Pre Clinical In Vitro Models

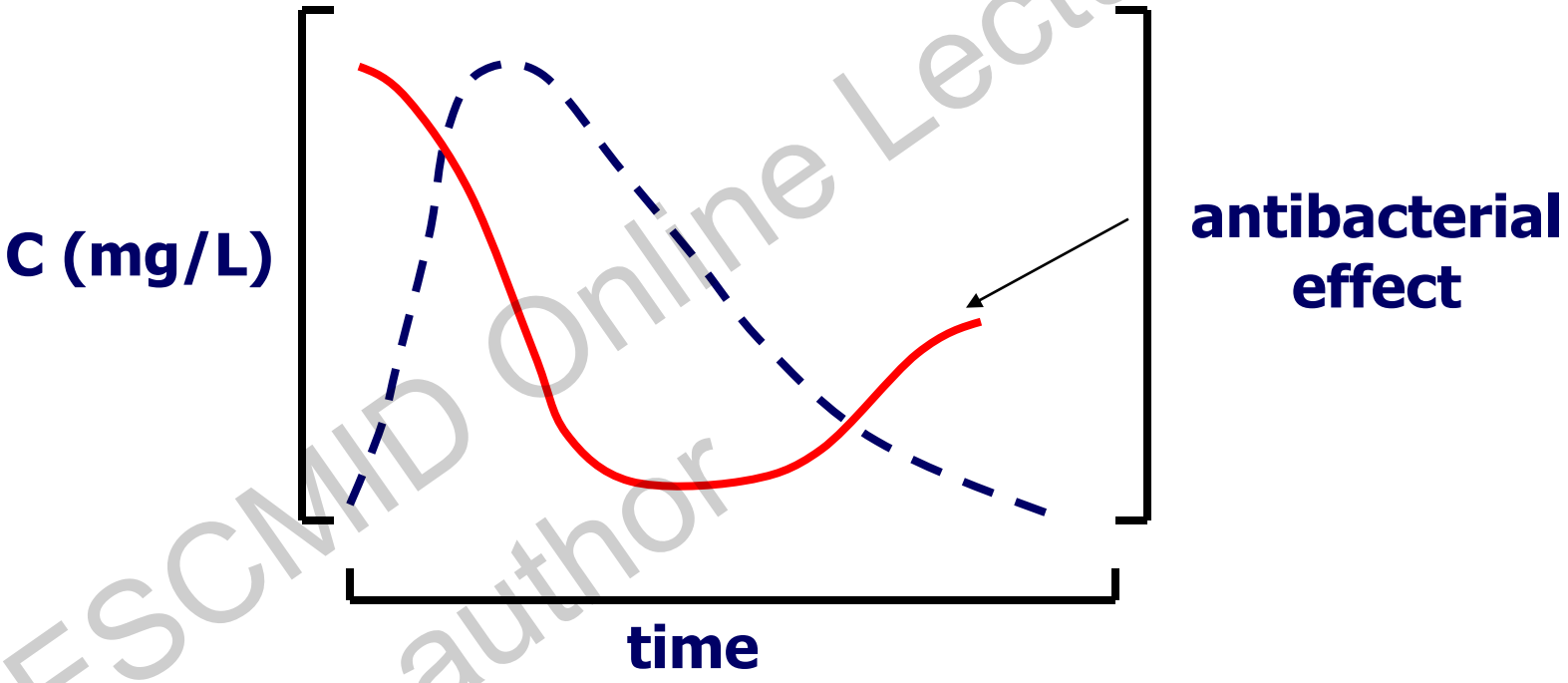
Karen Bowker
BCARE
Southmead Hospital
Bristol, UK

In vitro models

- What are they
- What they can't do
- What are the pk/pd outputs
- Resistance
- Combinations
- In vivo correlates

In vitro pK/pD modelling

study of changing drug concentration and antibacterial effect (ABE)



What can in vitro models do?

Use pre-clinical models to understand drug exposure in relation to

- species
- inoculum
- exposure; pattern & duration
- impact of combination therapy
- pre-existing resistance within drug class
- risk of emergence of resistance

Advantages v animals

- Easier to simulate human pk profile -free/total fractions
- Multiple sampling
- Extended duration of therapy
- High inoculum

- Strains difficult to grow in animals –Str.pneumoniae, H.influenzae
- Simulate diseases not established in animals eg TB, anthrax, Acinetobacter, anaerobes
- Combination therapies

What in vitro models can't do

- Simulate immune response
- Tissue concentrations - protein binding

Technical issues

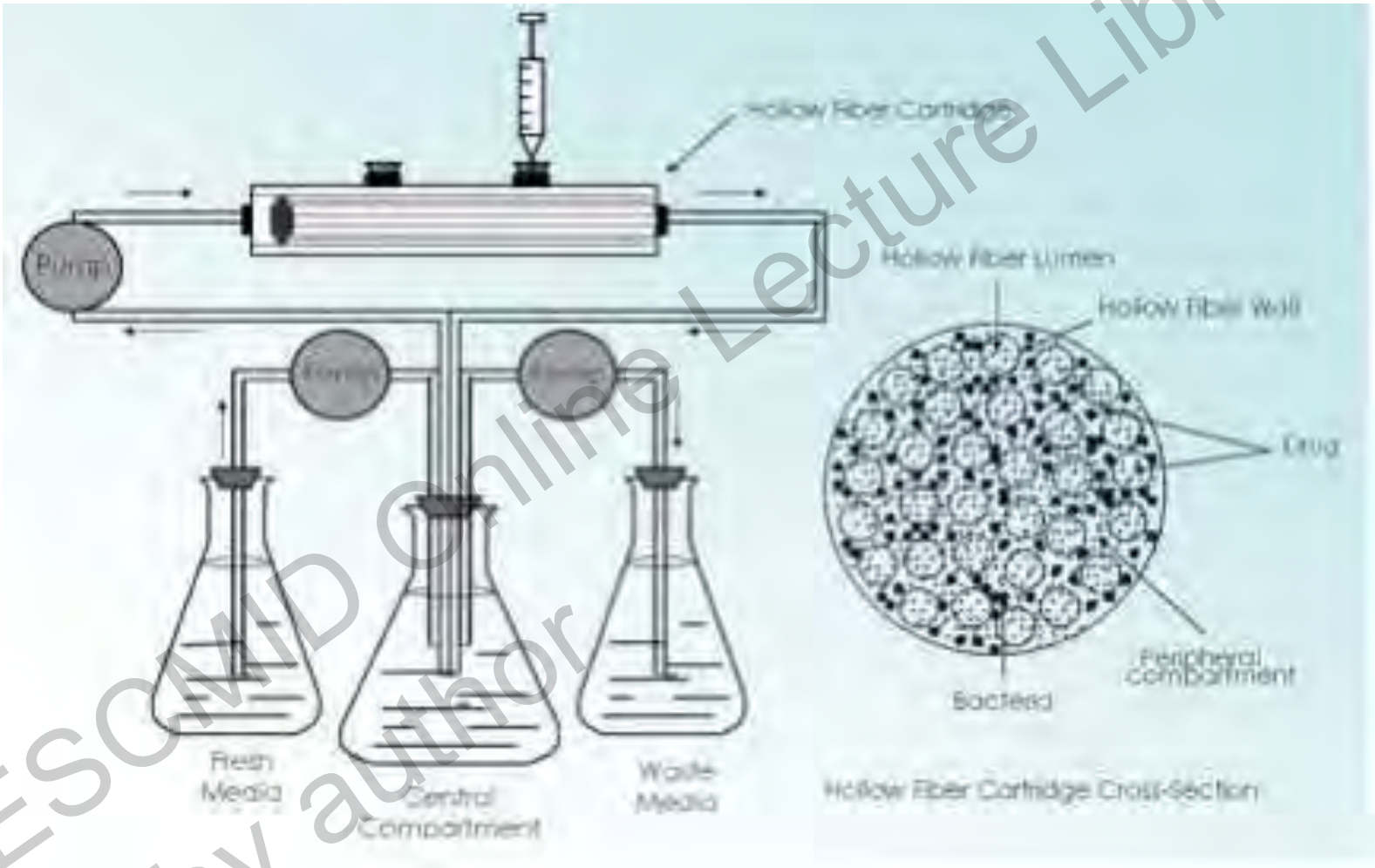
- Adherence
- Contamination
- Dialysis models - blocked filters
- Labour intensive

Types of IVMs

- Dilutional - with and without filter membrane (glass/plastic)
- Dialysis - hollow fibre
- Foreign body - Simulated Endocarditis Vegetations SEVs, Hanging Clot (fibrin)

- Intra-cellular
- Biofilms e.g. - mimic short and long term infection by S.pneumoniae/P.aeruginosa & pathophsiology of infection

