ESCMID MANAGING INFECTIONS PROMOTING SCIENCE

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

MIC Distributions and ECOFFs:

Their role in setting (clinical) breakpoints

Distributions vs Percentiles

- It is common practice to present MIC data as MIC_{50}s and MIC_{90}s or similar.
- This practice hides a lot of useful information, and is <u>not recommended</u>

0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64
1	29	357	48	5	0	0	3	37	199	177	24

MIC_{50} MIC_{90}

MIC Distributions

- Every bug-drug combination has an MIC distribution
  - i.e. not a single MIC value for each category of S, I or R
- This distribution consists of the MICs of both
  - Wild-type isolates
  - Isolates with acquired resistance mechanism(s)
- Wild-type isolates are those without acquired resistance (which is phenotypically-expressed)

MIC Distributions

- The wild-type distribution arises because there is
  - Assay variation: **the main cause**
  - Strain to strain (biological) variation: a minor cause
- Wild type distributions are log-normally distributed
  - Wild type MIC distributions are typically 3-5 two-fold dilutions wide

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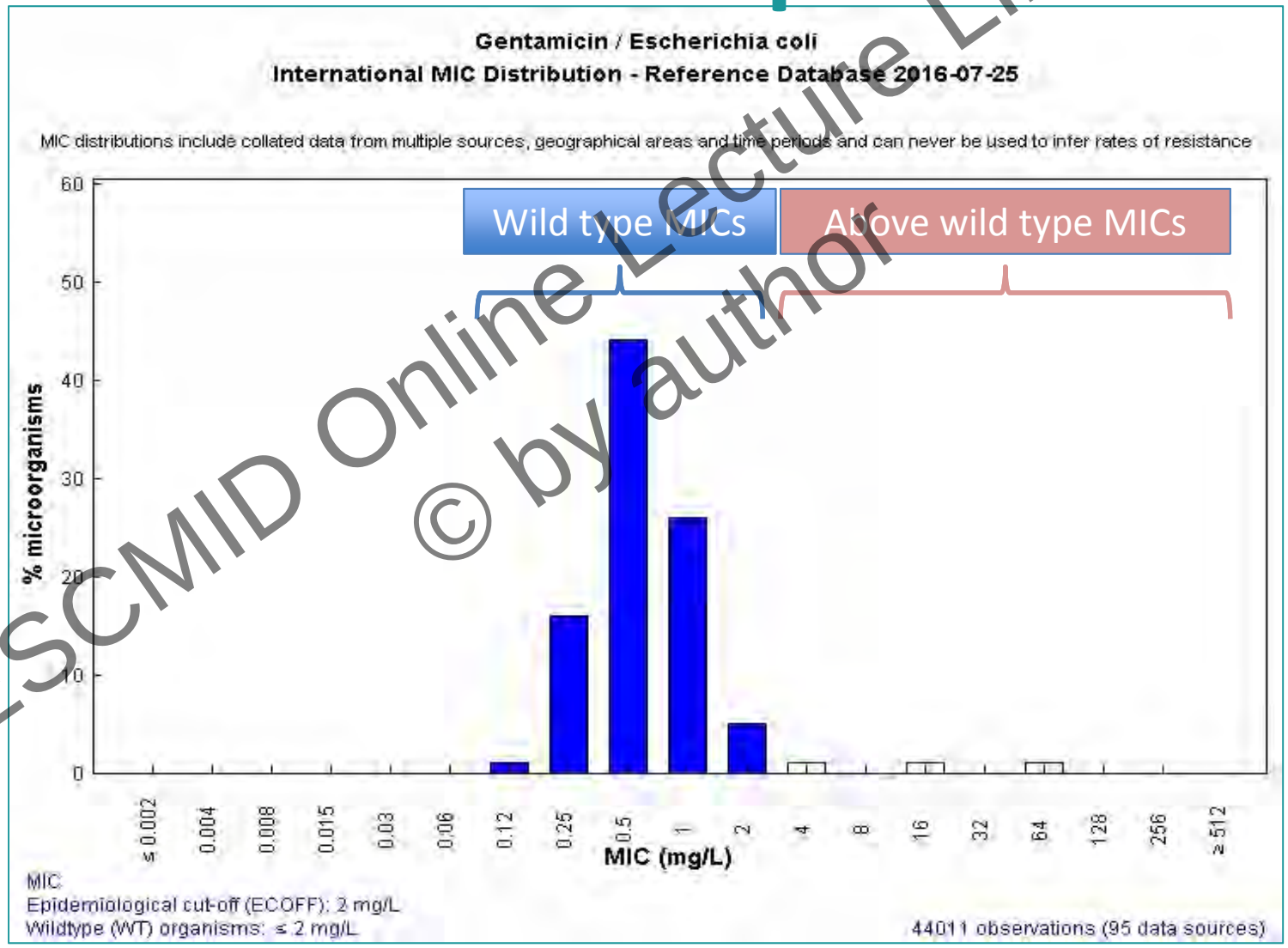
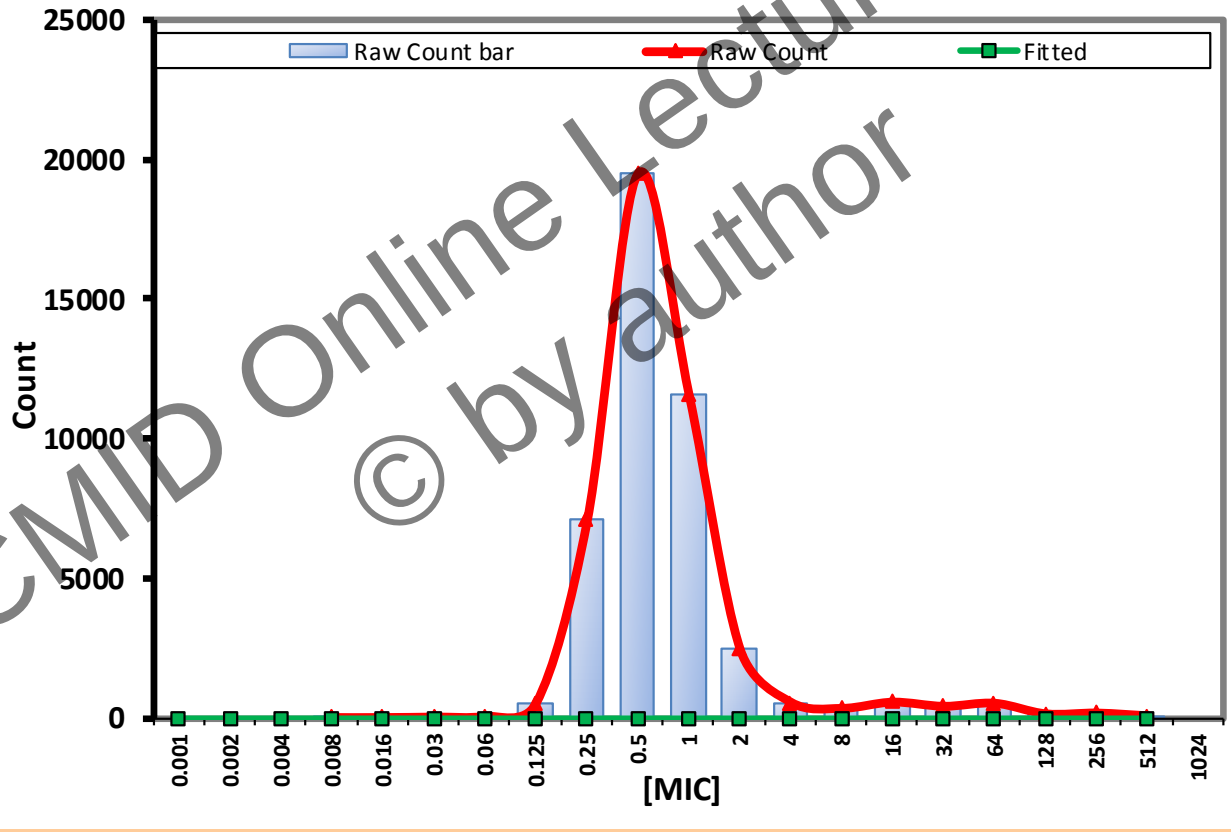
Examples

