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

När resistensbestämningen är inkonklusiv! S, I and R and the ATU

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Susceptibility categories S, I and R (2002 – 2018)

S = a microorganism is defined as susceptible by a level of antimicrobial activity associated with a high likelihood of therapeutic success.

R = a microorganism is defined as resistant by a level of antimicrobial activity associated with a high likelihood of therapeutic failure.

Susceptibility categories S, I and R (2002 – 2018)

S = a microorganism is defined as susceptible by a level of antimicrobial activity associated with a high likelihood of therapeutic success.

I = a microorganism is defined as intermediate by a level of antimicrobial agent activity associated with **uncertain therapeutic effect**. It implies that an infection due to the isolate may be appropriately treated in body sites **where the drugs are physiologically concentrated** or when a **high dosage of drug can be used**; it also indicates a **buffer zone that should prevent small, uncontrolled, technical factors** from causing major discrepancies in interpretations.

R = a microorganism is defined as resistant by a level of antimicrobial activity associated with a high likelihood of therapeutic failure.

(1) Uncertain therapeutic effect

– responsibility of EMA, EUCAST and the company

(2) Concentration at the site of infection

– responsibility of the clinician (dose, frequency of administration, route of administration).

(3) Buffer for uncontrolled technical factors

- uncertain result is the responsibility of the laboratory

EUCAST decided …..

- To keep S, I and R but change definitions to point out that phenotypic AST is quantitative
- To review and revise breakpoints to correspond to the new definitions.
- To emphasize the relationship between the concentration of the antimicrobial agent at the site of the infection (exposure) AND the breakpoints for categorisation (S, I and R).
- To task clinical laboratories with the responsibility for uncertain laboratory results, irrespective of origin and to identify and form strategies for difficult areas.

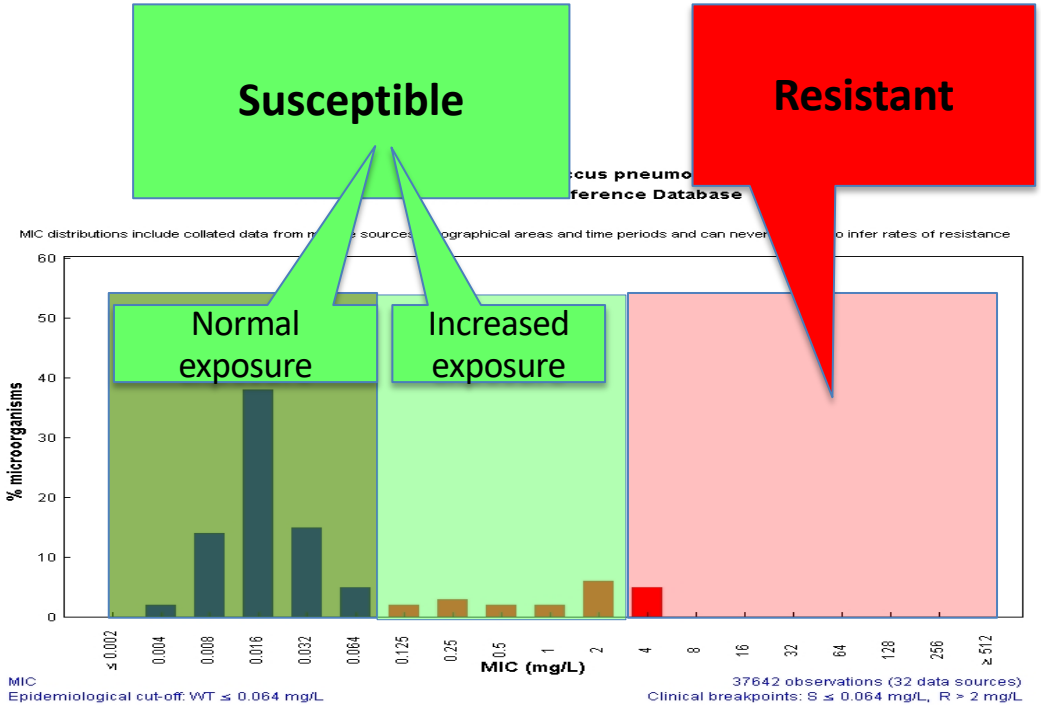
New definitions of S, I and R from 2019

S - Susceptible, standard dosing regimen: A microorganism is categorised as *Susceptible, standard dosing regimen,* when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.