Types of in vitro models - Hollow fibre



In vitro pK model - dilutional

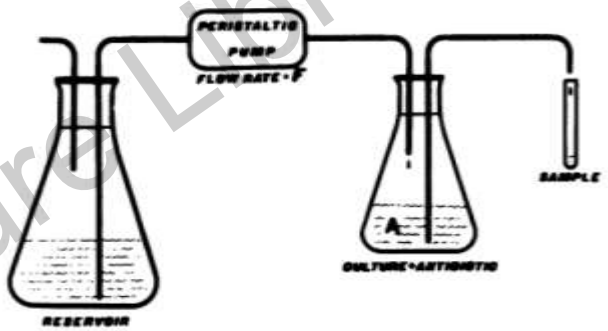


FIG. 1. *Model I apparatus for simulation of monoexponential decrease in antibiotic concentration.*

$C = C0 \times e^{-kt}$

C0 = conc in the vessel at Time 0

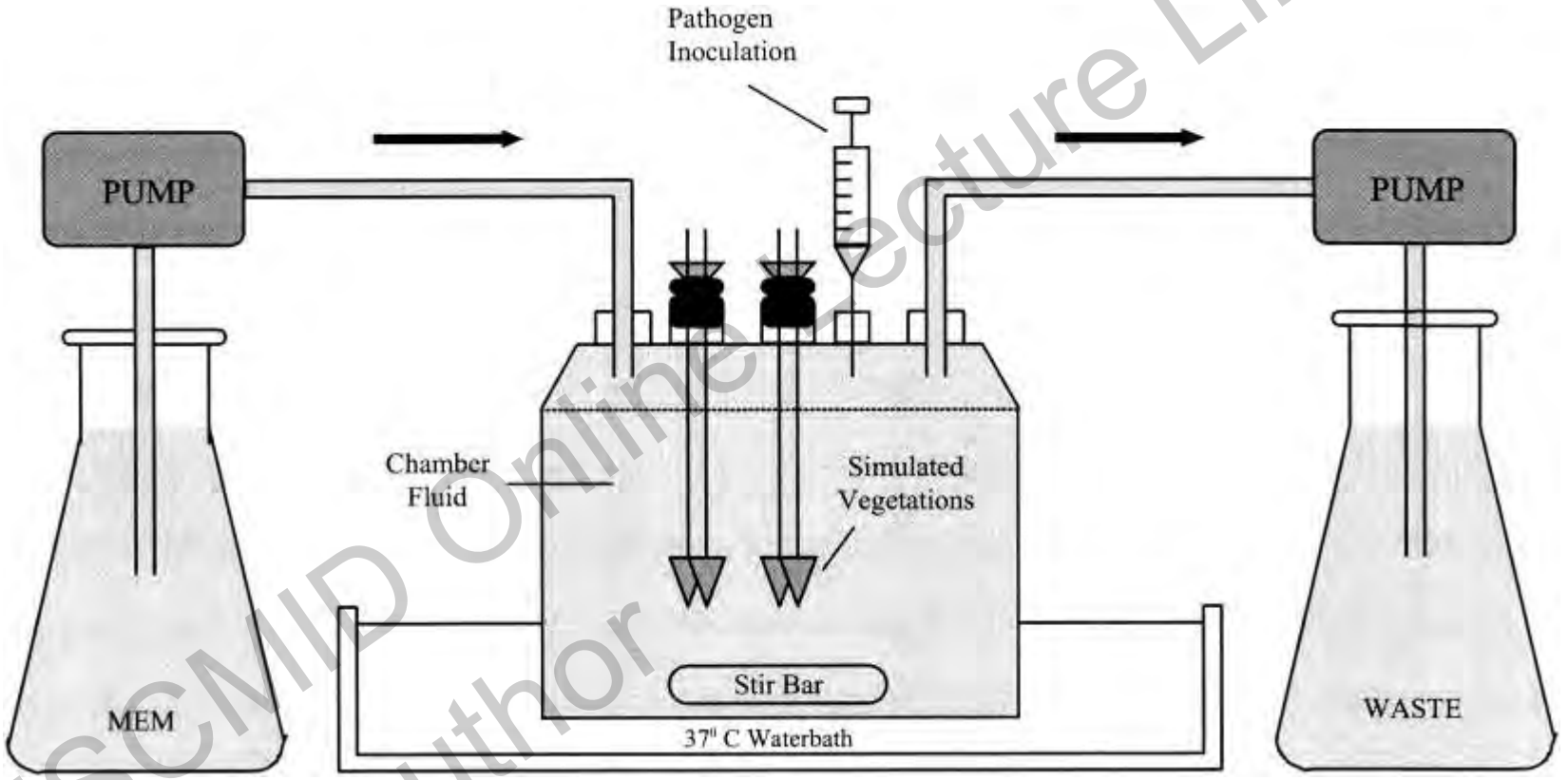
K= F/V

F= V x 0.693/t1/2

Grasso et al 1978

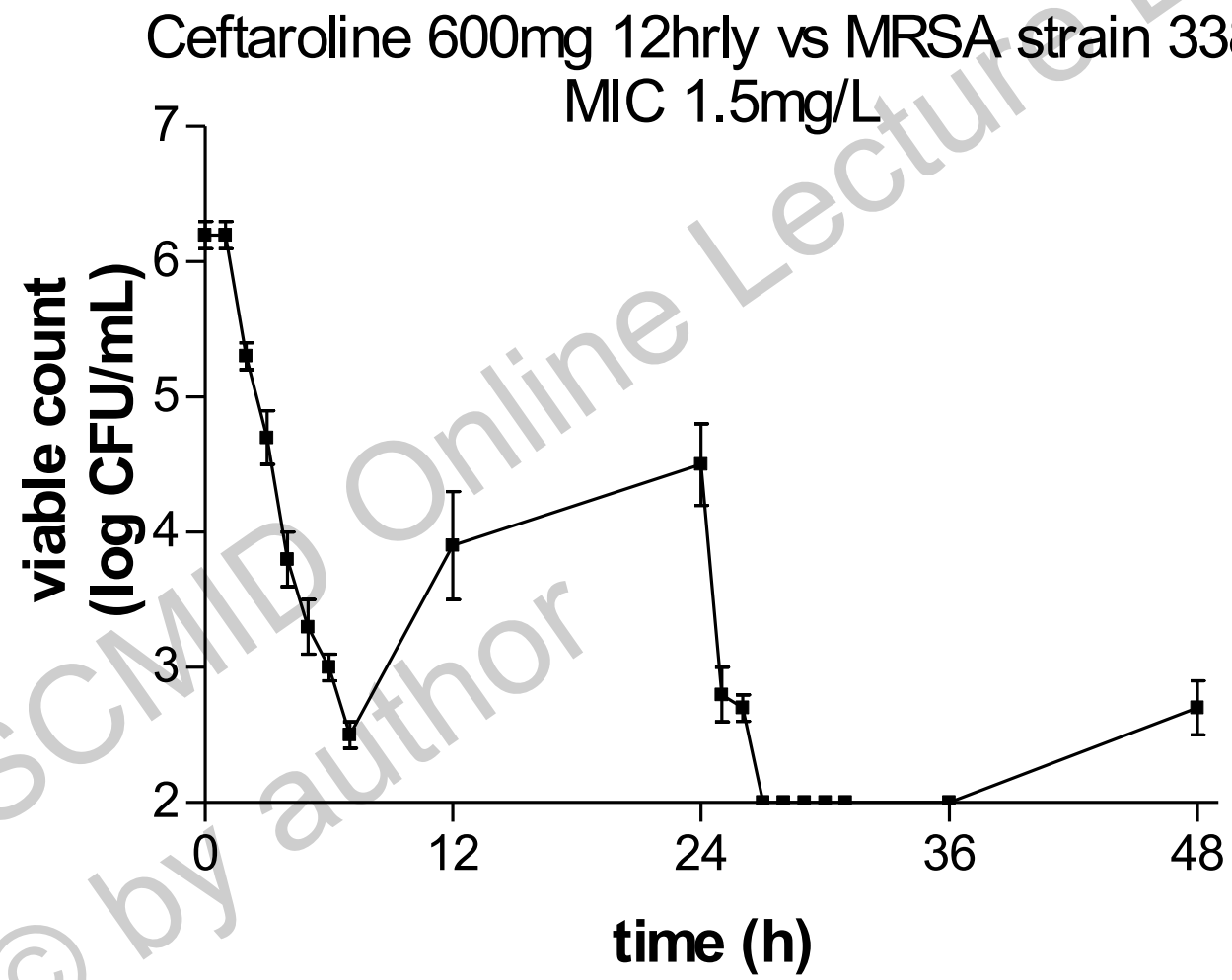
Bacteria are diluted at same rate of antibiotic – growth rate!

In vitro model of IE. PUMP, peristaltic pumps used to introduce fresh or remove exhausted medium.

