Automated AST – a curse?

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Is it a curse? Of course not!! Vincent is right! It is a blessing!

That is….. When the machine does what we want it to do, automatically, immediately, with the right output, is always updated with the latest changes in breakpoints or ”rules”, new antibiotics, with all species and drugs available, with no delays in development and …..

…then Vincent is right!

Interactive session

- Everyone in the audience with an automated AST device – raise a hand!

- Who has a Microscan – raise a hand!
- Who has a Phoenix – raise a hand!
- Who has a Vitek2 – raise a hand!
- Who has another automated AST device?

Now use the blue voting card!

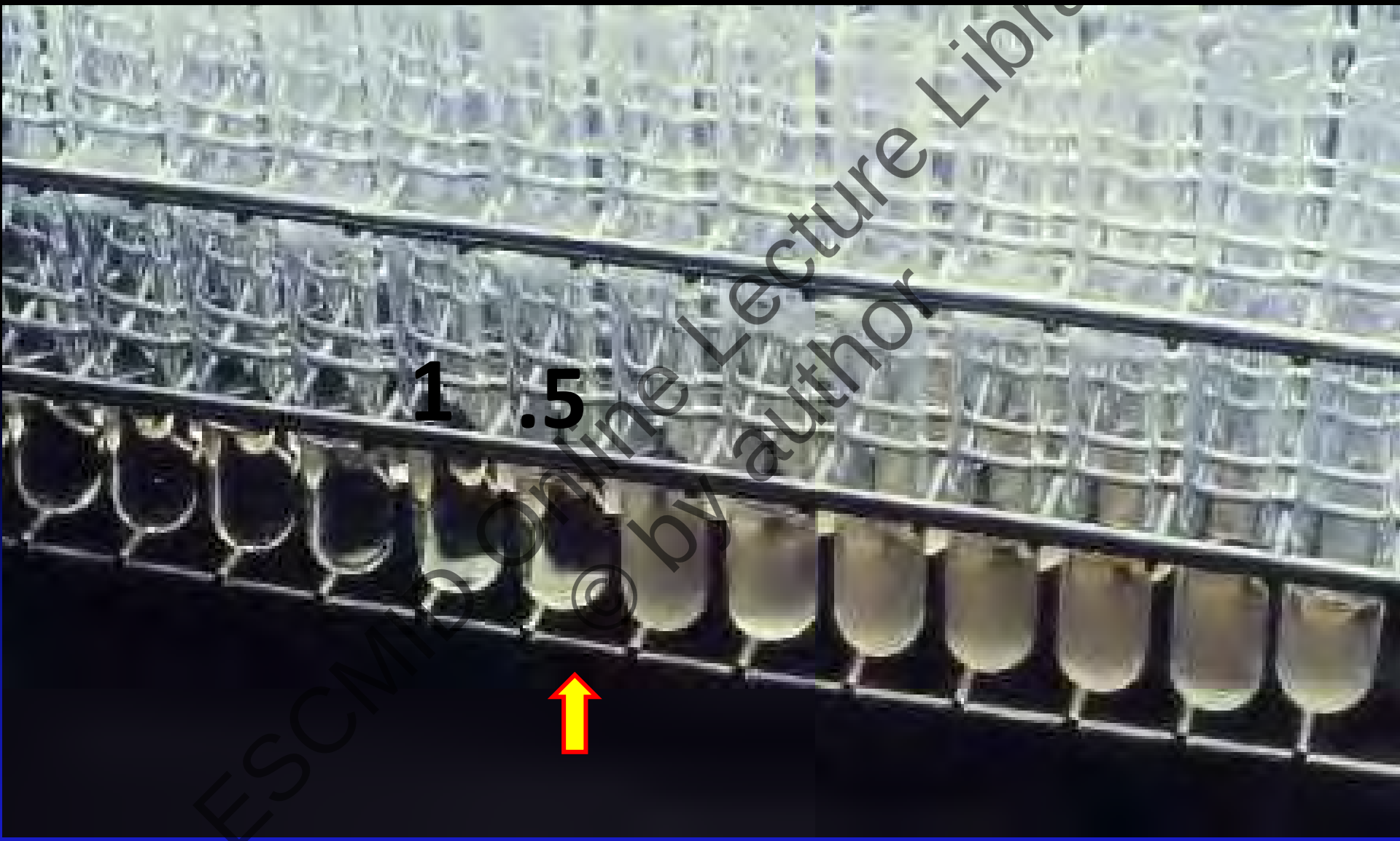
All who are happy with the flexibility of their automatic AST device

raise the **blue** card!