	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF
Corynebacterium amycolatum	0	0	0	0	0	0	1	1	0	0	0	2	4	4	0	0	0	0	0	ND
Corynebacterium jeikeium	0	0	0	0	0	2	0	0	0	0	0	0	0	0	10	0	0	0	0	ND
Corynebacterium pseudodiphtheriticum	0	0	5	1	1	2	1	0	0	0	1	0	0	0	0	0	0	0	0	ND
Corynebacterium spp	0	0	5	1	3	6	2	2	1	1	5	9	10	5	15	0	0	0	0	ND
Corynebacterium striatum	0	0	0	0	0	0	0	0	0	1	3	3	4	1	0	0	0	0	0	ND
Corynebacterium urealyticum	0	0	0	0	0	1	0	1	0	0	0	4	1	0	5	0	0	0	0	ND
Enterobacter aerogenes	0	0	0	0	3	0	12	155	823	677	179	52	36	10	3	12	3	16	48	2.0
Enterobacter agglomerans	0	0	0	0	0	1	5	43	5	0	0	0	0	0	0	0	0	0	0	ND
Enterobacter amnigenus	0	0	0	0	0	0	0	2	4	1	1	0	0	0	0	0	0	0	0	ND
Enterobacter cloacae	1	0	2	6	37	179	397	1238	3670	1389	163	94	126	96	19	104	50	52	60	2.0
Enterobacter dissolvens	0	0	0	0	0	0	0	11	8	0	0	0	0	0	0	0	0	0	0	ND
Enterobacter gergoviae	0	0	0	0	0	0	0	2	3	2	0	0	0	0	0	0	0	0	0	ND
Enterobacter intermedium	0	0	0	0	0	0	0	1	2	0	0	0	0	1	0	0	0	0	0	ND
Enterobacter sakazakii	0	0	0	0	0	0	0	6	14	7	3	0	1	0	0	0	0	0	0	ND
Enterobacter spp	0	0	0	0	0	1	7	219	667	298	34	14	5	6	5	11	7	4	75	2.0
	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF
Enterobacter taylorae	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	ND
Enterococcus faecalis	0	0	0	0	0	2	2	7	19	38	77	192	898	1829	720	64	59	74	802	32.0
Enterococcus faecium	0	0	0	1	0	0	1	4	9	22	91	598	1182	580	94	13	11	46	320	32.0
Enterococcus hirae	0	0	0	0	0	0	0	0	1	1	50	98	234	151	28	0	0	0	2	ND
Escherichia coli	0	0	9	7	27	52	526	7074	19520	11576	2456	553	338	561	412	519	143	168	70	2.0
Haemophilus influenzae	0	0	0	0	0	0	0	10	19	54	35	15	0	0	0	0	0	0	0	4.0
Hafnia alvei	0	0	0	0	0	0	3	11	13	1	0	0	0	0	0	0	0	0	0	ND
Klebsiella pneumoniae	0	0	4	4	11	13	174	2034	6242	2225	316	233	269	273	169	192	164	186	93	2.0
Klebsiella spp	0	0	0	1	5	23	83	456	595	208	11	5	10	4	5	5	5	4	0	2.0
Lactobacillus acidophilus	0	0	0	0	0	0	0	0	1	1	4	3	1	1	0	0	0	0	0	ND
Lactobacillus amylovorus	0	0	0	0	0	0	0	0	1	0	4	13	12	1	0	0	0	0	0	ND
Lactobacillus crispatus	0	0	0	0	0	0	0	0	0	2	0	3	1	1	0	0	0	0	0	ND
Lactobacillus delbrueckii	0	0	0	0	0	0	0	0	5	1	9	17	12	0	0	0	0	0	0	ND
Lactobacillus fermentum	0	0	0	0	0	0	0	0	0	0	0	15	6	0	0	0	0	0	0	ND
Lactobacillus gallinarum	0	0	0	0	0	0	0	0	2	1	4	1	0	0	0	0	0	0	0	ND