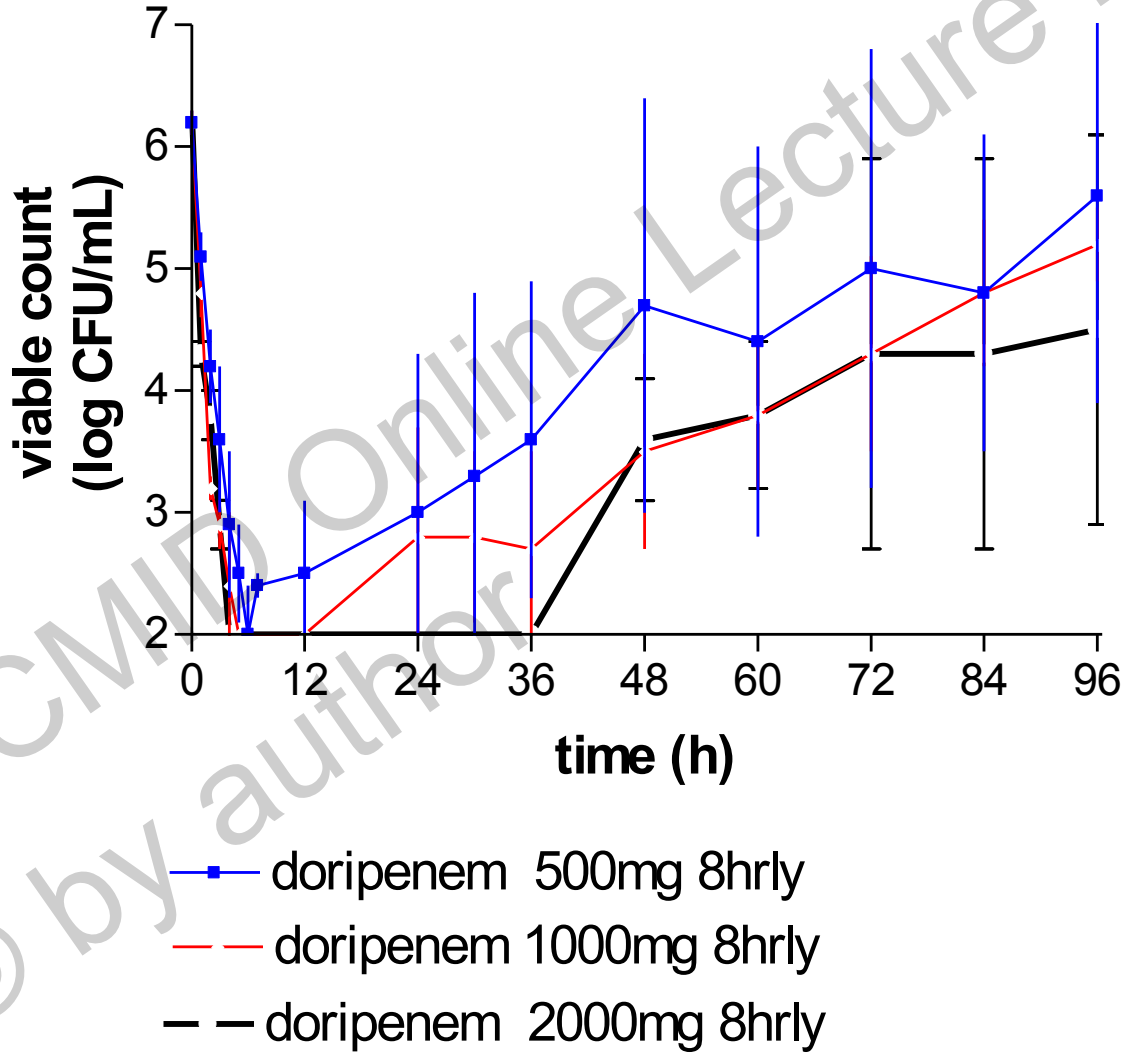


Mercier R et al. Antimicrob. Agents Chemother. 2004;48:2551-2557

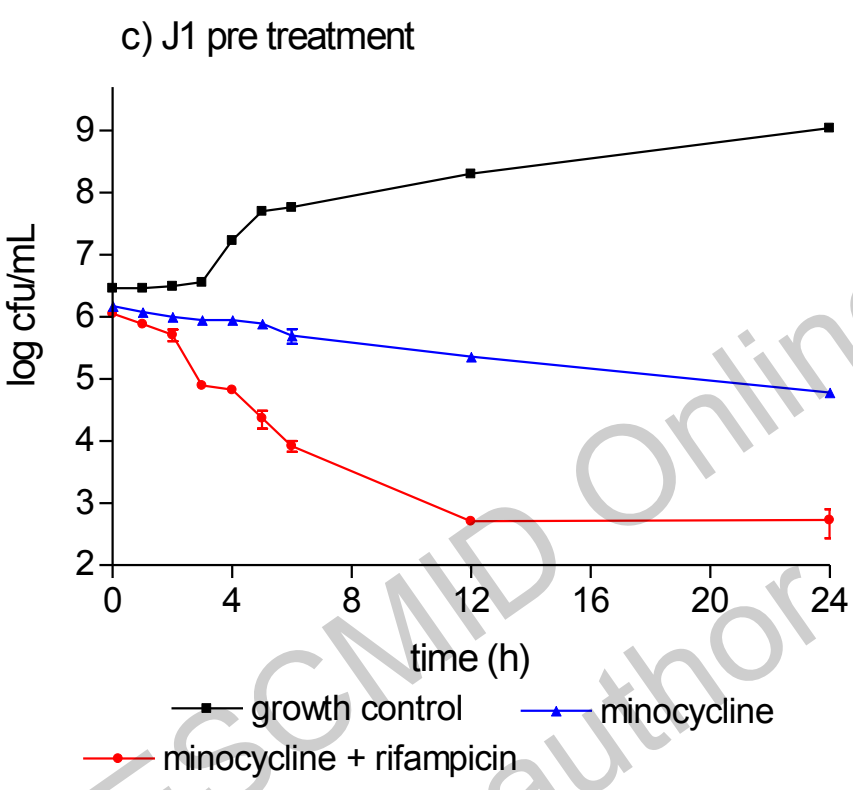
Descriptive studies - Ceftaroline

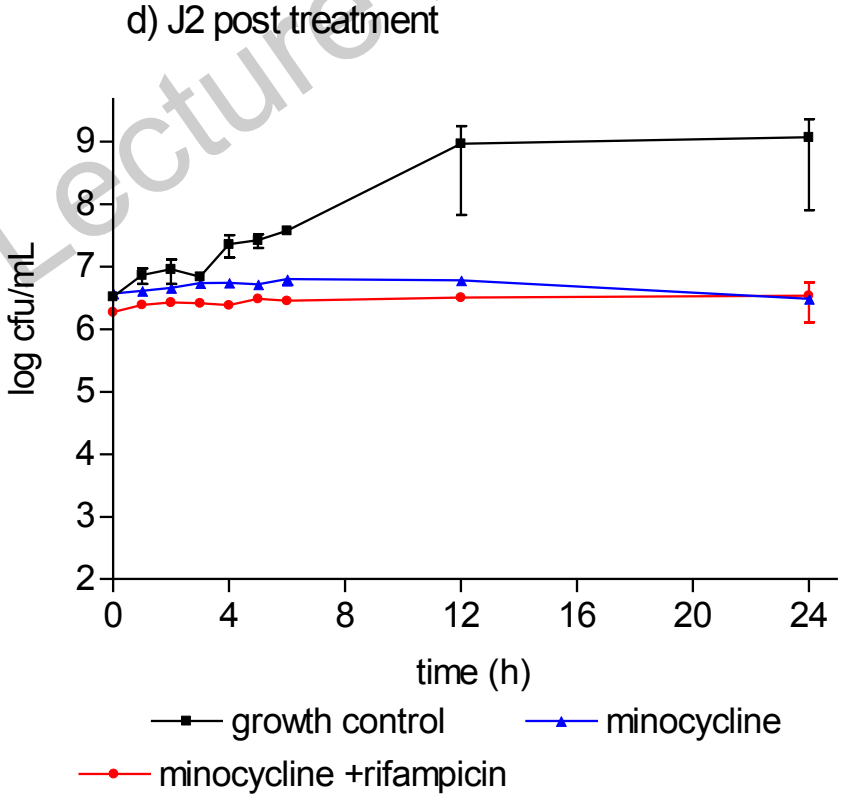


Descriptive studies - Doripenem



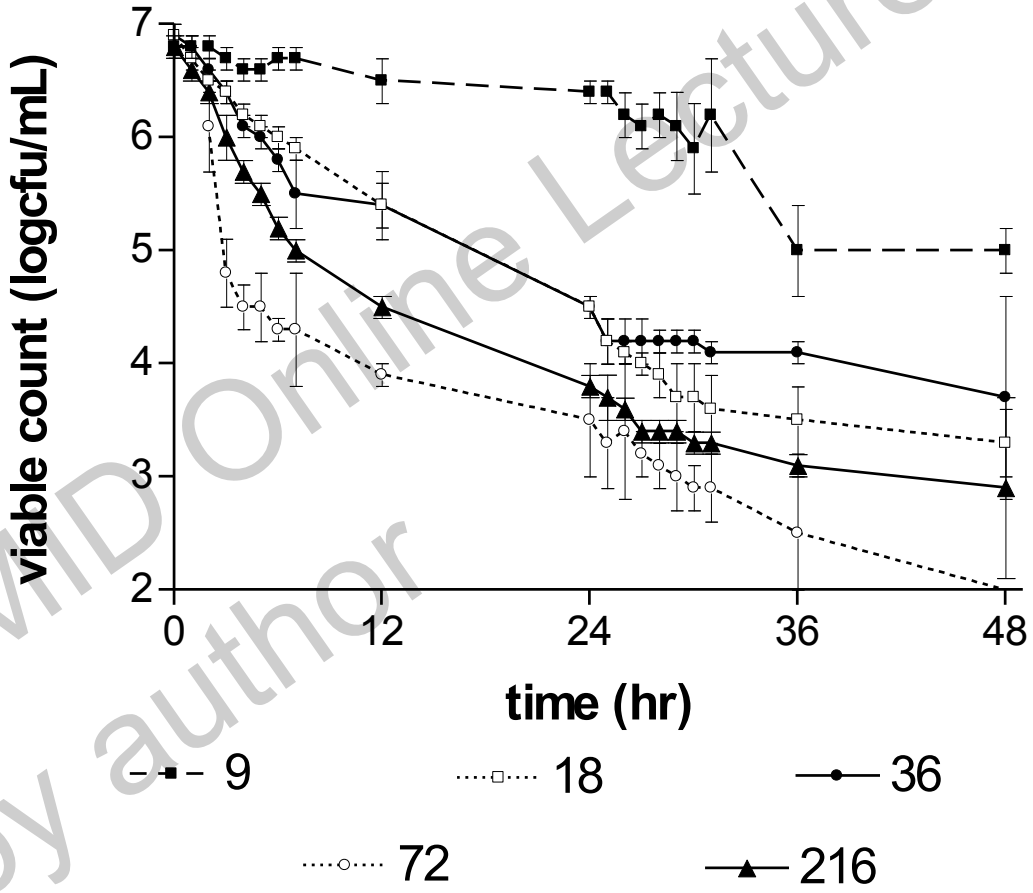
Descriptive studies - treatment failure





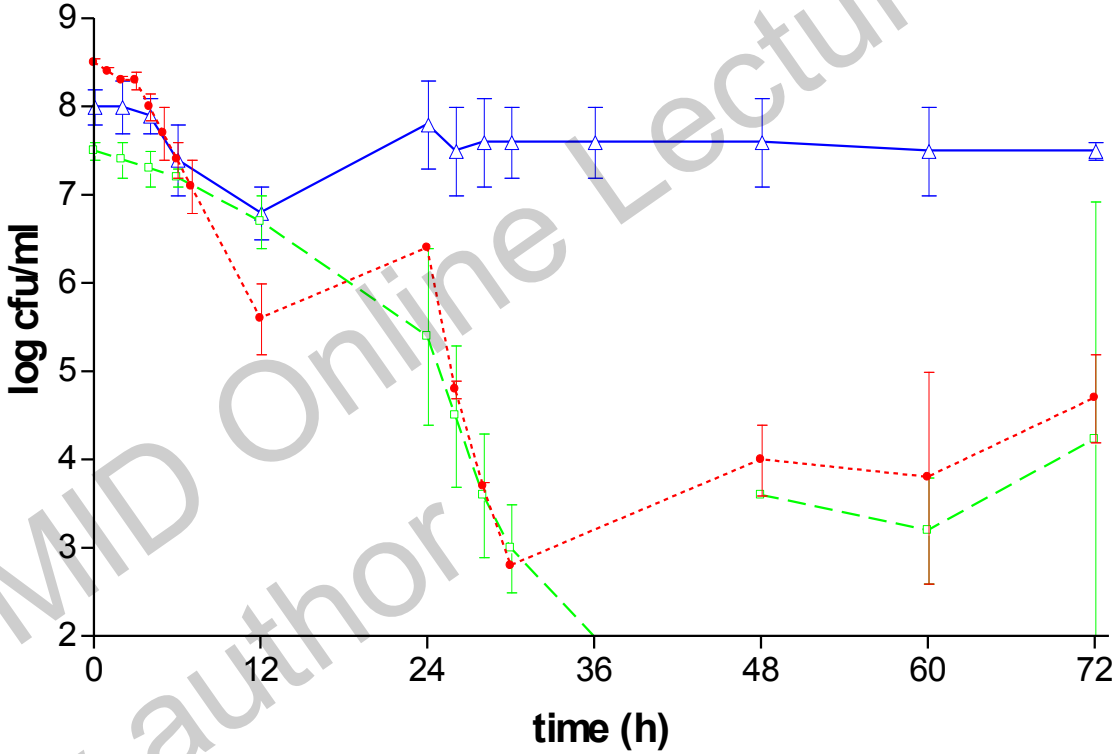