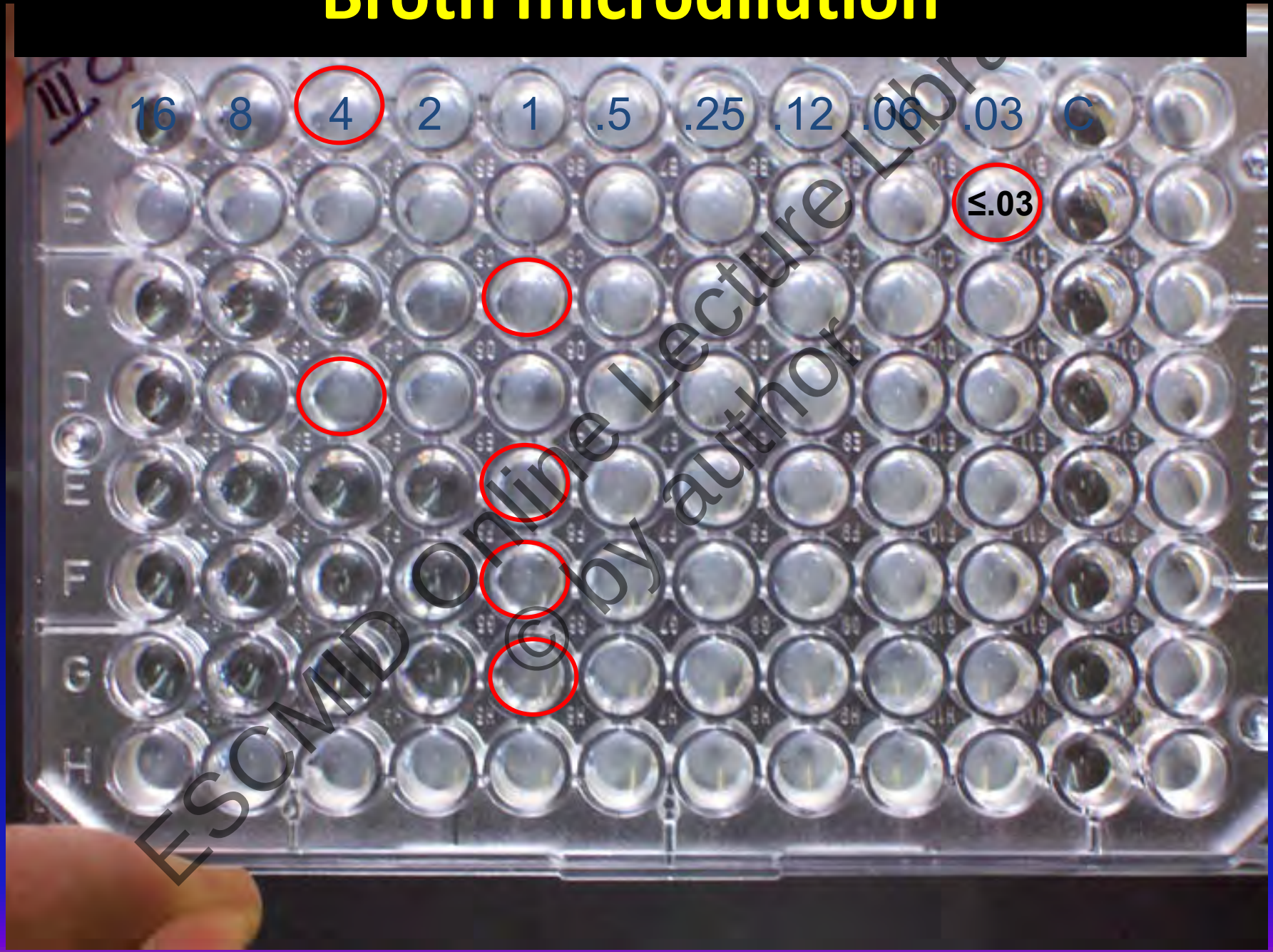
Methods for antimicrobial susceptibility testing

- Broth dilution
- Broth microdilution (BMD)
- Agar dilution
- Gradient tests
- Disk (or tablet) diffusion

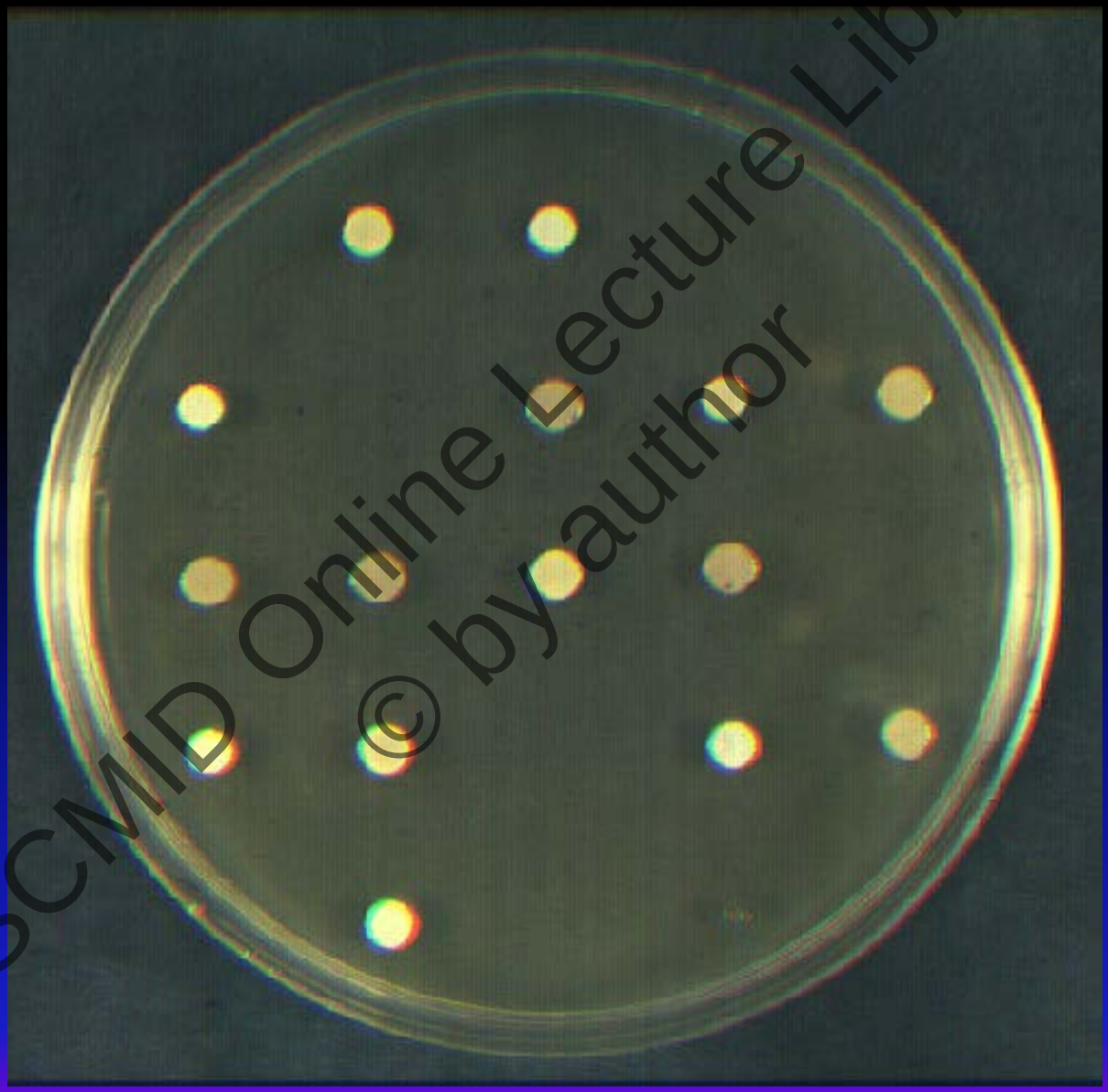
Broth dilution



Broth microdilution



Agar dilution



Gradient tests

Etest, M.I.C.E., MIC Test Strip

Disk diffusion AST

Semiautomated

Fully automated?
Future perspectives!