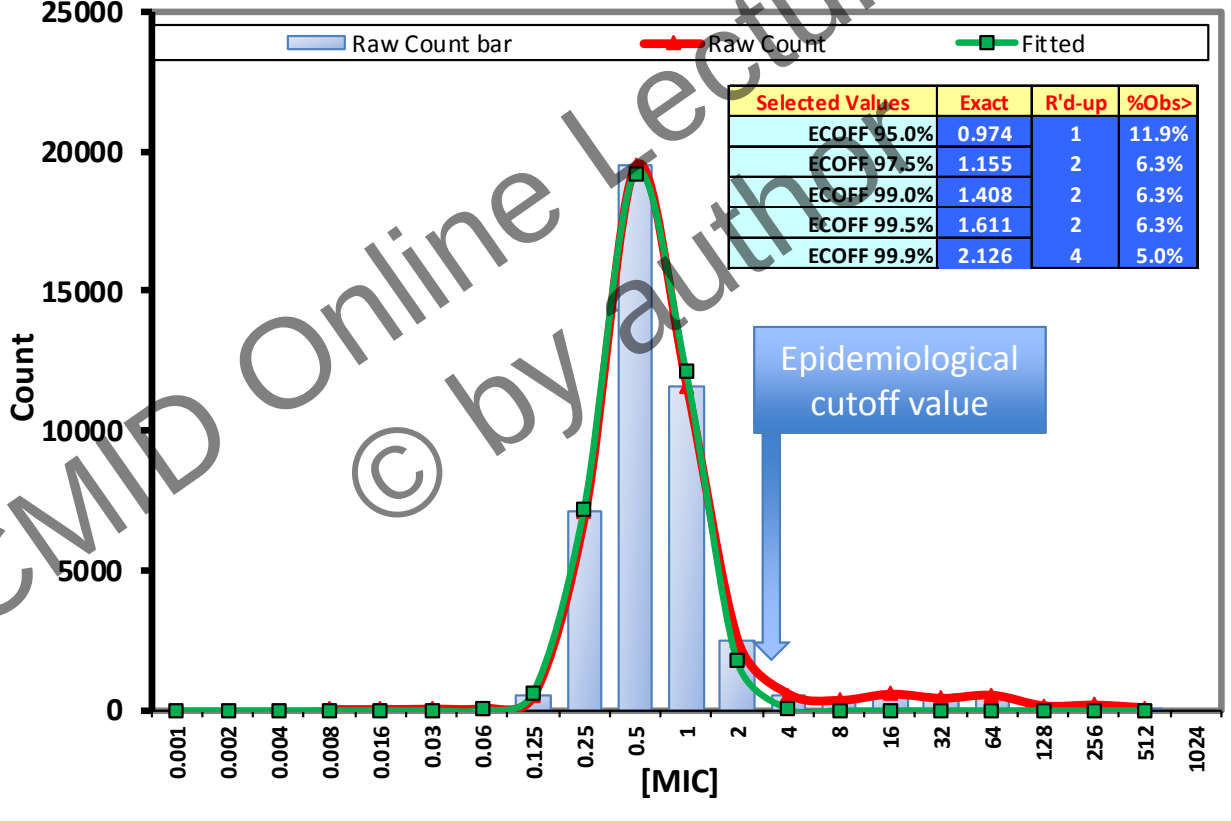
One example…



Wild type log-normal distribution



Wild type log-normal distribution



ECOFF (ECV) Definition

- An epidemiological cutoff value is the MIC (or other similar quantitative measure of bug-drug interaction) that has the highest probability of distinguishing the wild-type population from the non-wild-type population

Basic Assumptions

- WTs & ECOFFs are a feature of a single species
  - they cannot be applied or extrapolated to a genus or other larger grouping