I – Susceptible, increased exposure: A microorganism is categorised as *Susceptible, Increased exposure** when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.

R - Resistant: A microorganism is categorised as *Resistant* when there is a high likelihood of therapeutic failure even when there is increased exposure.

SIR – new definitions 2019



Susceptible, increased exposure

- Change from oral to intravenous
- Increase the individual dose
- Increase frequency of dosing (for some agents)
  - Long or continuous iv infusion of betalactams
- Pharmacokinetics of some agents, eg. concentration in urinary tract

Increased exposure

Significantly increased exposure gains 1 – 3 MIC-dilutions:

- Benzylpenicillin vs. S. pneumoniae S≤0.06 R>2 mg/L (many dilutions)
- Piperacillin-tazobactam vs. Enterobacterales S≤8 R>16 mg/L (one dilution)
- Ceftazidime vs. Enterobacterales S≤1 R>4 mg/L (two dilutions)
- Meropenem vs. Enterobacterales S≤2 R>8 mg/L (two dilutions)
- Ciprofloxacin vs. Enterobacterales S≤0.25 R>0.5 mg/L (one dilution)

Exposure cannot be increased – no I-category

- Gentamicin vs. Enterobacterales S≤2 R>2 mg/L
- Vancomycin vs. Staphylococci S≤2 R>2 mg/L
- Colistin vs. Enterobacterales S≤2 R>2 mg/L

EUCAST – dosing and administration of antibiotics and the relationship to breakpoints.

Phenoxymethylpenicillin	0.5-2 g x 3-4 oral	None	
Oxacillin			
Cloxacillin			
Dicloxacillin			
Flucloxacillin			
Mecillinam	None	None	0.2 - 0.4 g x 3 oral
Cephalosporins	**Standard dose**	**High dose**	**UTI, uncomplicated**
Cefaclor	0.25-1 g x 3 oral depending on species and/or infection type	None	
Cefadroxil	0.25 -1 g x 3 oral	None	0.25-1 g x 3 oral
Cefalexin	0.5 -1 g x 2 oral	None	0.5-1 g x 2 oral
Cefazolin	1 g x 3 iv	2 g x 3 iv	
Cefepime	1 g x 3 or 2 g x 2 iv	2 g x 3 iv	
Cefixime	0.2-0.4 g x 2 oral	None	0.2 - 0.4 g x 2 oral
Cefotaxime	1 g x 3 iv	2 g x 3 iv	
Cefpodoxime	0.1-0.2 g x 2 oral	None	0.1 - 0.2 g x 2 oral
Ceftaroline	0.6 g x 2 iv over 1 hour	0.6 g x 3 iv over 2 hours	
Ceftazidime	1 g x 3 iv	2 g x 3 iv or 1 g x 6 iv	
Ceftazidime-avibactam	(2 g ceftazidime + 0.5 g avibactam) x 3 over 2 hours		
Ceftibuten	0.4 g x 1 oral	None	

Konsekvenser

- Flera I-kategorier avskaffas
  - Om man inte tydligt kan öka exponeringen av bakterien ges ingen I-kategori
- Flera nya I-kategorier introduceras.
  - För vissa arter måste antibiotika ges så att högsta möjliga exponering av mikroorganismen alltid garanteras. De får i resistensbeskedt aldrig ett ”S”.
- I-kategorin som metodologisk buffert är avskaffad.
  - Ökar ansvaret på laboratoriet och tillverkare av material och apparater.

Pseudomonas in Table 2020

Pseudomonas spp.

EUCAST Clinical Breakpoint Tables v. 10.0, valid from 2020-01-01

Expert Rules and Intrinsic Resistance Tables

For several agents, in v. 10.0 of the breakpoint tables, EUCAST has introduced breakpoints which categorise wild-type organisms (organisms without phenotypically detectable acquired resistance mechanisms to the agent) as "Susceptible, increased exposure (I)" instead of "Susceptible, standard dosing regimen (S)". In v. 9.0, these are listed as agent^{HE} to emphasize the need for high exposure (HE). Following efforts to explain and inform colleagues in clinical microbiology, colleagues involved in treatment and in forming antimicrobial policies and stewardship, laboratories are encouraged to implement the new standard as soon as possible but no later than at the end of 2020. During the transition, it is possible to continue to use breakpoints in table v. 9.0 for breakpoints highlighted in light green in v. 10.0.