Automated (semi-) AST systems

- **MIC-based**
  - Broth microdilution (cards or microtiter trays)
    Phoenix (BD)
    Microscan (Siemens)
    Sensititre (Trek, Thermo Scientific)
  - Growth curve algorithm
    Vitek2 (bioMerieux)
- **Disk Diffusion**
  - Biomic (Giles Scientific)
    Sirscan (Oriana)
    Osiris (Biorad)

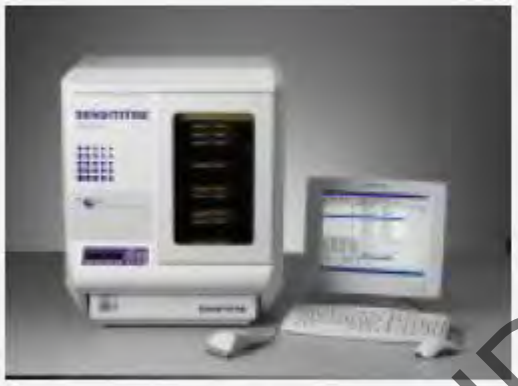
Limited concentrations series

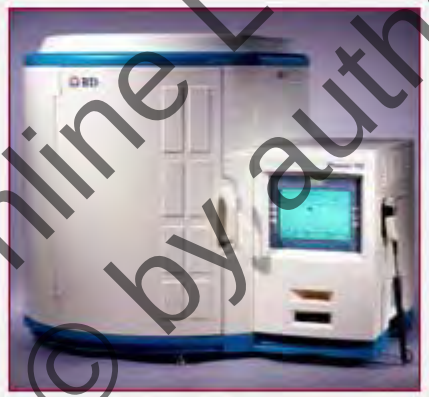
- Microscan
- Phoenix
- Vitek2

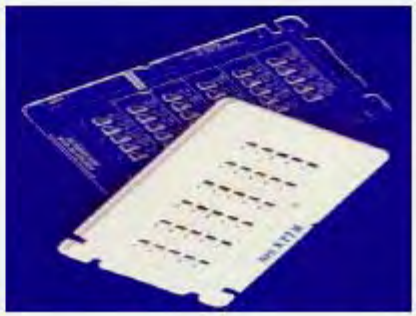
Flexible concentration series

- Sensititre

MIC based automatic systems







AST and MIC devices

- **AST devices**
  - Test output: category S, I, R (IE or ”-”)
  - Microscan, Phoenix, Vitek2
  - Dependent on match between limited concentration series, breakpoints and expert rules
  - Assessed by categorical agreement
- MIC determination devices
  - Test output: MIC (mg/L)
  - Sensititre (dependent on correct dilution series)
  - Gradient tests (Etest, M.I.C.E., MIC Test Strip) depend on correct calibration to broth microdilution
  - Independant of breakpoints and expert rules
  - Assessed by essential agreement

Disadvantages of most of current automation in antimicrobial susceptibility testing

- Limited capacity
- Black box technique
- Much of system outside user control
- "Rapid" results are not more rapid than a gradient test or a disk diffusion

Do not fully meet EUCAST standards..
and are slow in getting there

- Does not cover all tests – require supplementary systems
- User variation in setup
- "Expert rules systems" require certain antibiotics present on card.
- Some resistance mechanisms not reliably detected
- Commercial decisions affect presentation
- More expensive on consumables! Savings on labour?
- Lab tied to one system for a protracted period (through capital investment or lease contract)

Problems with machines

What they do, they do well.
The problems are with what they do not do!

- Missing species
- Missing drugs
  - New drugs take time to develop
  - New drugs take time to approve
  - Existing drugs not always suitable (pivmecillinam, piperacillintazobactam)
  - Some drugs necessary to make ”expert rules work”
- Incomplete MICs (truncated dilution series)
  - too many ≤ and ≥
- EUCAST
  - terminology out of whack (R> and R≥)
  - ”IE” not handled (report value without interpretation)
  - ”–” not handled (intrinsic resistance: report ”R” or not at all)
- Approval process focused on CA instead of EA – next slide

Missing species

Phoenix

H. influenzae
M. catarrhalis
N. gonorrhoeae
N. meningitidis
Gram-positive anaerobes
Gram-negative anaerobes

MicroScan

Streptococcus A, C and G
S. pneumoniae
S. viridans (test available only for *S. bovis*)
H. influenzae
M. catarrhalis
N. gonorrhoeae
N. meningitidis
Gram-positive anaerobes
Gram-negative anaerobes

Vitek2

H. influenzae
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Gram-positive anaerobes

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Missing drugs

Phoenix

Breakpoints Not Available	Antibiotics Not Available
Rifampicin (Staphylococci) Trimethoprim (Enterococci) Cotrimoxazole (Enterococci)	None

MicroScan

Breakpoints Not Available	Antibiotics Not Available
Trimethoprim Chloramphenicol Fusidic acid Rifampicin	Tigecycline/Gram-positive organisms Roxithromycin Telithromycin Doxycycline

Vitek2

Breakpoints Not Available	Antibiotics Not Available
Ampicillin-sulbactam Rifampicin Netilmicin (Staphylococci) Trimethoprim (Enterococci) Trimethoprim-sulfa (Enterococci) Gentamicin (Enterococci) Ofloxacin (Pneumococci)	Cefadroxil Ceftibuten Azithromycin Roxithromycin Ampicillin (Pneumococci) Cefepime (Pneumococci) Cefpodoxime (Pneumococci) Cefuroxime (Pneumococci) Teicoplanin (Pneumococci) Doxycycline (Pneumococci) Minocycline (Pneumococci)

