mg/kg IM q12h 60%	100	99.7	98.51	93.82	76.65	37.73	5.47	0.08	0
22 mg/kg IV q12h 40%	100	100	100	100	99.3	92.05	59.35	13.5	0.05
22 mg/kg IM q12h 40%	100	100	100	100	100	99.54	97.39	77.71	23.84

VET 02-A4 Guidelines

Table C10: Decision Table When Only 2 Cutoff Values Available (CO_{WT} and [CO_{PD} or CO_{CL}])

Abbreviations: CL, clinical cutoff value (CO_{CL}); PD, pharmacodynamic cutoff value (CO_{PD}); WT, wild type cutoff value (CO_{WT}).

Enterobacteriaceae

Streptococcus

Staphylococcus

Ranking of Cutoffs	Suggested Breakpoint	Comments
WT > PD	PD	Could accept CO_{WT} as breakpoint if CO_{WT} only 1 dilution higher than CO_{PD}
PD > WT	PD	
WT = PD	WT = PD	
WT > CL	CL	Could accept CO_{WT} as breakpoint if CO_{WT} only if 1 dilution higher than CO_{CL}
CL > WT	CL	
WT= CL	WT= CL	

Conclusions

Conclusions

- For *f* T>MIC 40% of dose interval:
  - High probability of target attainment (PTA) for MIC ≤ 0.25 μg/mL at dose of 6.6 mg/kg, IM, or IV
- For *f* T>MIC 60% of dose interval:
  - High probability of target attainment (PTA) for MIC ≤ 0.25 μg/mL at dose of 6.6 mg/kg, IM, but not IV
- For *f* T>MIC 60% of dose interval:
  - High probability of target attainment (PTA) for MIC ≤ 0.25 μg/mL at dose of 22 mg/kg, IM, and IV

Proposal from Generic Drug Working Group

Proposed action: VET 08, Table 2

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	MIC Breakpoints and Interpretive Categories (µg/mL)			Comments
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Penicillins							
Horses							
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Proposed action: VET 08, Table 2

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	MIC Breakpoints and Interpretive Categories (µg/mL)			Comments
				S	I	R	
Penicillins							
Horses							
A	Respiratory Skin, Soft Tissue	Ampicillin	*Staphylococcus aureus*	≤ 0.25	0.5	≥ 1	Ampicillin breakpoints were determined from an examination of MIC distributions of isolates and PK-PD analysis of ampicillin in horses after administration at a dose of 22 mg/kg IM or IV every 12 hours (x) *E. coli* and other *Enterobacteriaceae* will test resistant to ampicillin.
A	Respiratory Skin, Soft Tissue	Ampicillin	*Enterobacteriaceae*	≤ 0.25	0.5	≥1	

Similar comment as dog and cat:

Additional Recommendations:

1. Retain Ampicillin (Horses) in Table 1A, Group A Vet08
2. Add dose for administration (22 mg/kg)
3. Add Staphylococcus aureus and Enterobacteriaceae
4. Add comment "*E. coli* and other Enterobacteriaceae will test resistant to ampicillin."

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Thank you.

Any Questions?

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