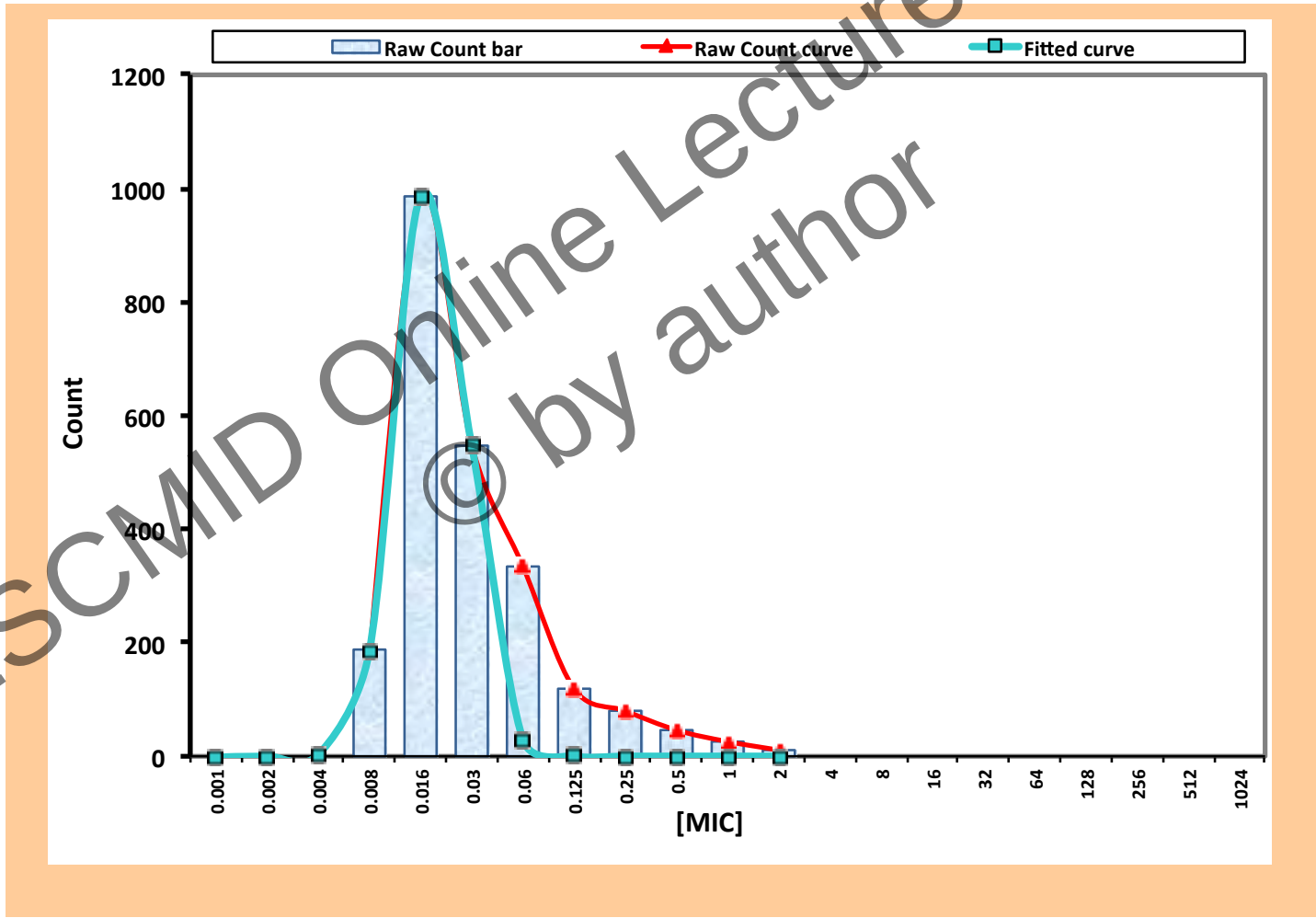
- WTs & ECOFFs are "the same everywhere"; they do not vary
  - over time
  - geographically
  - between sources: humans, animals, environment

Basic Features

- Wild type distributions are log-normally distributed
  - If they are not, then there is likely to be overlap with a non-wild type population, or there is a problem with speciation
- There is MIC assay variation both
  - Within laboratories (intra-laboratory variation)

    and
  - Between laboratories (inter-laboratory variation)

Skewed distribution example



Intra- and Inter-laboratory variation

Intra-laboratory

Inter-laboratory

	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64
Enterobacter cloacae	0	0	51	98	241	167	87	37	38	32	8	2	1	1	0	1
Enterobacter cloacae	2	8	13	15	2	1	2	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	2	5	25	14	6	3	1	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	1	19	26	22	16	5	1	2	0	1	1	0	0	0	0	0
Enterobacter cloacae	2	20	24	14	3	2	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	6	17	11	8	1	0	0	0	0	1	0	0	0	0	0	0
Enterobacter cloacae	2	13	37	12	5	2	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	13	3	21	33	13	3	1	2	1	0	0	0	0	0	0	0
Enterobacter cloacae	3	11	27	27	13	4	3	0	1	1	0	0	0	2	0	0
Enterobacter cloacae	0	4	24	28	22	7	2	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	0	1	37	24	20	9	3	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	0	0	7	11	5	2	1	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	2	12	12	5	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	2	4	13	6	5	0	3	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	2	7	9	4	2	1	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	6	22	8	5	0	0	0	0	0	0	0	0	0	0	0	0

Basic Features

- Intra-laboratory and Inter-laboratory variation can be reduced but never entirely eliminated
- Due to assay variation **there will never be perfect correlation** between the absence/presence of molecular resistance data and ECOFFs
- For mutational resistance it is essential to distinguish between polymorphisms and mutations generating phenotypic ‘resistance’

EUCAST Data Requirements

- The MICs should ideally have been measured with a **reference** method or one calibrated to it
  - ISO 20776-1 in the case of bacteria
  - ISO 16256 in the case of yeasts
  - Other reference methods as they are developed and agreed upon internationally

EUCAST Data Requirements

- All the MICs for the putative wild-type population should, as far as is feasible, be **on-scale**
  - a small proportion of wild-type population values could be included as "≤"
    - ideally it should be <5%, although it is possible to provide a reasonable estimate of ECOFFs if the mode is not also the lowest concentration tested
  - ">" and "≥" values are acceptable in the data set provided they are clearly separated from the wild-type population

EUCAST Data Requirements

- The data must have an obvious single mode in the putative wild-type population
- There are at least 15 MIC values in the putative wild-type population
- At least 5 acceptable MIC distributions should be available before setting an ECOFF
- The modes of the acceptable distributions should be within one two-fold dilution of the most common putative wild-type mode
- If one distribution contributes more than 50% of values, additional distributions should be sought

Data Pooling

- Because there is known variation between laboratories, as well as within laboratories, data from **several laboratories** are required for estimation of ECOFFs to ensure variation is accounted for
- The predictive power of ECOFFs increases as the number of laboratories increases
  - a working rule at the present is a minimum of **5 labs** preferably with at least 35 presumptive wild-type values although a total of **≥100** overall is usually satisfactory