Problems with machines

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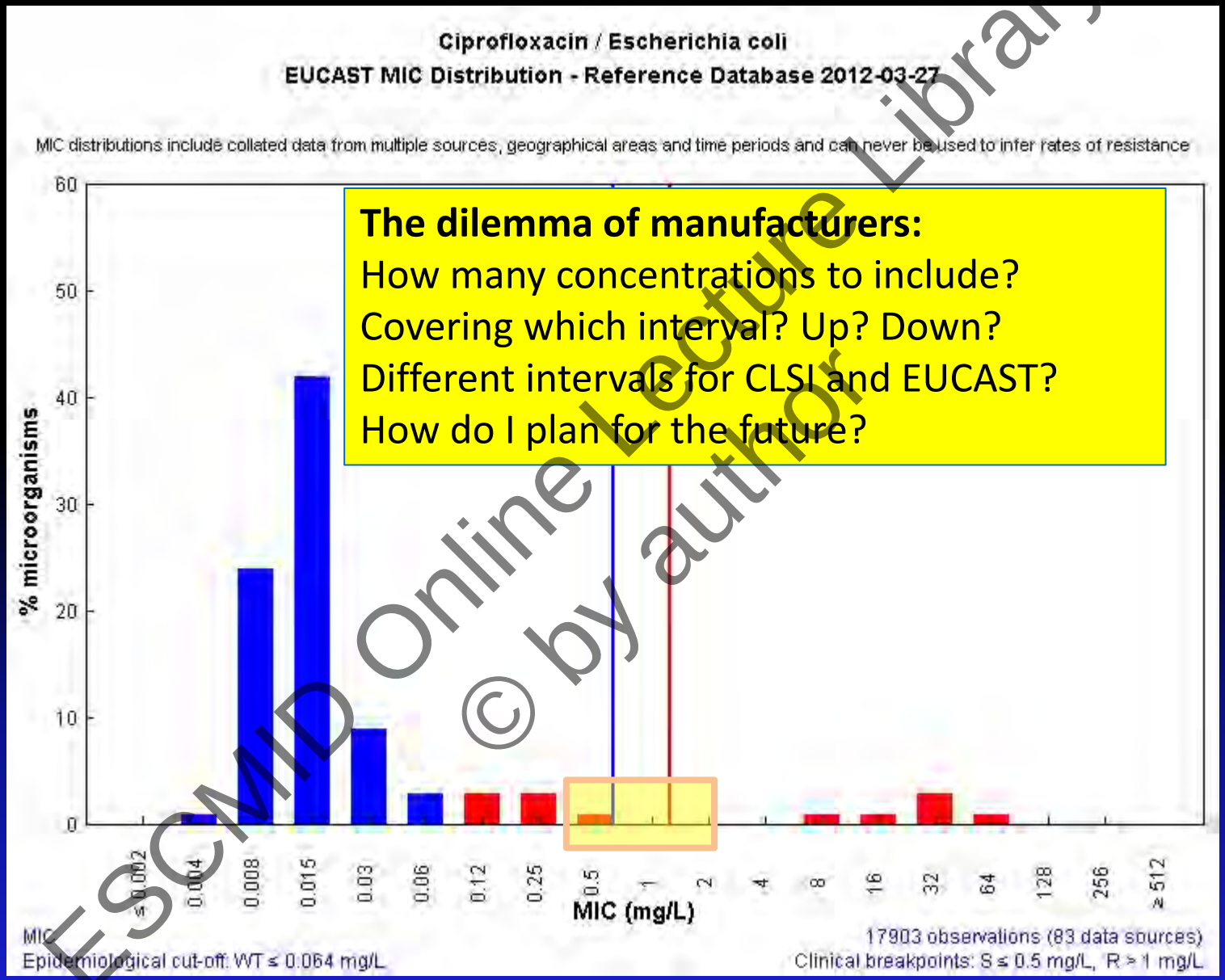