Descriptive studies - Anaerobes

Figure 1 Antibacterial effect of moxifloxacin at AUC/MIC ratios in the range 9-216 against B fragilis



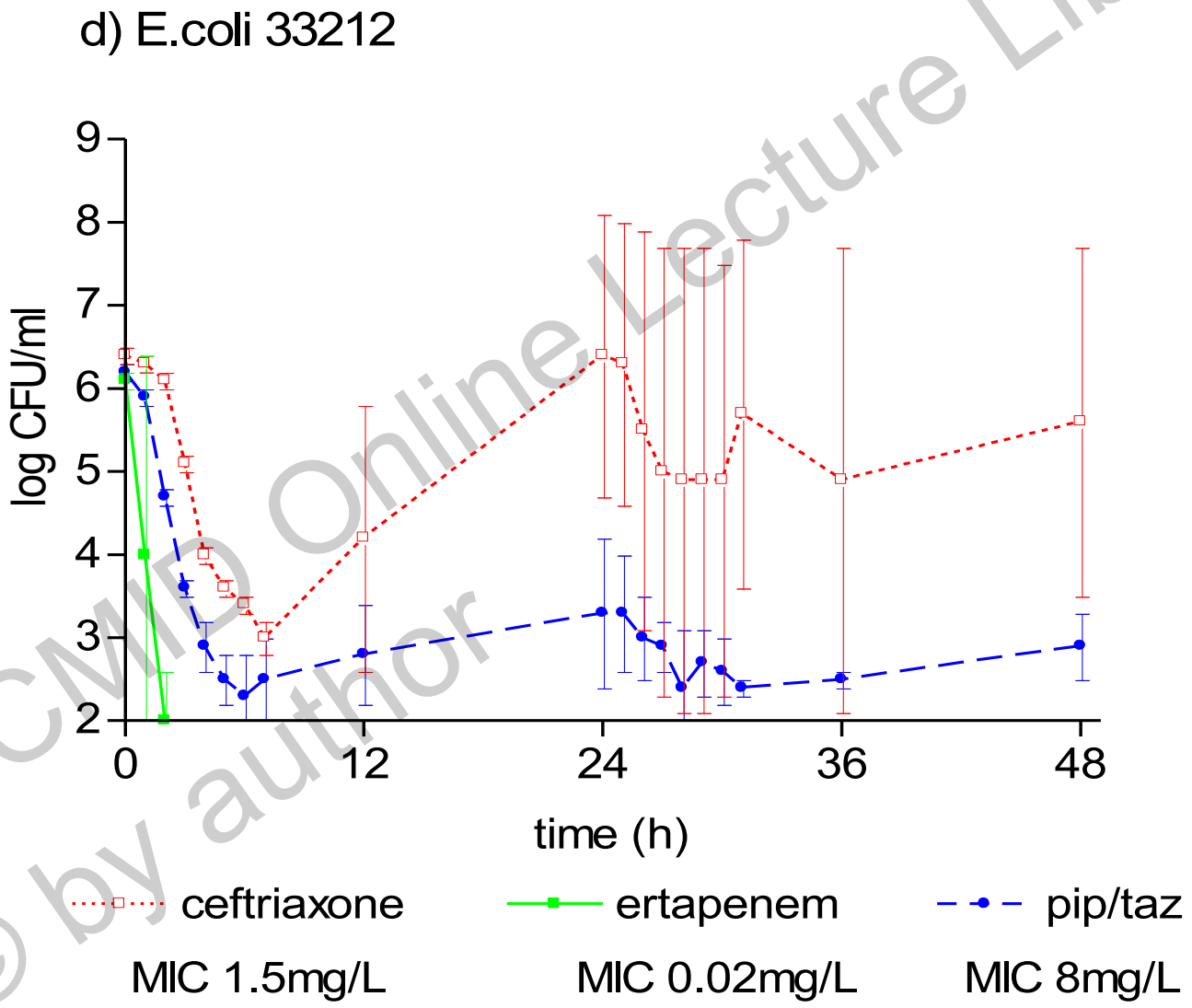
MacGowan 2004

Activity against defined resistance mechanisms - MIC does not drive outcome (gemifloxacin)



Bowker & MacGowan, 2004

Activity against defined resistances - MIC drives outcome (ESBL)