MIC determination (broth microdilution according to ISO standard 20776-1 except for fosfomycin where agar dilution is used)
Medium: Mueller-Hinton broth
Inoculum: $5x10^5$ CFU/mL
Incubation: Sealed panels, air, 35±1°C, 18±2h
Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth.
Quality control: *Pseudomonas aeruginosa* ATCC 27853. For agents not covered by this strain and for control of the inhibitor component of beta-lactam inhibitor combinations, see EUCAST QC Tables.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1°C, 18±2h
Reading: Unless otherwise stated, read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.
Quality control: *Pseudomonas aeruginosa* ATCC 27853. For agents not covered by this strain and for control of the inhibitor component of beta-lactam inhibitor-combination disks, see EUCAST QC Tables.

***Pseudomonas aeruginosa* is the most frequent species of this genus. Other less frequent *Pseudomonas* species recovered in clinical samples are: *P. fluorescens* group, *P. putida* group and *P. stutzeri* group.**

Penicillins	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	-	-			-	-		1. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Ampicillin iv	-	-			-	-		2. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Ampicillin-sulbactam	-	-			-	-		
Amoxicillin	-	-			-	-		
Amoxicillin-clavulanic acid	-	-			-	-		
Piperacillin	0.001	16		30	50	18	18-19	
Piperacillin-tazobactam	0.001[1]	16[1]		30-6	50	18	18-19	
Ticarcillin	0.001	16		75	50	18		
Ticarcillin-clavulanic acid	0.001[2]	16[2]		75-10	50	18		
Temocillin	-	-			-	-		

Cephalosporins	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor	-	-			-	-		**1.** For susceptibility testing purposes, the concentration of avibactam is fixed at 4 mg/L.
Cefadroxil	-	-			-	-		**2.** For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Cefalexin	-	-			-	-		
Cefazolin	-	-			-	-		
Cefepime	0.001	8		30	50	21		
Cefixime	-	-			-	-		
Cefotaxime	-	-			-	-		
Cefoxitin	NA	NA			NA	NA		
Cefpodoxime	-	-			-	-		
Ceftaroline	-	-			-	-		
Ceftazidime	0.001	8		10	50	17		
Ceftazidime-avibactam, *P. aeruginosa*	8[1]	8[1]		10-4	17	17	16-17	
Ceftibuten	-	-			-	-		
Ceftobiprole	IE	IE			IE	IE		
Ceftolozane-tazobactam, *P. aeruginosa*	4[2]	4[2]		30-10	24	24		
Ceftriaxone	-	-			-	-		
Cefuroxime iv	-	-			-	-		
Cefuroxime oral	-	-			-	-		

Carbapenems	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ertapenem	-	-			-	-		**1.** For susceptibility testing purposes, the concentration of vaborbactam is fixed at 8 mg/L.
Imipenem	0.001	4		10	50	20		
Meropenem	2	8		10	24	18		
Meropenem-vaborbactam, *P. aeruginosa*	8[1]	8[1]		IP	IP	IP		

Monobactams	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam	0.001	16		30	50	18		

Fluoroquinolones	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	0.001	0.5		5	50	26		

Pseudomonas in Table 2020

Table 1b. The following agents for *Pseudomonas* are not affected by the proposal:

		Previous	New	SIR for WT
Pseudomonas	Ceftazidime-avibactam	8/8	8/8	S (standard dose corresponds to high dose ceftazidime)
Pseudomonas	Ceftolozane-tazobactam	4/4	4/4	S (only high dose available)
Pseudomonas	Meropenem	2/8	2/8	S
Pseudomonas	Meropenem-vaborbactam	8/8	8/8	S (standard dose corresponds to high dose meropenem)

Information till kliniker

- Ni har 2020 på er. De nya brytpunkterna markerade i ”mörkgrönt” inför ni ett datum mellan den 1 jan och den 31 dec 2020.
  Var tydliga till vården när ni inför förändringen.
  Skicka med en kommentar på alla svar.