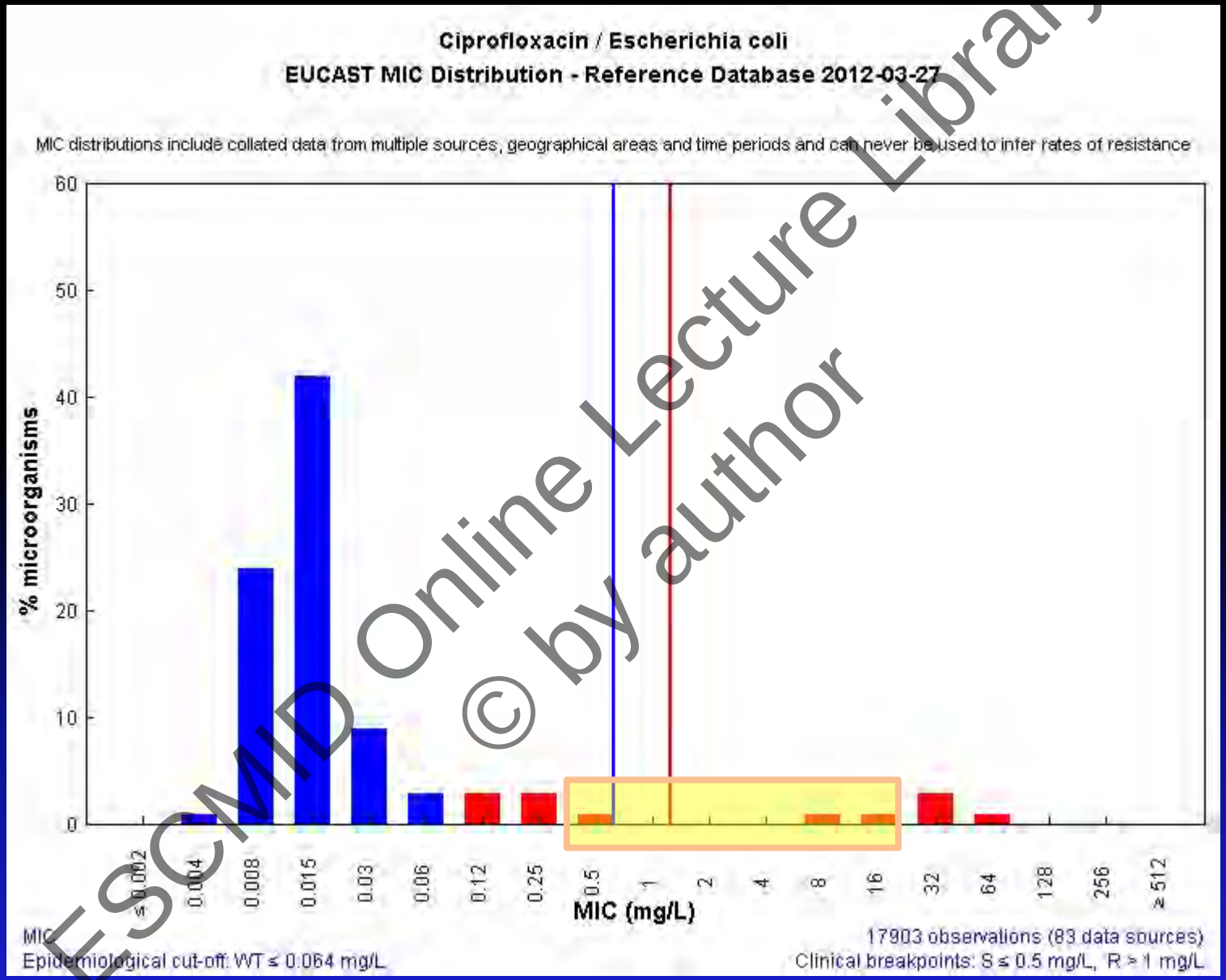

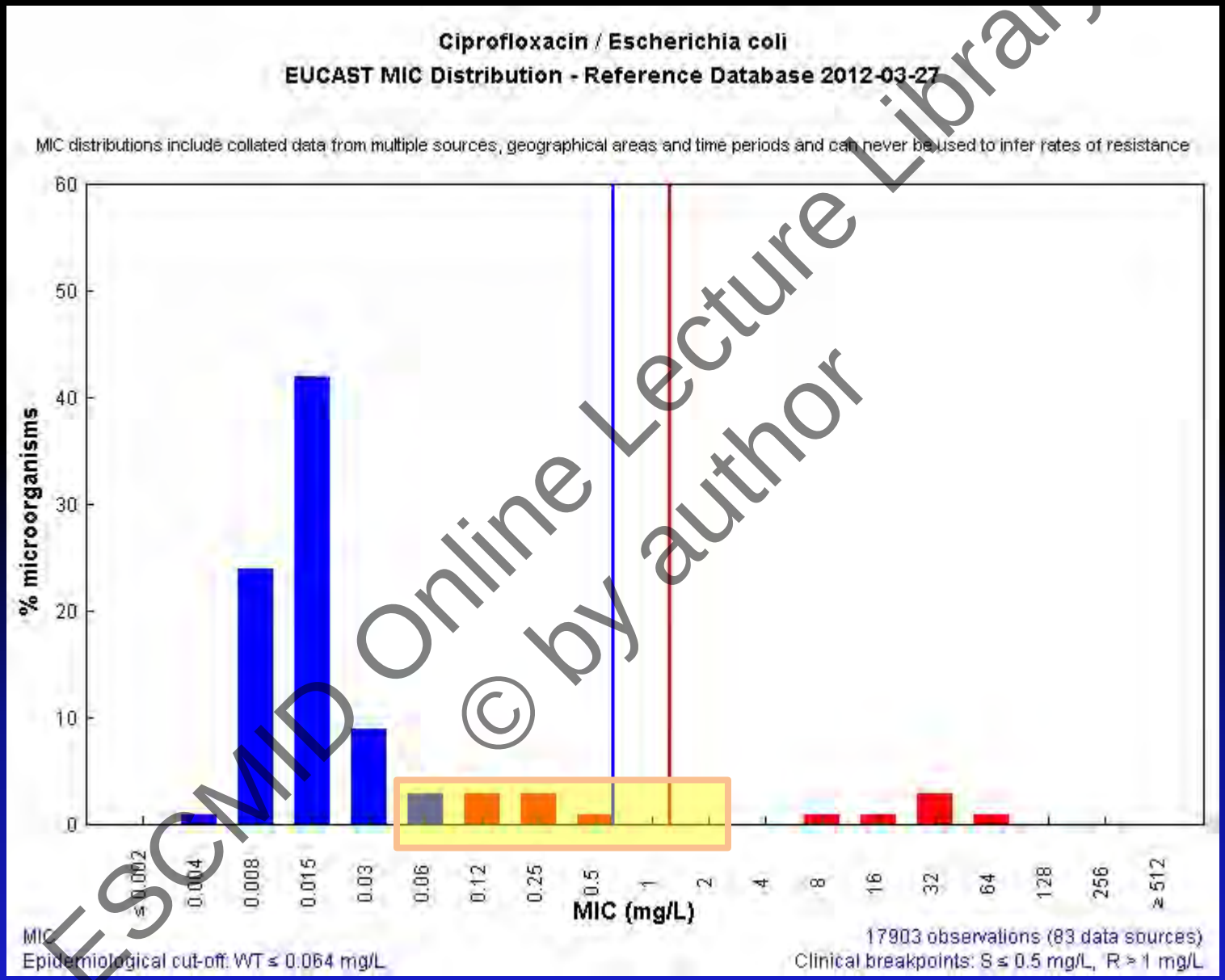

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EUCAST terminology lacking

Phoenix

S ≤	R >	-	IE
Yes Yes	No (R ≥) Yes (converted to R ≥)	Yes Yes*	Yes Yes*

MicroScan

S ≤	R >	-	IE
Yes Yes	Yes Yes	No Most "-" do not have interpretations reported	No No

Vitek2

S ≤	R >	-	IE
Yes Yes	No No	No* No* * not reported or reported R	No** No** ** not reported

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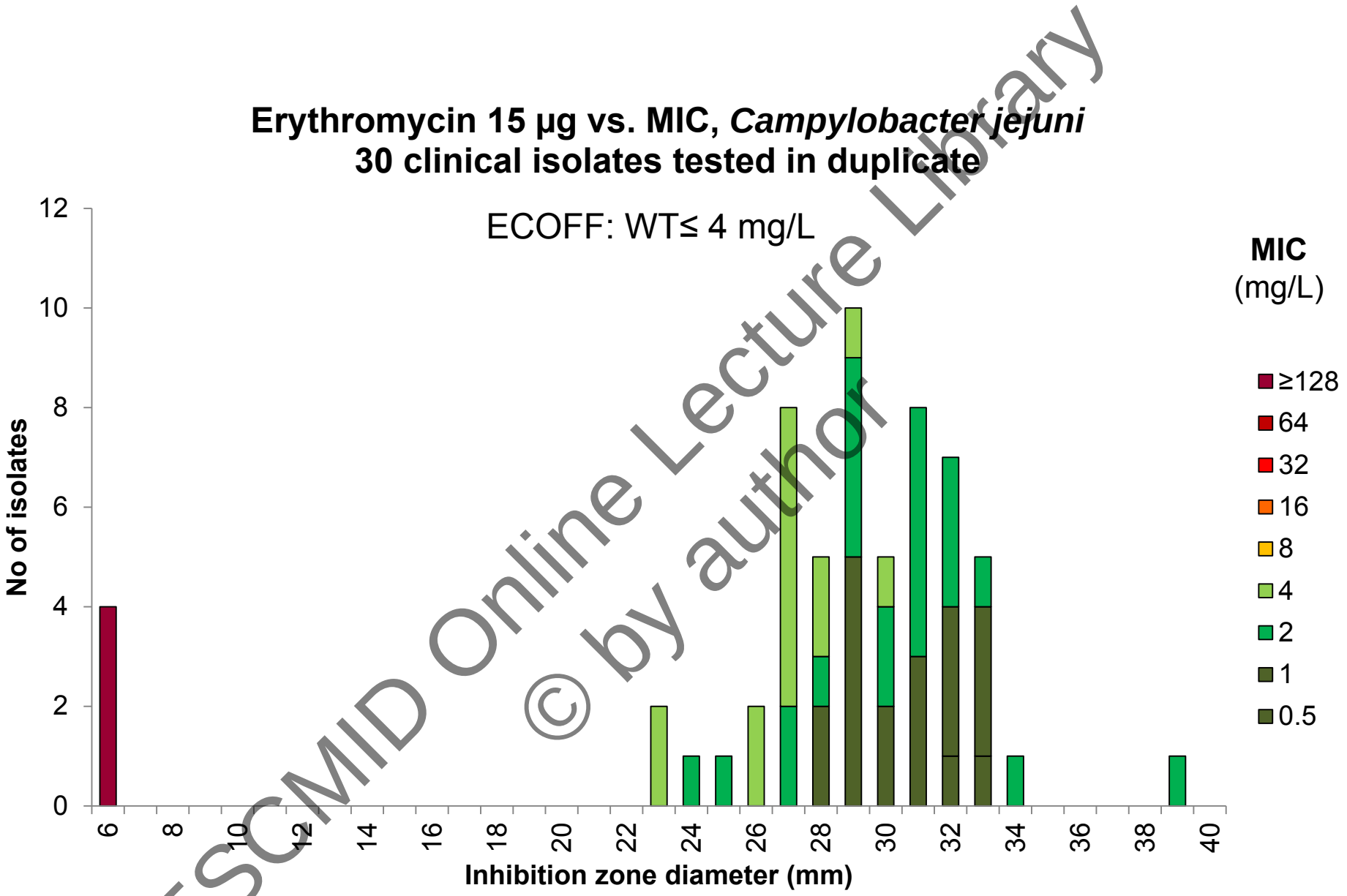
Successfull CA depends on…