Issues with Pooling

CRNEO	Amphotericin B										
MIC	Log_2MIC	Gua	Cue	Os	An	Fal	Ami	Ter	Br	Mi	Con
0.0009765625	-10										
0.001953125	-9										
0.00390625	-8										
0.0078125	-7										
0.015625	-6										
0.03125	-5		2				1	1			
0.0625	-4		22				2	2	31		9
0.125	-3		59	2	6		34	4	25	2	25
0.25	-2	2	83	12	102	9	176	31	222	21	10
0.5	-1	7	21	10	97	50	84	41	300	267	
1	0	3	17	53	45	14	5	43	438		
2	1		1	9	4	2		2	13		
4	2		1							2	
8	3										
16	4										
32	5										
64	6										
128	7										
256	8										
512	9										
	Total	12	206	24	267	108	313	84	623	743	44
	Log_2 Mean	-1.4167	-2.7282	-2.1667	-1.6610	-1.0926	-2.2540	-2.0238	-2.0104	-0.9011	-3.4773
	Log_2 SD	0.6686	1.1440	0.6370	0.8844	0.6976	0.7805	0.8709	0.8873	0.6038	0.6643
	Log_2 Mode	-1	-2	-2	-2	-1	-2	-1	-1	0	-3
	Log_2 Median	-1	-2	-2	-1	-1	-2	-1	-1	0	-3
	Group	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non

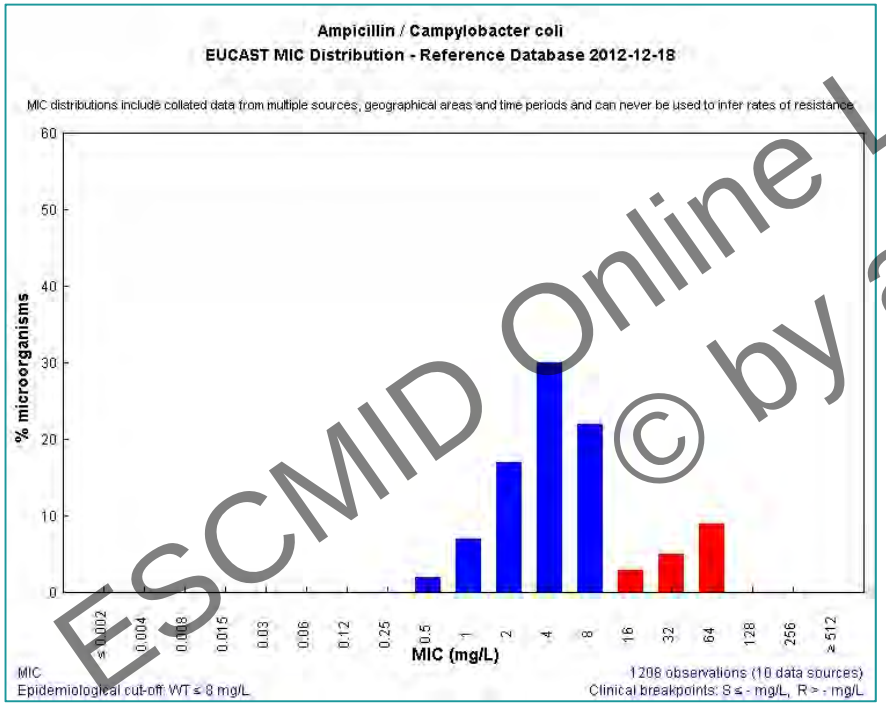
Issues with Pooling

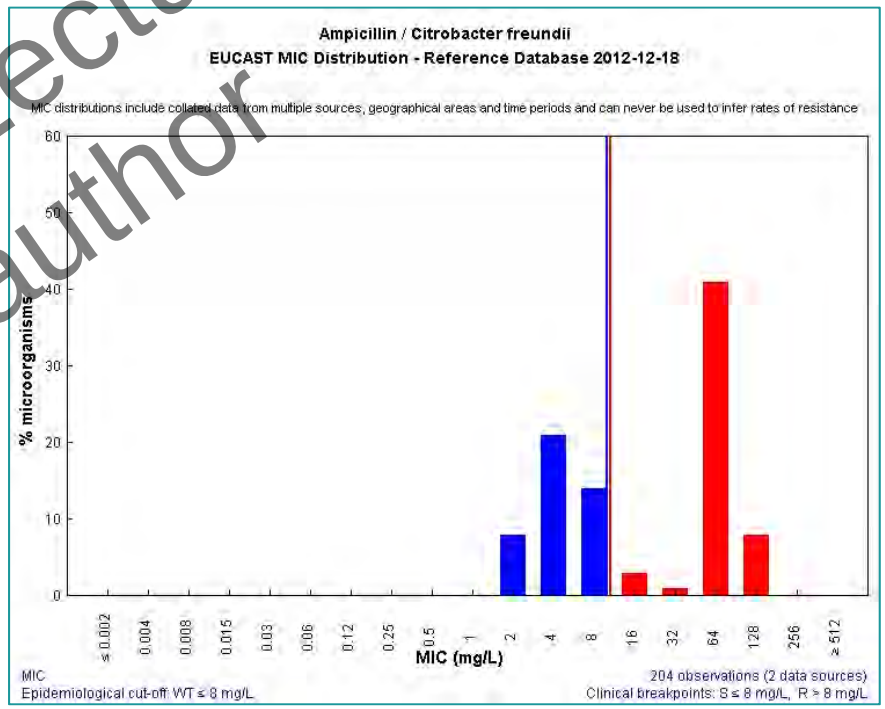
CRNEO	Amphotericin B										
MIC	Log_2MIC	Gua	Cue	Os	An	Ful	Ann	Ter	Br	Mi	Con
	t-test p=	0.3302	0.0000	0.0315	0.4687	0.0000	0.0000	0.0062	0.0000	0.0000	0.0000
Comp Gp	`	2412	2218	2400	2157	2316	2111	2340	1801	1681	2380
	Mean	-1.2106	-1.1145	-1.2046	-1.2151	-1.2379	-1.1284	-1.1979	-1.1049	-1.5663	-1.1765
	SD	1.0407	0.9767	1.0416	1.0569	1.0437	1.0486	1.0432	1.0675	0.9901	1.0164
	t-test p=	0.4833	0.0000	0.0000	0.0000	0.1523	0.0000	0.0000	0.0000	0.0000	0.0000
	ECV 95.0		0.5		1	1	0.5		1	1	
	ECV 97.5		1		1	2	0.5		1	2	
	ECV 99.0				2	2	1		1	2	
	ECV 99.9		2		2	2	1		2	2	
	% 95.0		9.2		3.4	3.7	5.1		0.3	2	
	% 97.5		1.0		3.4	0	5.1		0.3	0.3	
	% 99.0		1.0		0	0	0.6		0.3	0.3	
	% 99.9		0.5		0	0	0.6		0	0.3	
	Mean est		-2.8		-1.75	-1.08	-2.32		-1.93	-0.87	
	SD Est		0.991		0.835	0.652	0.586		0.719	0.461	