- Prio 1: infektionsläkare informeras i god tid. De måste hjälpa er att föra ut kunskapen till resten av vården
- Prio 2: primärvård – skriftlig och muntlig information NordicAST hjälper till.

Konsekvenser

- Flera I-kategorier avskaffas
  - Om man inte tydligt kan öka exponeringen av bakterien ges ingen I-kategori
- Flera nya I-kategorier introduceras.
  - För vissa arter måste antibiotika ges så att högsta möjliga exponering av mikroorganismen alltid garanteras. De får i resistensbeskedt aldrig ett ”S”.
- I-kategorin som metodologisk buffert är avskaffad.
  - Ökar ansvaret på laboratoriet och tillverkare av material och apparater.

AST is the responsibility of the laboratory

- **Some tests** have problems with aminoglycosides, others with trimethoprimsulfa
- **Some types of tests** will not cope with some agents/bacteria (vancomycin, colistin, fosfomycin)
- **Some agents** are difficult (piperacillintazobactam, colistin, vancomycin…)
- **Some devices** are generally problematic.
- **Material from some manufacturers** is problematic

- EUCAST helps to identify problematic areas.
- Daily QC helps identify problems

Materials and devices

- **Disks**
- **Media (powders)**
- **Gradient tests** (cave several - EUCAST Warnings)
- Media (prepoured, commercially distributed)
- Semiautomated devices (Vitek2, Phoenix, MicroScan etc)

Clin Microbiol Infect. 2019 Mar;25(3):346-352. doi: 10.1016/j.cmi.2018.05.021. Epub 2018 Jun 7.

The quality of antimicrobial discs from nine manufacturers-EUCAST evaluations in 2014 and 2017.

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Abstract

OBJECTIVES: Antimicrobial discs for susceptibility testing can be obtained from many manufacturers. We evaluated the quality of discs from nine manufacturers in 2014 and 2017.

METHODS: Antimicrobial discs of 16 agents from nine manufacturers were evaluated using EUCAST criteria. Discs were tested in triplicate on Müller-Hinton medium against EUCAST quality control (QC) strains. Mean values were compared with targets and ranges in the EUCAST QC tables.

RESULTS: Three manufacturers (Becton Dickinson, Mast and Oxoid) demonstrated excellent and consistent disc quality both in 2014 and 2017. Manufacturers with discs of inadequate quality improved their results between the two periods. Overall, 92% (795/861) versus 97% (1038/1071) of zone diameter readings were within QC ranges and 58% (497/861) versus 75% (806/1071) were within the QC target ± 1 mm, for the first and second studies, respectively. One manufacturer (HiMedia) had major quality problems with 33% (26/78) of readings out of range in the first study and 17% (20/120) in the second study. Discs from some manufacturers showed unexpected variation in inhibition zone diameters (4-9 mm) for discs within the same vial.

CONCLUSIONS: Antimicrobial discs from three of nine manufacturers exhibited excellent and reproducible quality. The discs of the other six manufacturers demonstrated various quality issues, some of which were severe. After presenting the results to manufacturers and users, all managed to improve the quality. Our study points to the need for more stringent criteria for disc manufacturing. Criteria should not only address the nominal potency of discs but also define the end result.

*S. pneumoniae vs. b*enzylpenicillin MIC 1 – 4 mg/L
Broth microdilution vs. Gradient tests