- Collection of isolates
  - Isolates with only low and high MICs are easier to deal with
- Some breakpoints more difficult to deal with than others
  - Breakpoints which divide wild type distributions
  - Narrow or no I-category

Cipro 5µg Salmonella

	CIP MIC								
Zone diam	0,02	0,03	0,06	0,12	0,25	0,5	1	2	Total
21							2		2
22						5	6		11
23						9	13	2	24
24						11	17	1	29
25						19	17	1	37
26					1	12	6		19
27					4	3	2		9
28					14	6	1		21
29				2	16	10			28
30				3	10	1			14
31		3		2	3				8
32		8		1	2				11
33		21	2						23
34	1	35	4		2				42
35	6	25	2						33
36	4	10							14
37	7	6							13
38	6	4							10
39	1								1
40	3								3
Total	28	112	8	8	52	76	64	4	352

Breakpoint for susceptibility in M100



Future phenotypic AST (6 – 8 - 16h)

- Automated **MIC testing**
  - Fully automatic? Provide inoculum?
  - ”Full” MIC-ranges (see next slide)!
    - 96 wells? 196 wells? 1196 wells? etc
    - Incubation and variable reading after 6, 8, 10 etc …20h depending on species and antibiotic.
  - 6 – 8 h (!?) – new techniques for measuring growth may reduce time.
  - Software downloading breakpoints and ECOFFs from internet.
  - **Output**: MIC + Clinical S, I or R + WT or NWT

Cefotaxime and enterobacteriaceae
Breakpoints: S≤1 / R>2 mg/L
ECOFFs 0.12 – 1 mg/L

	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		S≤	R>	ECOFF≤
Citrobacter freundii	0	0	0	0	1	3	42	5	5	2	3	4	3	18	18	5	1	0	0		1.0	2.0	0.5
Citrobacter spp	0	0	0	0	17	35	19	11	10	9	2	4	3	4	4	3	0	0	0		1.0	2.0	0.5
Enterobacter aerogenes	0	0	0	0	7	28	33	9	3	3	4	2	8	15	8	9	6	11	0		1.0	2.0	0.5
Enterobacter cloacae	0	0	1	6	19	64	213	189	129	44	26	19	35	53	90	59	56	58	12		1.0	2.0	0.5
Enterobacter spp	0	0	0	1	19	47	79	45	29	22	7	10	4	20	11	4	0	5	0		1.0	2.0	0.5
Escherichia coli	0	5	40	282	1656	4953	2591	485	194	100	60	56	46	58	106	134	26	35	28		1.0	2.0	0.25
Klebsiella oxytoca	0	1	7	55	155	80	19	22	9	12	13	14	9	4	11	11	0	1	0		1.0	2.0	0.125
Klebsiella pneumoniae	0	2	12	99	523	745	271	84	65	19	18	36	54	43	49	68	105	199	20		1.0	2.0	0.25
Klebsiella spp	0	5	21	86	103	73	34	13	16	11	4	1	2	1	0	1	1	0	0		1.0	2.0	0.125
Morganella morganii	0	8	27	35	24	18	12	8	6	2	7	10	4	2	2	0	0	1	0		1.0	2.0	ND
Proteus mirabilis	0	1	107	212	71	11	2	3	3	1	1	1	2	1	2	4	1	1	0		1.0	2.0	0.064
Proteus vulgaris	0	0	14	22	27	23	20	11	3	0	0	0	4	2	1	0	0	4	0		1.0	2.0	0.125
Salmonella spp	0	0	0	0	3	436	10648	1281	184	16	2	6	7	35	11	0	0	0	0		1.0	2.0	0.5
Serratia marcescens	0	0	0	0	0	3	51	35	34	44	12	19	33	25	24	9	1	48	0		1.0	2.0	1.0
Serratia spp	0	0	0	0	1	7	42	43	43	26	13	5	20	35	7	6	1	1	0		1.0	2.0	1.0

Catch the end of relevant wild types!

Catch both breakpoints!

Catch future breakpoints!

Catch relevant MICs!