Estimation Methods

- The "visual" method (Kahlmeter)
- The 95% rule (Pfaller)
- The Normalised Resistance Interpretation (Kronvall)
- The iterative statistical method (Turnidge)
- Multimodal analysis (Meletiadis)
- Cluster analysis (Cantón)

The "Visual" Method





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Iterative Statistical Method

ORIGINAL ARTICLE 10.1111/j.1469-0691.2006.01377.x

Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values

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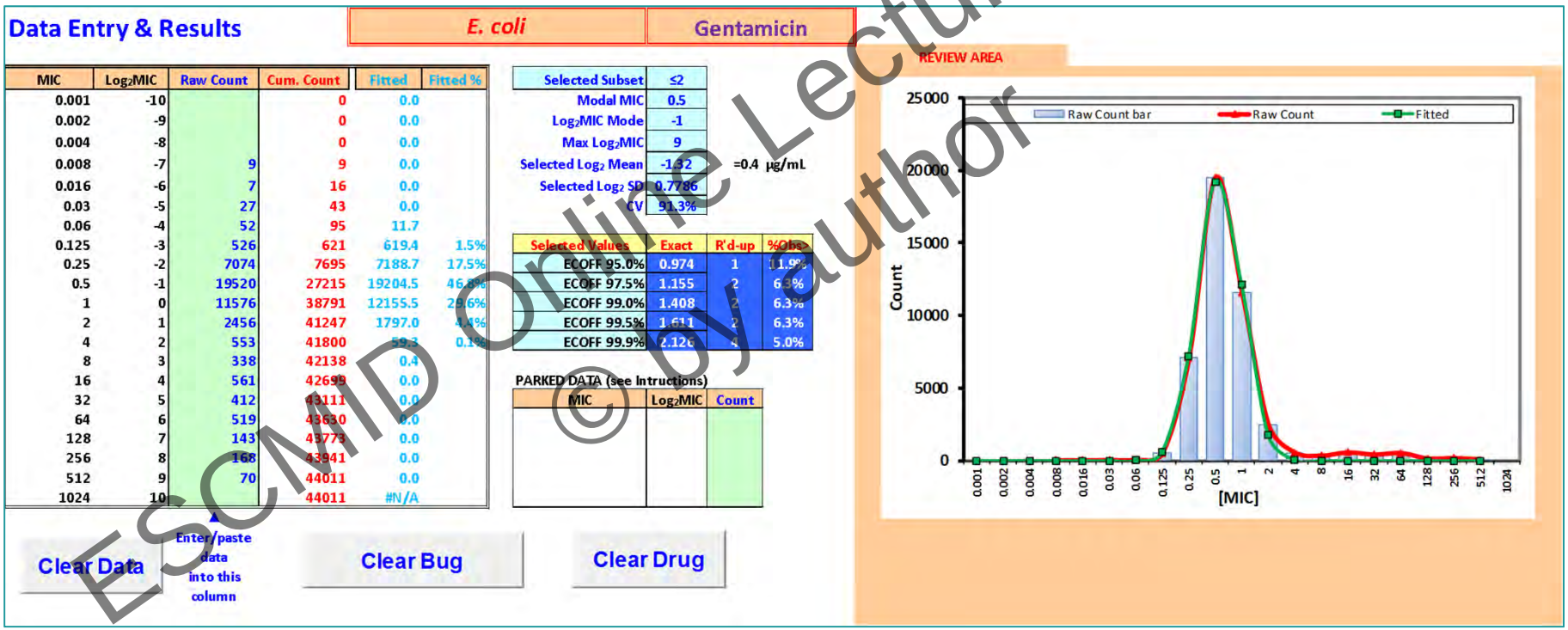
Iterative Statistical Method



Why we need ECOFFs

- To give us a 'definition' of the wild-type population
- To assist in the detection of emerging mechanisms of resistance
- To provide an anchor point for breakpoint setting