Producing Variability in pK/pD Parameters

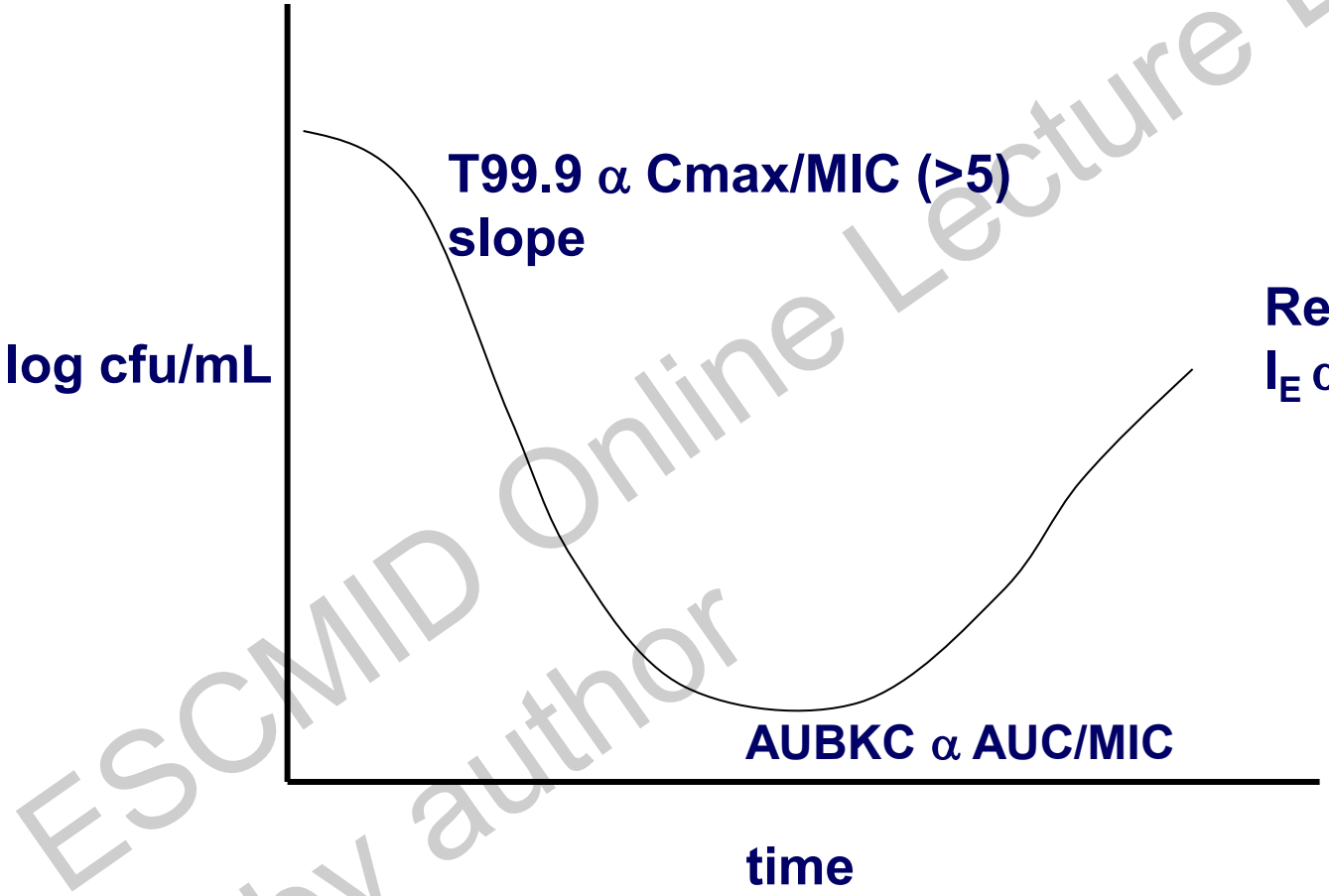
dose escalation
dose fractionation

MIC differences

ranges in pK/pD parameters
(AUC/MIC; Cmax/MIC; T > MIC)

Dose fractionation studies – pK/pD targets

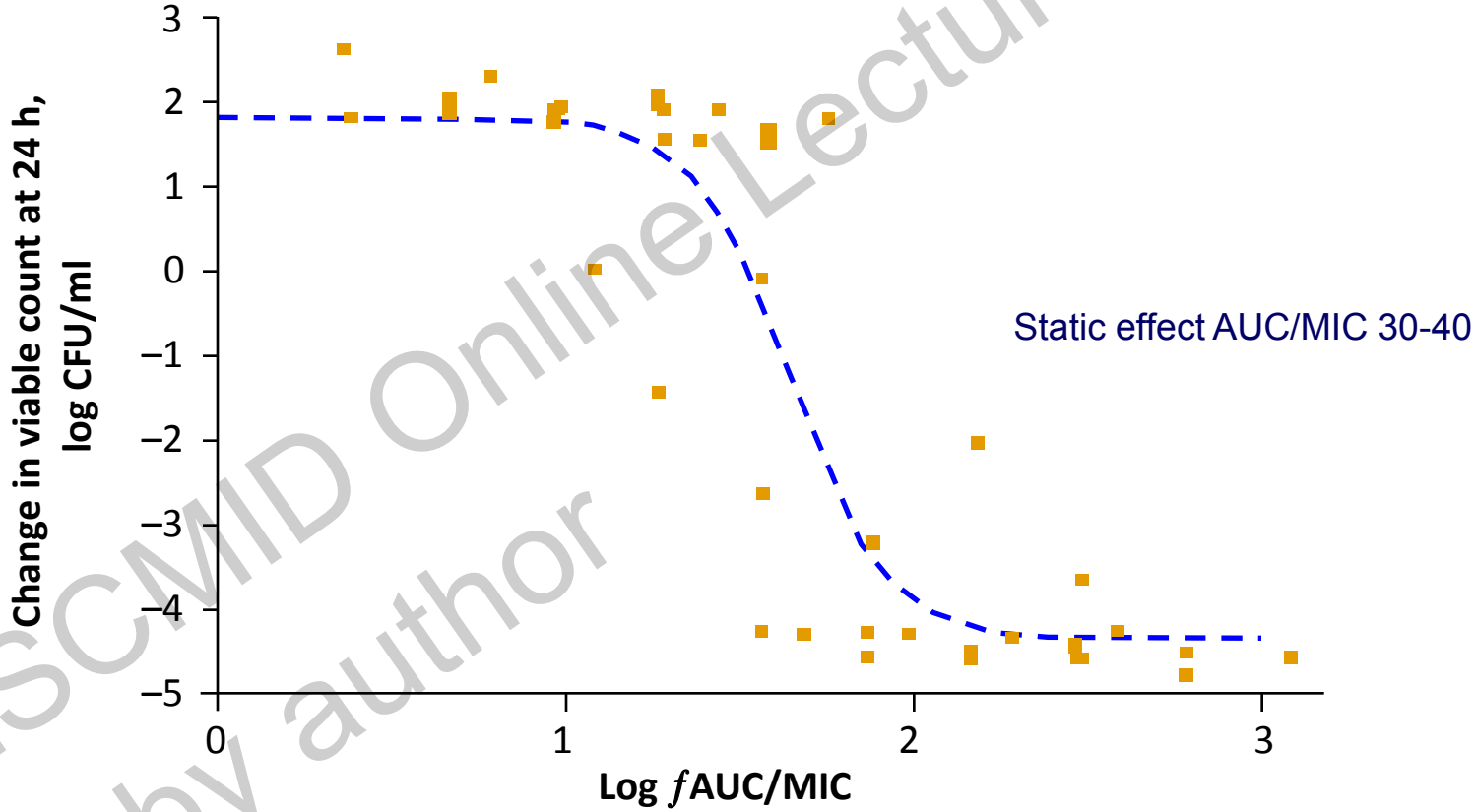
Effect of antibacterial effect measure on dominant pK/pD parameter e.g. fluoroquinolones



	Spearman rank Correlation (95% CI)
AUC/MIC v Cmax/MIC	0.77 (0.42 - 0.92)
AUC/MIC v T>MIC	0.87 (0.60 - 0.96)
Cmax/MIC v T>MIC	0.42 (0.14 - 0.77)

Exposure – response relationship

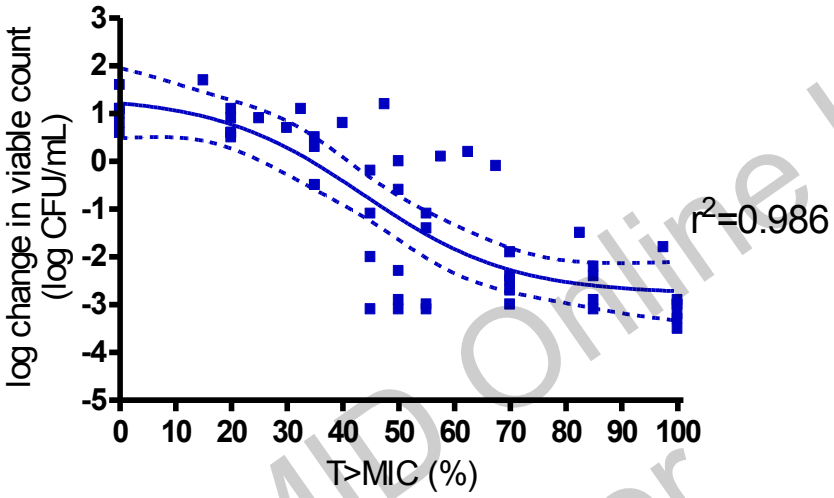
Daptomycin *f*AUC/MIC ratio and ABE for *S. aureus*