Routine ATCC control strains may still not detect problems

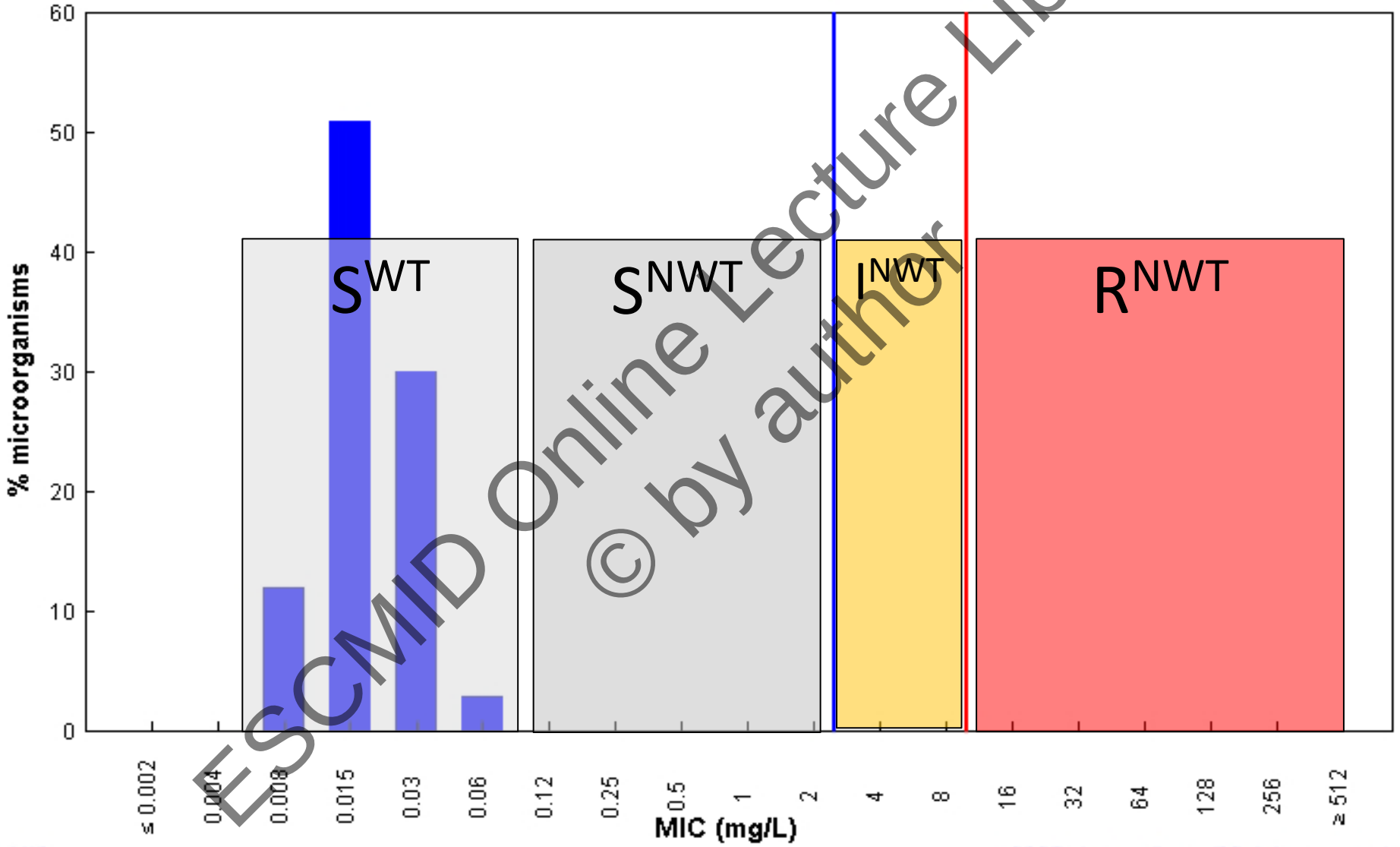
Future phenotypic AST (6 – 8 h)

- Automated **disk diffusion testing**
  - Fully automatic (provide inoculum):
    - plate inoculation
    - disk application
    - incubation
    - ”variable” zone diameter reading: automatic reading at 6, 8, 12 and 18h.
    - and zone diameter distribution databases to match.
  - Database consisting of zone diameter distributions related to incubation time and with calibrated breakpoints.
  - **Output**: Zone diameter + Clinical S, I or R + WT or NWT

Meropenem / Escherichia coli

EUCAST MIC Distribution - Reference Database 2012-04-01

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC

Epidemiological cut-off: WT ≤ 0.125 mg/L

8005 observations (68 data sources)

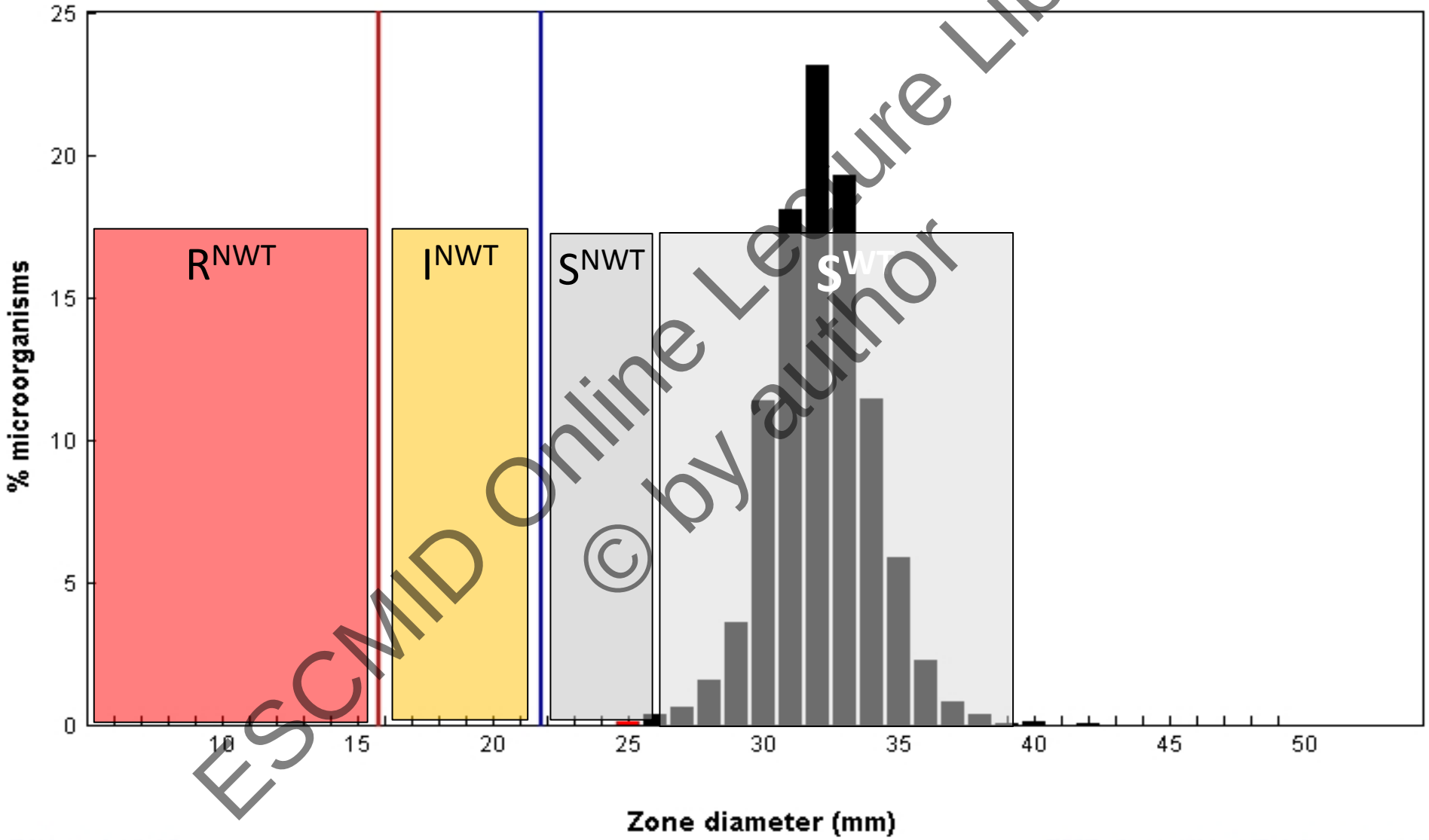
Clinical breakpoints: S ≤ 2 mg/L, R > 8 mg/L