Setting Breakpoints

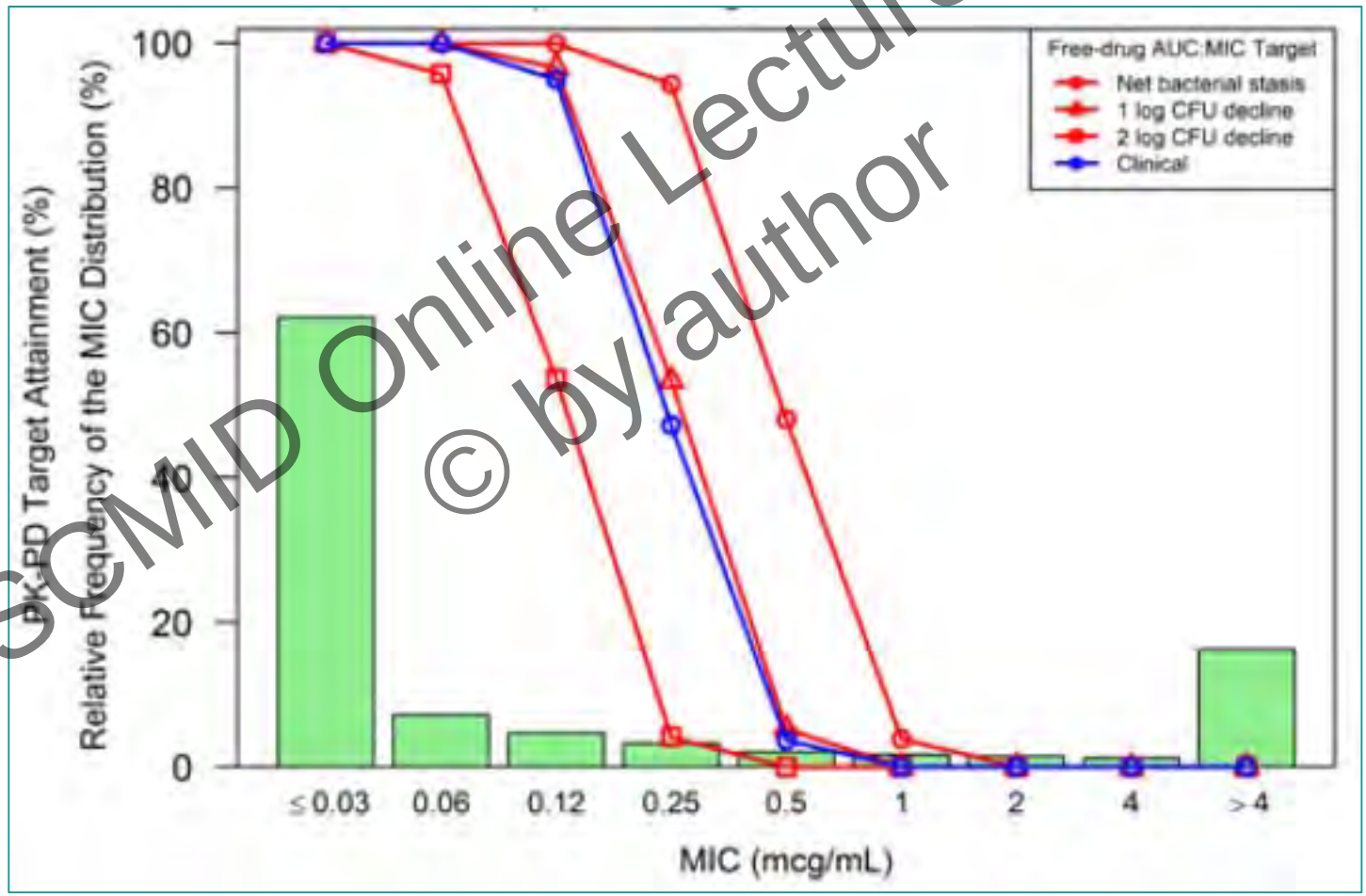
CLINICAL MICROBIOLOGY REVIEWS, July 2007, p. 391–408 Vol. 20, No. 3
0893-8512/07/$08.00+0 doi:10.1128/CMR.00047-06

Setting and Revising Antibacterial Susceptibility Breakpoints

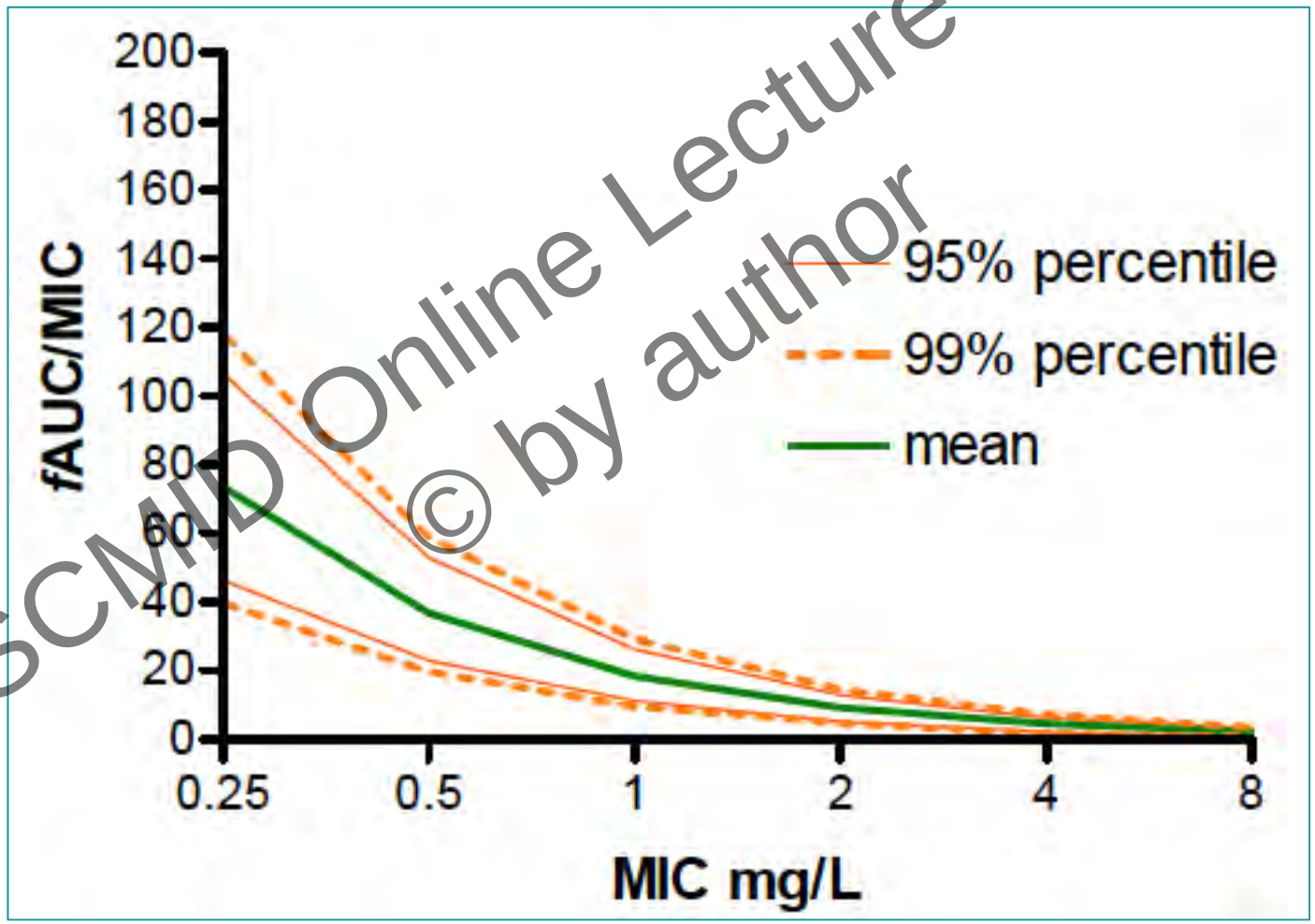
John Turnidge[1]* and David L. Paterson[2,3,4]