Bowker KE *et al. J Antimicrob Chemother* 2009;64:1044–1051

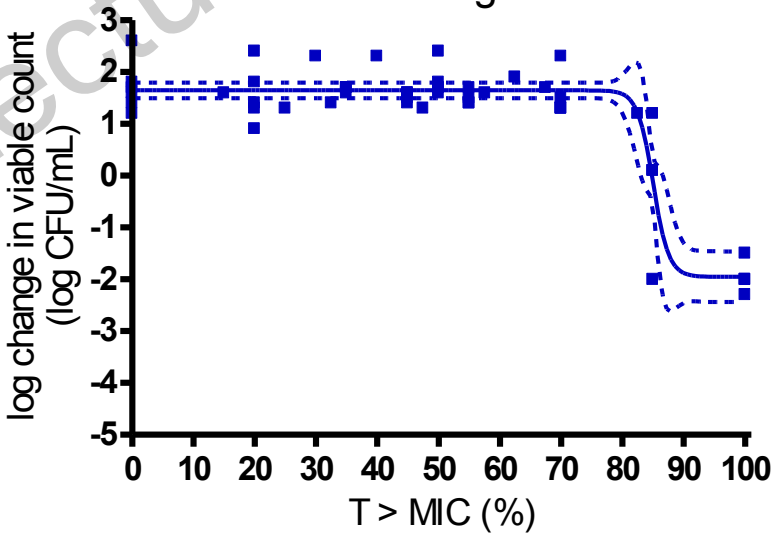
Effect of exposure

Piperacillin-tazobactam fT>MIC relationship to antibacterial effect at 24 h for P aeruginosa



Static effect, T>MIC 40%

fT>MIC relationship to antibacterial effect at 72h for P aeruginosa



Static effect, T>MIC >80%

Bowker et al, 2012

Effect of bacterial species: Ceftaroline

		T>MIC % for 24hr			
Species	n	static effect	-1 log drop	-2 log drop	-3 log drop
S.aureus					
MSSA	4	27 ± 10	31±12	28 ± 6	33 ± 9
MRSA	4	22 ± 9	25 ± 7	27 ± 6	32 ± 8
E.coli	4	35 ± 6	37 ± 7	38 ± 8	40 ± 10
K.pneumoniae	5	36 ± 8	44 ± 9	52 ± 13	85 ± 15
Citrobacter sp	2	47	49	52	54
Serratia sp	1	65	66	70	>100
*P.mirabilis**	3	39 ± 15	40 ± 26	41 ± 25	61 ± 15

*5 - >75

Noel et al, 2012, Bowker et al 2013

Inoculum - Vancomycin

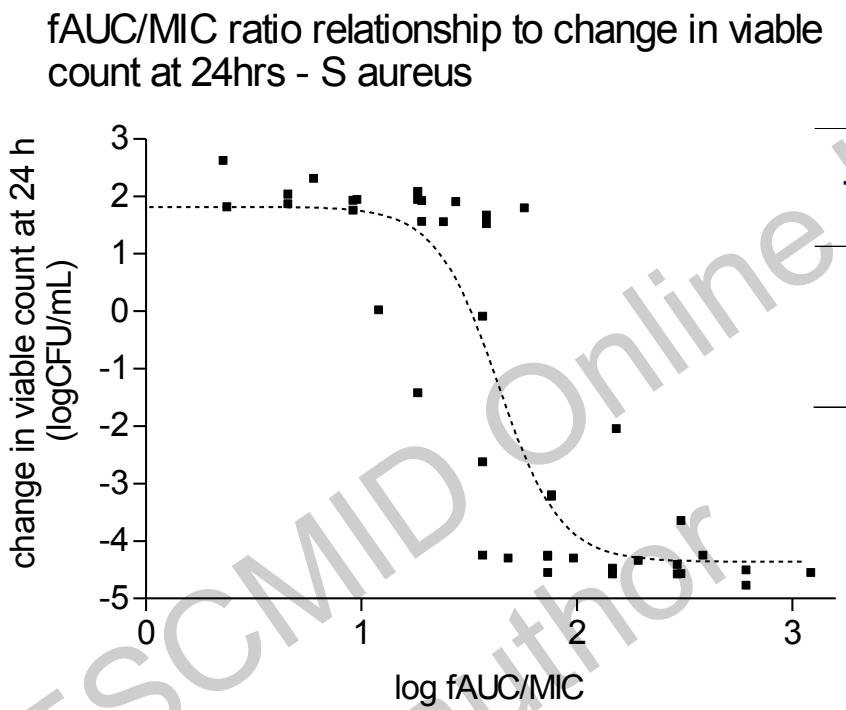
UK MRSA 15 and 16

antibacterial effect log drop at 24h	initial inoculum 10^6 CFU/ml		10^8 CFU/ml	
	dose g/d	free drug AUC/MIC	dose g/d	free drug AUC/MIC
static	0.05	17	-	-
-1 $\log_{10}$	0.1	33	0.67	213
-2 $\log_{10}$	0.4	127	0.9	294
-3 $\log_{10}$	0.95	303	>4	>1274
-4 $\log_{10}$	>4	>1274	>4	>1274

MacGowan et al, 2008

Effect of drug exposure

Daptomycin and *S.aureus*

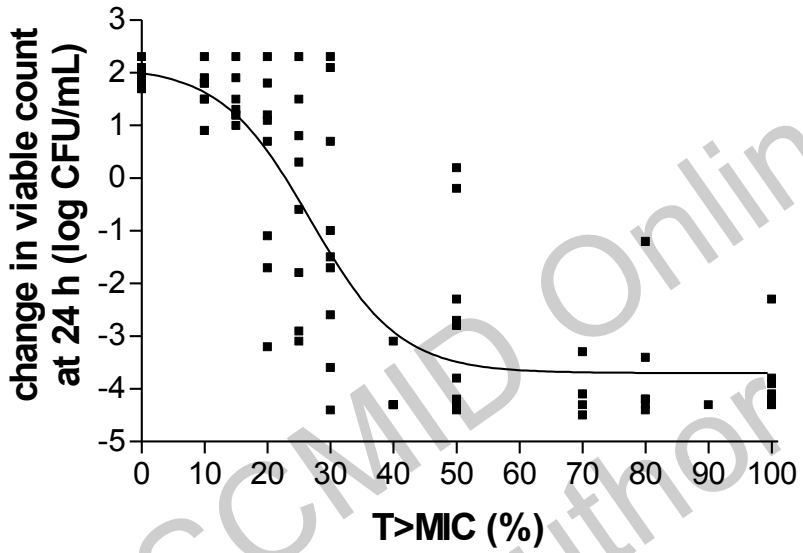


fAUC/MIC	number of experiments	% exps with growth on MICx4 plates	bacterial count on MICx4 plates
0.5-10	11	73(8)	4.3 ± 1.3
10-30	5	60(3)	3.9 ± 0.8
30-40	3	67(2)	3.6
>40	6	17(1)	4.7

Bowker et al, 2009

Effect of drug exposure

Ceftaroline and *S.aureus*
fT>MIC relationship to antibacterial effect and changes in population profile



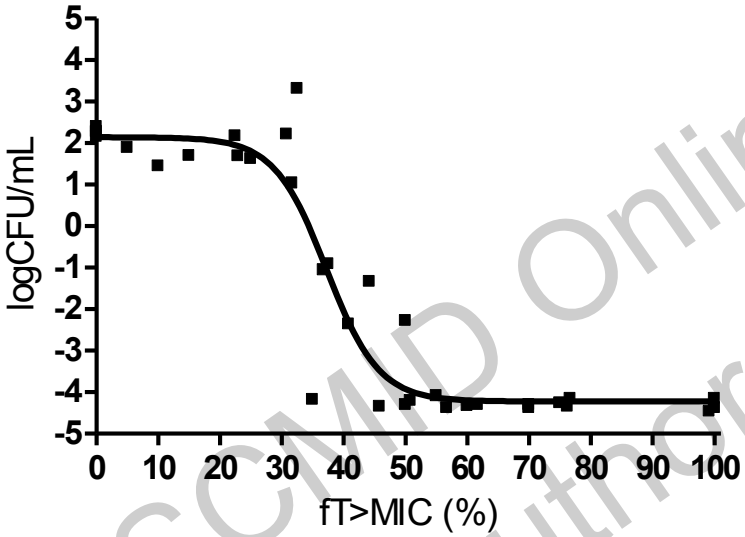
fT>MIC (%)	Number of experiments	% Exps with growth on MICx2 plates	Bacterial count on MICx2 plates
1-10	7	71(5)	6.4 ± 2.3
15-25	15	73(11)	5.9 ± 2.2
25-40	16	69(11)	6.3 ± 1.2
40-50	5	60 (3)	3.4 ± 1.6
≥50	29	7(2)	2.5