Meropenem / Escherichia coli

EUCAST zone diameter distribution - Reference database 2012-04-01

EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

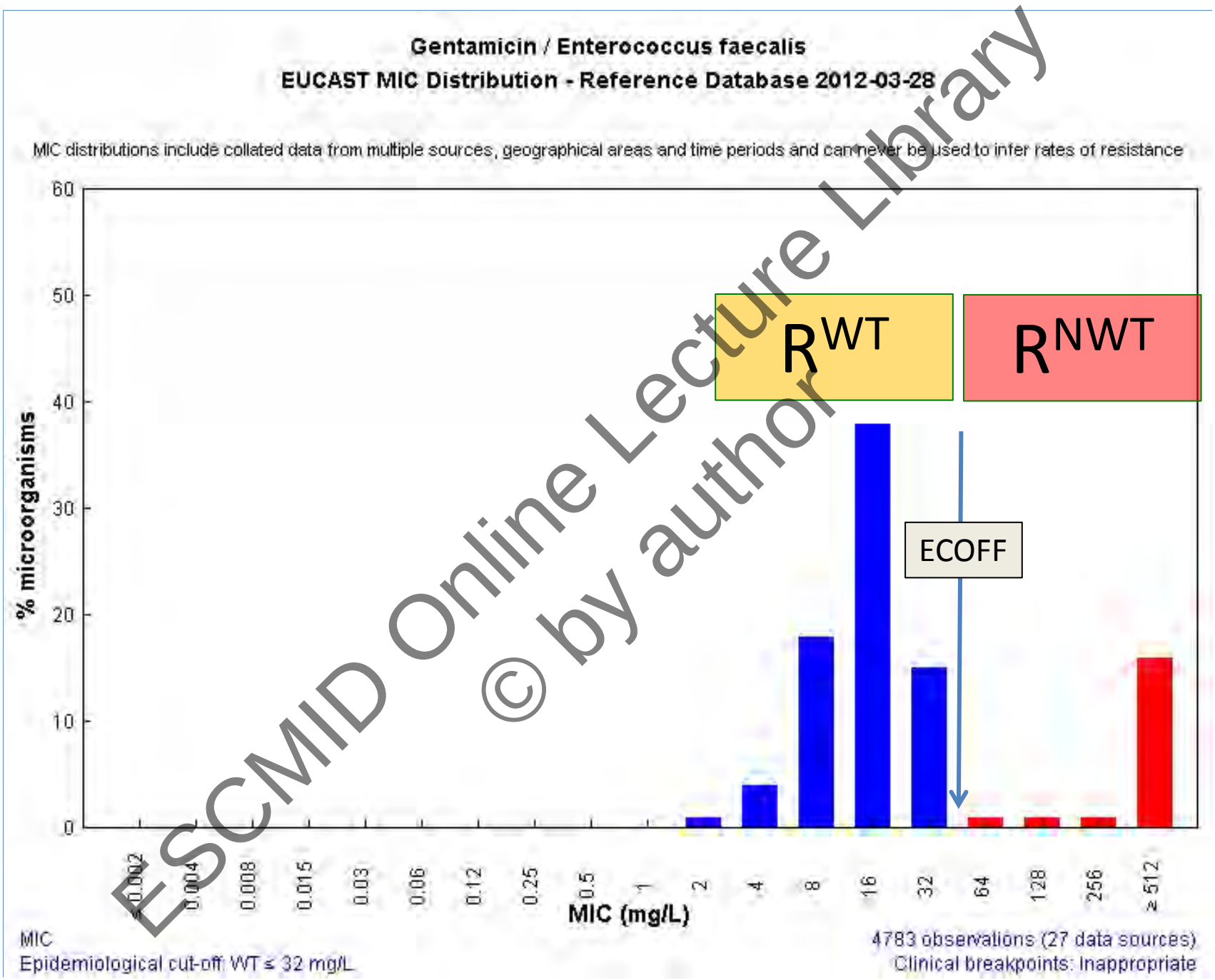


Disk content: 10

7027 observations (7 data sources)

Epidemiological cut-off: WT ≥ 26 mm (MIC ≤ 0.125 mg/L)

Clinical breakpoints: S ≥ 22 mm, R < 16 mm (S ≤ 2 mg/L, R > 8 mg/L,)



MIC testing and disk diffusion are hard to beat!

Disk /MIC testing

- Manual
- 16 – 20h (8h possible)
- Direct testing possible on pure cultures (blood, urine)
- Flexible and adaptable
- Little dependency
- Truly quantitative
- Standard lab equipment
- Cheap consumables
- Know-how in AST
- QC (regular ATCC)

Automated AST

- Semiautomated
- 8 – 16 – 20 h
- Direct testing possible on pure cultures (blood cultures)
- Non-flexible
- Dependency on manufacturer
- Blackbox/trapdoor results
- Expensive dedicated equipment
- Expensive consumables
- Know-how in machine
- QC (suppl MIC-range)

Rapid change

Flexibility

Flexibility

Flexibility