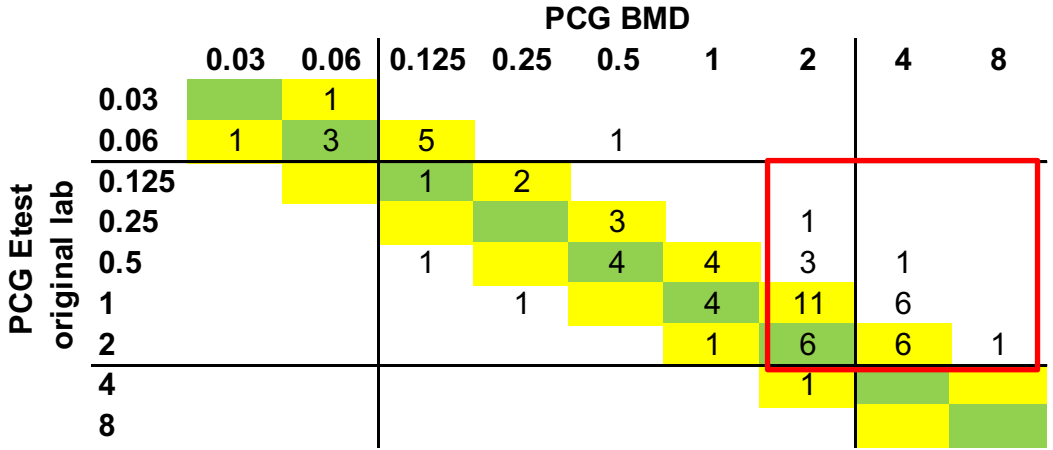
	Below target (%)	On target (%)	Above target (%)
Etest, BD MH-F	63	37	0
Etest, Oxoid MH-F	89	22	0
Etest, consecutive	81	17	3
MTS, BD MH-F	89	11	0
MTS, Oxoid MH-F	100	0	0
French data (Etest) (MICs from WT to 2 mg/L)	70	25	5

Gradient test MICs* from other laboratories vs. EDL BMD

* Most likely Etest

>2 dilutions lower	**3**
2 dilutions lower	**10**
1 dilution lower	**32**
Identical	**18**
1 dilution higher	**3**
2 dilutions higher	**2**
>2 dilutions higher	**0**

66 % below target
27 % on target
7 % above target



Warning

Determination of benzylpenicillin MIC in *Streptococcus pneumoniae* using gradient tests.

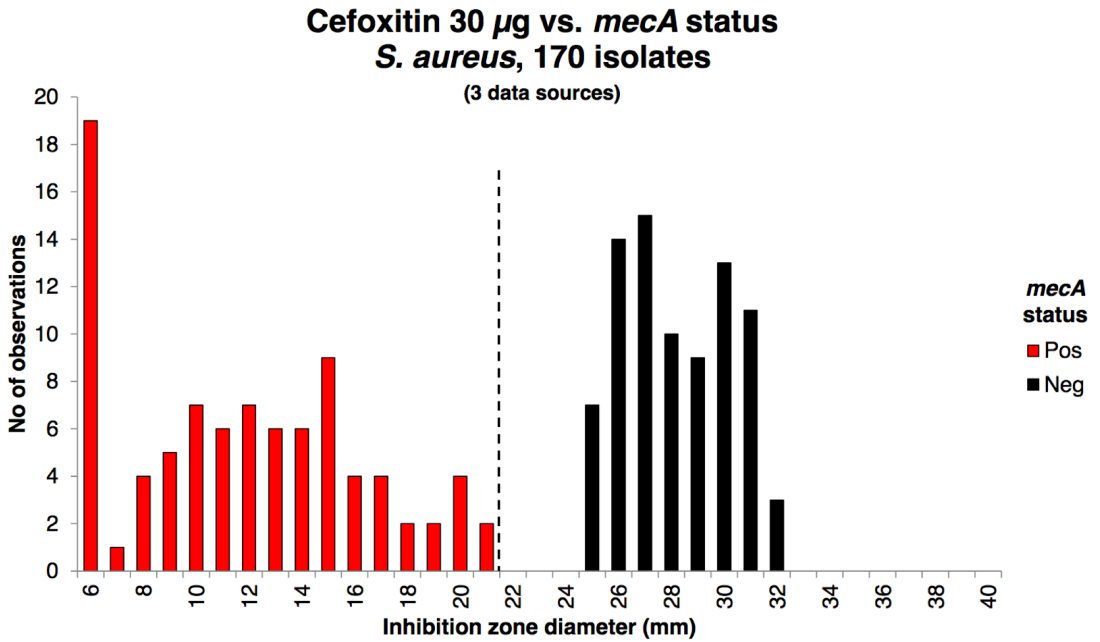
- EUCAST benzylpenicillin breakpoints in *Streptococcus pneumoniae* are S≤0.06 mg/L, R>2 mg/L. Isolates which are screen positive (with the oxacillin 1 µg disk) have MIC-values above 0.06 mg/L and are either "Susceptible, increased exposure", in which case dosing can be related to the MIC value, or resistant (R>2 mg/L), in which case these should not be treated with benzylpenicillin.. Laboratories must be able to perform correct MIC determination on screen positive isolates and this is never more important than in the area 0.5 – 4 mg/L.