MacGowan et al, 2013

Effect of drug exposure:

Ceftaroline and E.coli
fT>MIC relationship to antibacterial effect and changes in population profiles

Relationship between fT>MIC and antibacterial effect for *E.coli* at 24h



fT>MIC	Number of experiment	%Experiment with growth on MICx4 plate	Bacterial count on MICx4 plates
1-10	2	50% (1)	2.9
11-20	3	67% (2)	3.2
21-30	3	100% (3)	4.8 ± 0.9
31-40	5	66% (3)	4.5 ± 1.2
41-50	5	20% (1)	3.4
51-60	4	25% (1)	3.4
≥61	9	0	-

Bowker et al, 2013

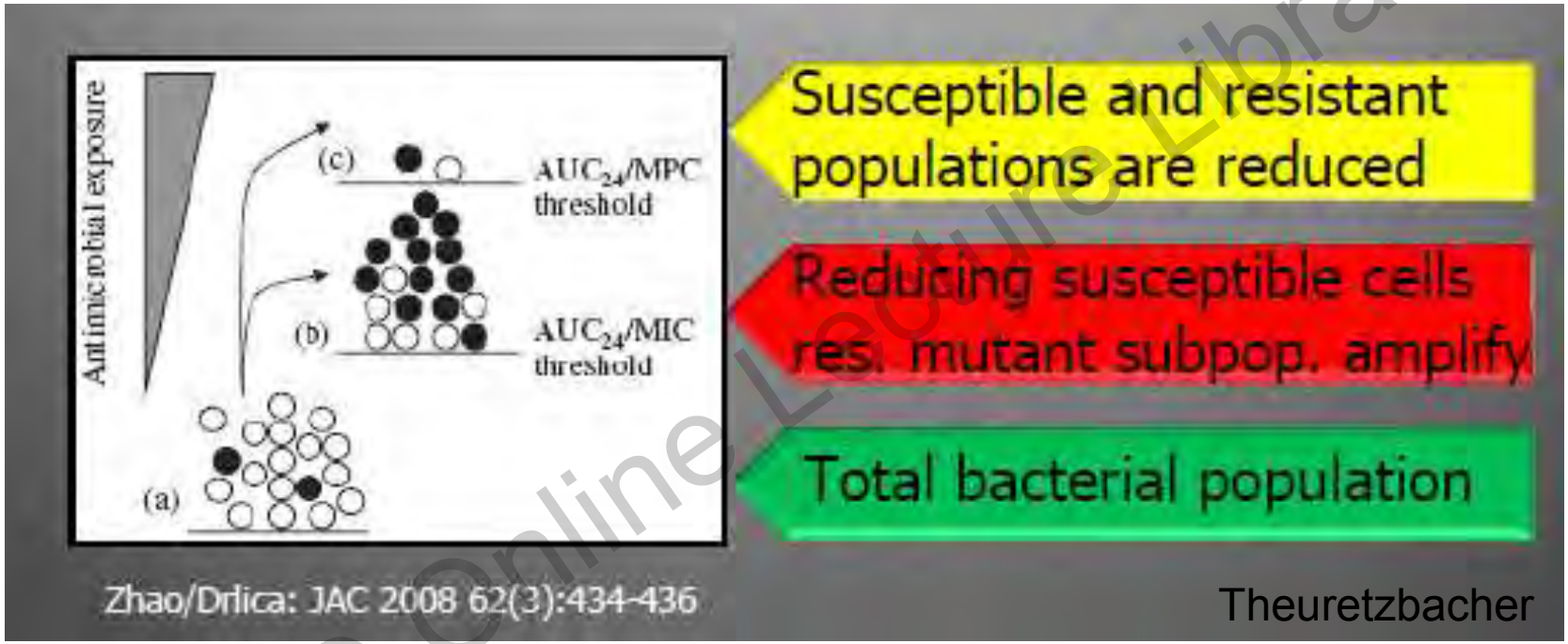
Role of in vitro models in resistance

Critical factors for selection of subpopulations

- Pathogen species/strain - heterogeneity
- Growth of organism
- Pre existing resistance mechanism (which?) - mutation ?spontaneous ?frequency
- Mixtures of S and R strains, same species
- Inoculum high inocula (10^{7-8} cfu/mL) + 360mL volumes $\Rightarrow$ large bacterial exposure 10^{10-12} cfu/mL

- Drug exposure, any drug class - how much? how often? how long?
- Flexible drug exposure; $\Rightarrow$ free drug concs associated with standard drug doses
  $\Rightarrow$ dose ranging and fractionation
- Combination therapy
- Clinical correlates - S.aureus & vancomycin

Prevention of resistant sub populations

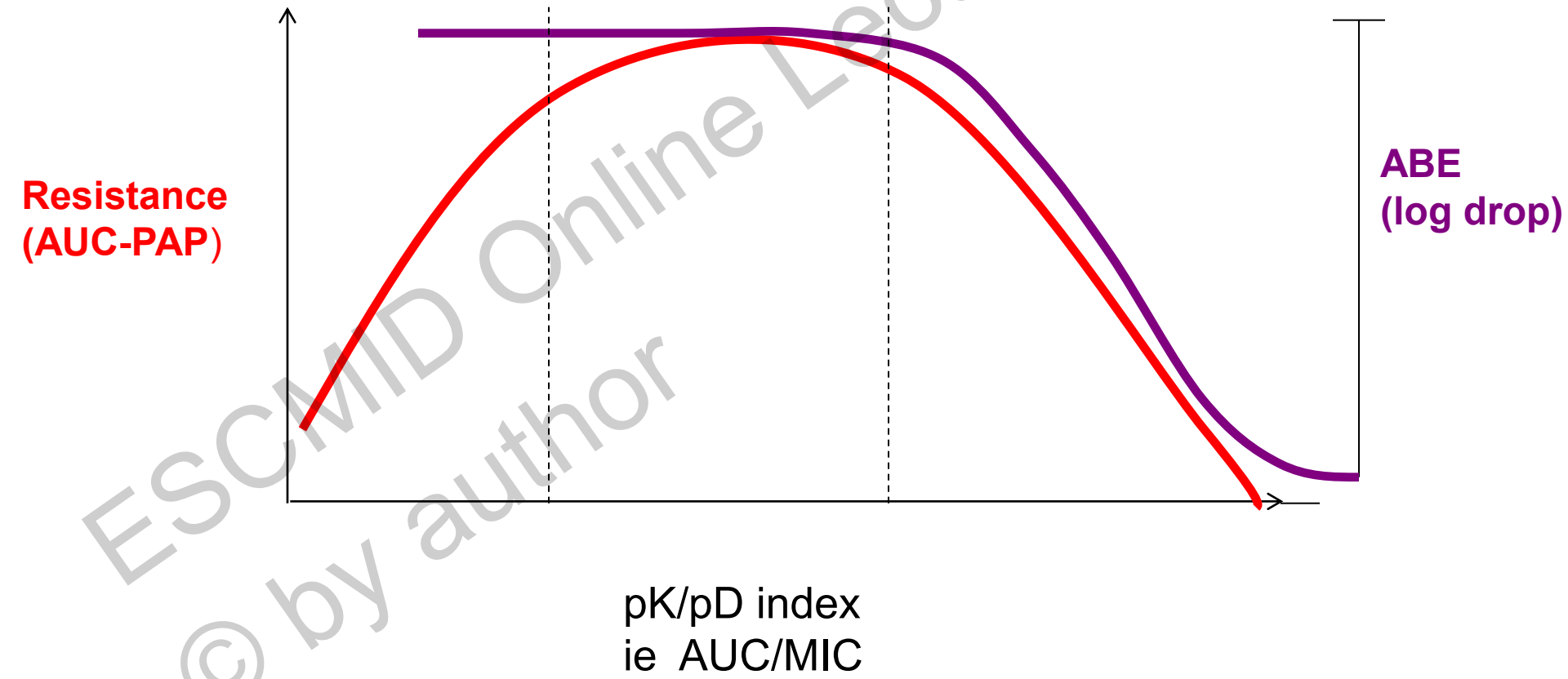


End points to measure resistance

- increase in MIC (agar dilution, gradient strip, etc.)
- population analysis profiles (PAPs)
- absolute bacterial count on plates containing antibiotic (MIC multiple; absolute concentration)