- Data requirements for breakpoints
  - MIC distributions and ECOFFs
  - PK-PD cutoffs for animal model data and Phase I/II PK studies in humans – target attainment
  - Clinical cutoffs
    - MICs versus outcomes
    - Full PK-PD study of exposures versus outcomes

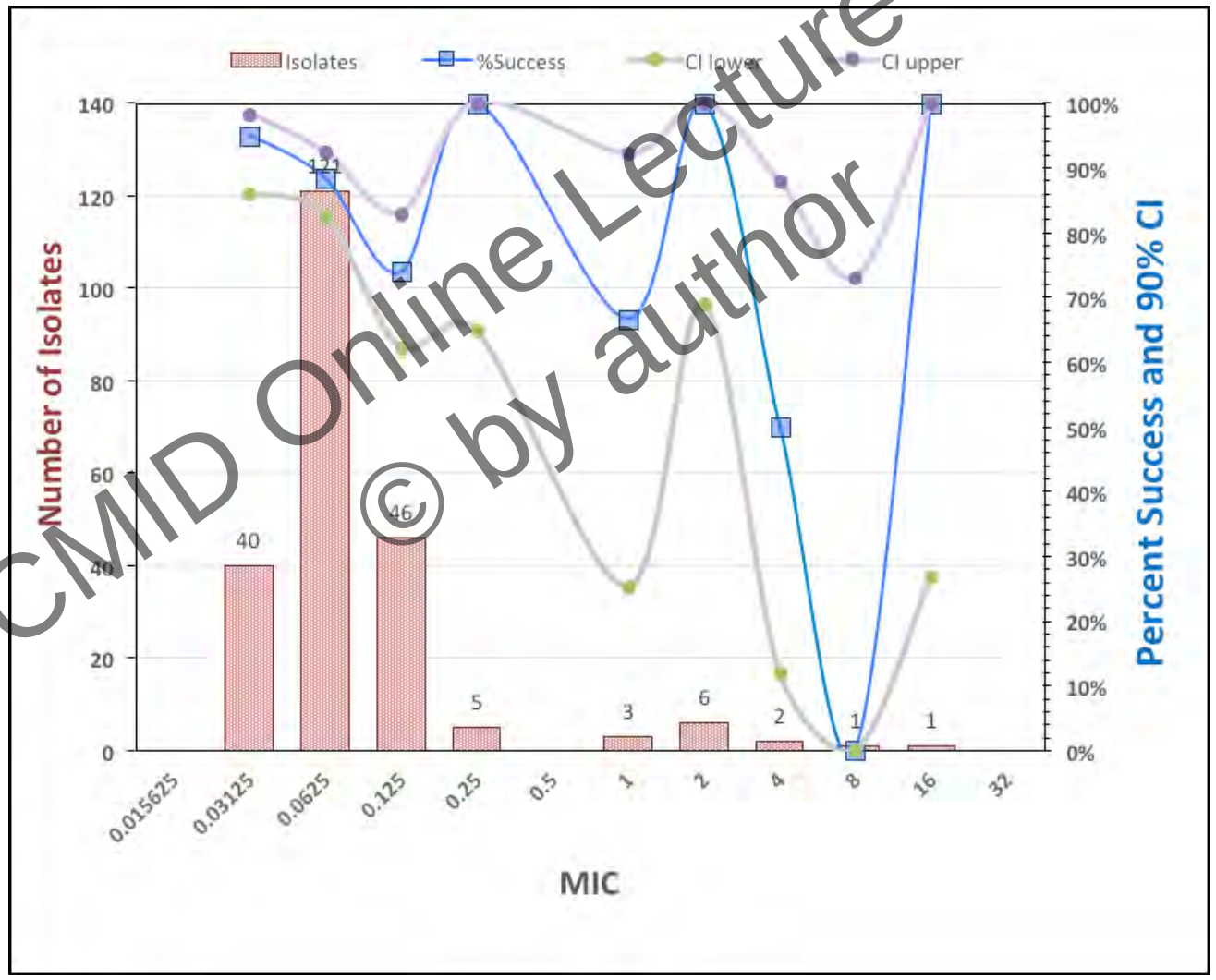
PK/PD Target Attainment – CLSI style



PK/PD Target Attainment – EUCAST style



Clinical Outcome vs MIC



Setting Breakpoints

- Examine each of the cutoffs and decide where the breakpoint should be set
  - No fixed way of achieving this, but…
- Wherever possible, avoid setting a breakpoint below the ECOFF!
  - Assay variation will create significant problems for the laboratory in interpreting results correctly when result is at or near that breakpoint

Importance of ECOFF

- Susceptibility testing and detecting emerging resistance cannot be done without it
- It is the lowest possible breakpoint!