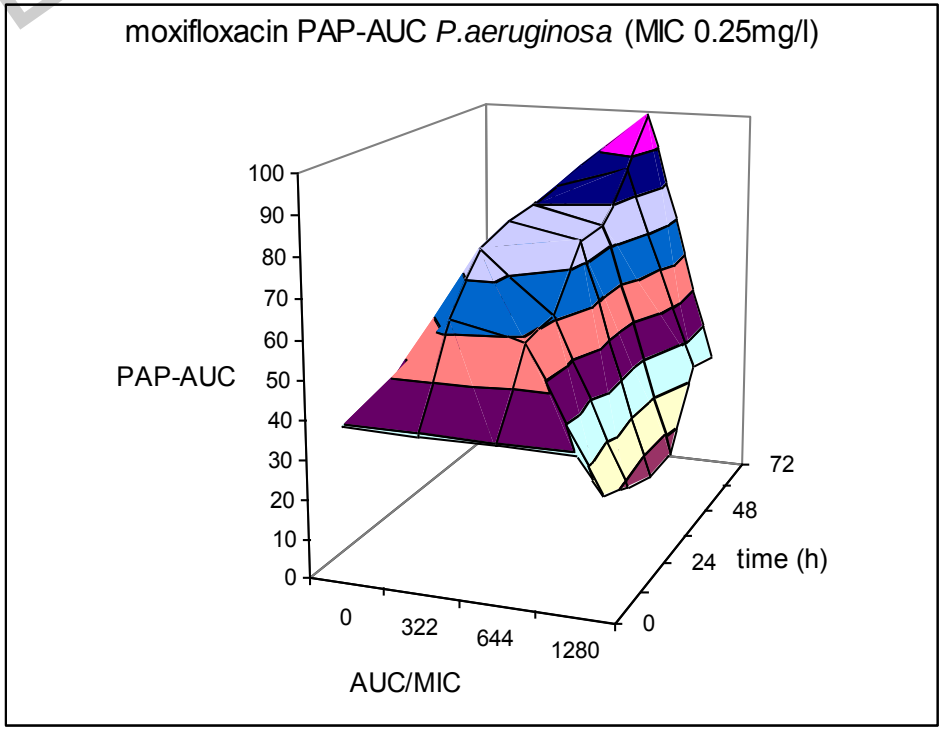
Emergence of resistance

No clearance	No clearance	clearance
No EOR	EOR	No EOR



Effect of drug exposure: emergence of resistance

dose (mg)/ MIC (mg/L)	AUC / MIC	PAP-AUC (log cfu/ml.mg/L) at 72hr
0/0.25 & 1.0	0	34 ± 17 (pre exposure)
400 12hr/1.0	320	95
800 24h/1.0	320	88 ± 13
200 24h/0.25	322	84 ± 1
400 24h/0.25	664	99 ± 2
400 12h/0.25	1280	34 ± 29
800 12h/0.25	1280	28 ± 14



Effect of inoculum and mechanism of resistance

S.pneumoniae and fluoroquinolones: Emergence of resistance after 96h on MICx4 plates

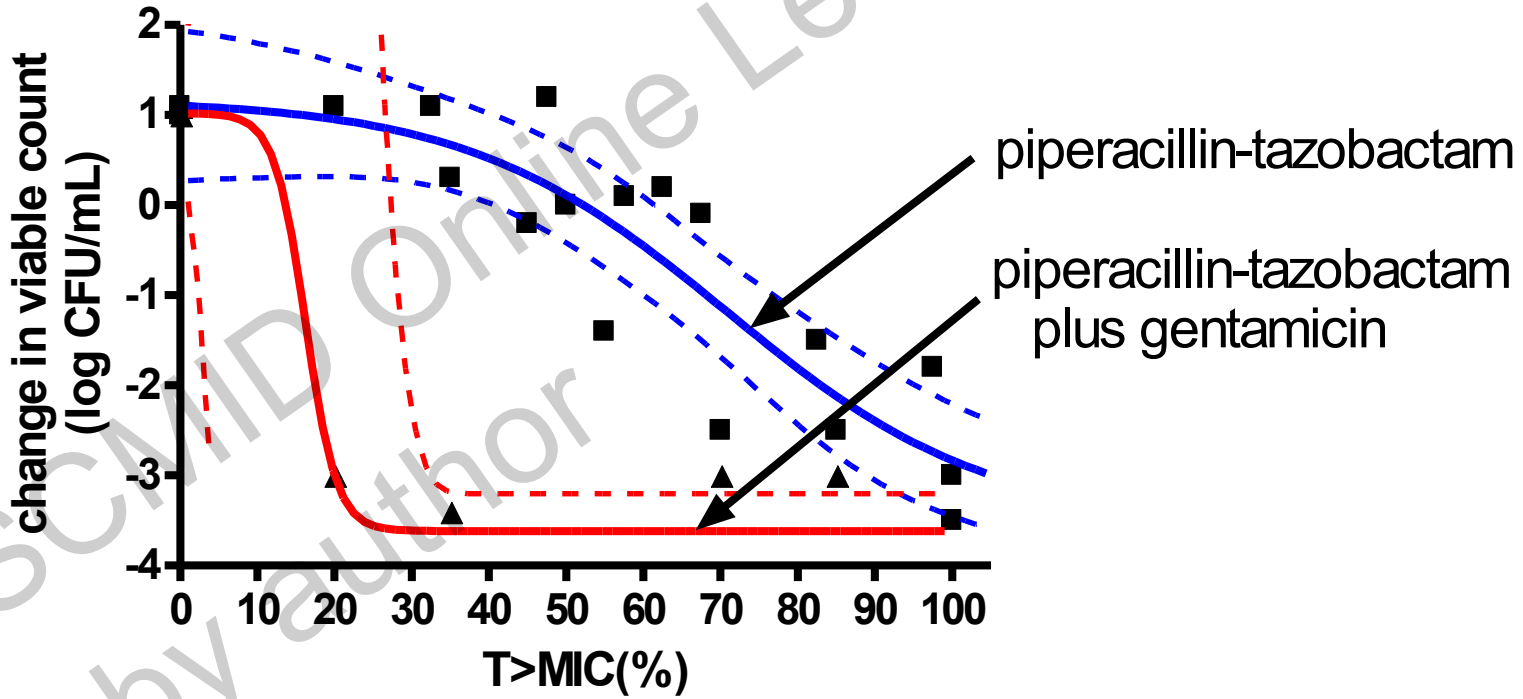
Strains/dose simulations	fAUC/MIC	innoculum (CFU/ml) 10^6 positive experiments	10^6 counts	10^8 positive experiments	10^8 counts
Strain 21843 wild type					
levo 1g/day	56	0/3	-	0/3	-
levo 0.75g/day	42	0/3	-	3/3	2.8±3.9
moxi 0.4g/day	63	0/3	-	0/10	-
Strain 21850 efflux					
levo 1g/day	112	2/3	4.5	1/3	7.2
levo 0.75g/day	84	0/5	-	4/4	7.9±0.2
moxi 0.4g/day	63	0/5	-	0/4	
Strain 21812 (par C)					
levo 1g/day	56	3/3	7.0±0.1	3/3	3.5±0.4
levo 0.75g/day	42	2/2	4.1	1/4	2.8
moxi 0.4g/day	48	3/3	6.9±1.5	3/7	4.4±2.9

Bowker et al, 2013

Effect of combination antimicrobial chemotherapy

Piperacillin-tazobactam – dose ranging for fT>MIC 0-100%, plus gentamicin 12mg/L.h

P.aeruginosa 46042 T>MIC for piperacillin-tazobactam alone and plus gentamicin (AUC 12mg/l.h)



Cochrane, unpublished

Correlation with in vivo models

	in vitro *f* T>MIC			in vivo *f* T>MIC		
	static	-1log drop	-2log drop	static	-1log drop	-2log drop
P.aeruginosa						
doripenem	25 ± 11	30 ± 11	35 ± 11	29 ± 5	-	44.7 ± 7
ceftolozane	24.9	26.6	31.2	24.0 ± 3.3	31.5 ± 3.9	-
E.coli						
ceftolozane	27.8 ±5.6	33 ± 5.6	39.4 ± 8.9	28	32.6	-
ceftaroline	35 ± 6.3	36.8 ± 7.1	38.3 ± 8.3	28 ± 9	41 ± 11	54.3 ±21
S.aureus						
ceftaroline	24.5 ± 8.9	27.8 ± 9.5	27.7 ± 5.7	26 ± 8	33 ± 9	45 ± 13

Andes & Craig

Conclusions

- Simple descriptive effects of simulated doses
- Allow identification of dominant pD index and size for effect
- pK/pD provides a rational framework for deciding drug doses, dosing regimens target, pathogens and clinical breakpoints
- pK/pD allows for prediction of therapeutic effect and provides a rationale to explain drug performance pK/pD may allow for performance of small therapeutic trials to establish efficacy (single RCT per indication)
- pK/pD can justify drug use in rare or difficult pathogens/indications (getting clinical cases very difficult)
- pK/pD of antibacterials can be applied to antivirals/antifungals
- a knowledge of a drugs pK/pD is essential in terms of drug licensing