- Following questions from NEQAS, EARS-Net and EUCAST participants, the EDL investigated the accuracy of benzylpenicillin gradient tests (Etest™, MTS™; M.I.C.E™ not available on the market) where broth microdilution was used as the reference. The gradient tests were found to be fairly accurate among wild type isolates (S≤0.06 mg/L), but for isolates with higher MIC-values both Etest™ and MTS™systematically underestimated MIC-values by one or more dilutions. In the area around the R-breakpoint (0.5 – 4 mg/L), and with some variation between the MH-media used and the two tests, 0 – 37% of values were on target, 63 – 100 % were below target and 0-10 % of the values above the target value. Conclusion: Etest™ and MTS™ systematically underestimate benzylpenicillin MIC-values in the important area close to the R-breakpoint.

ATU

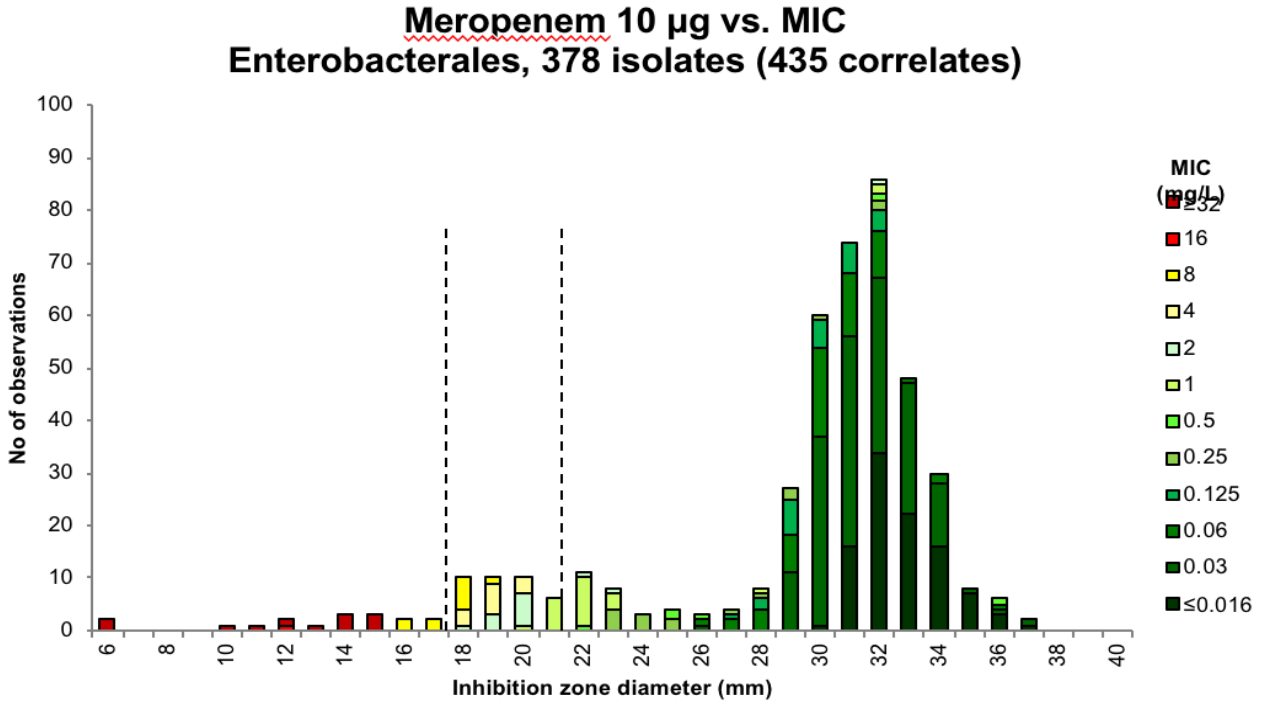
The Area of Technical Uncertainty

Most AST is unproblematic

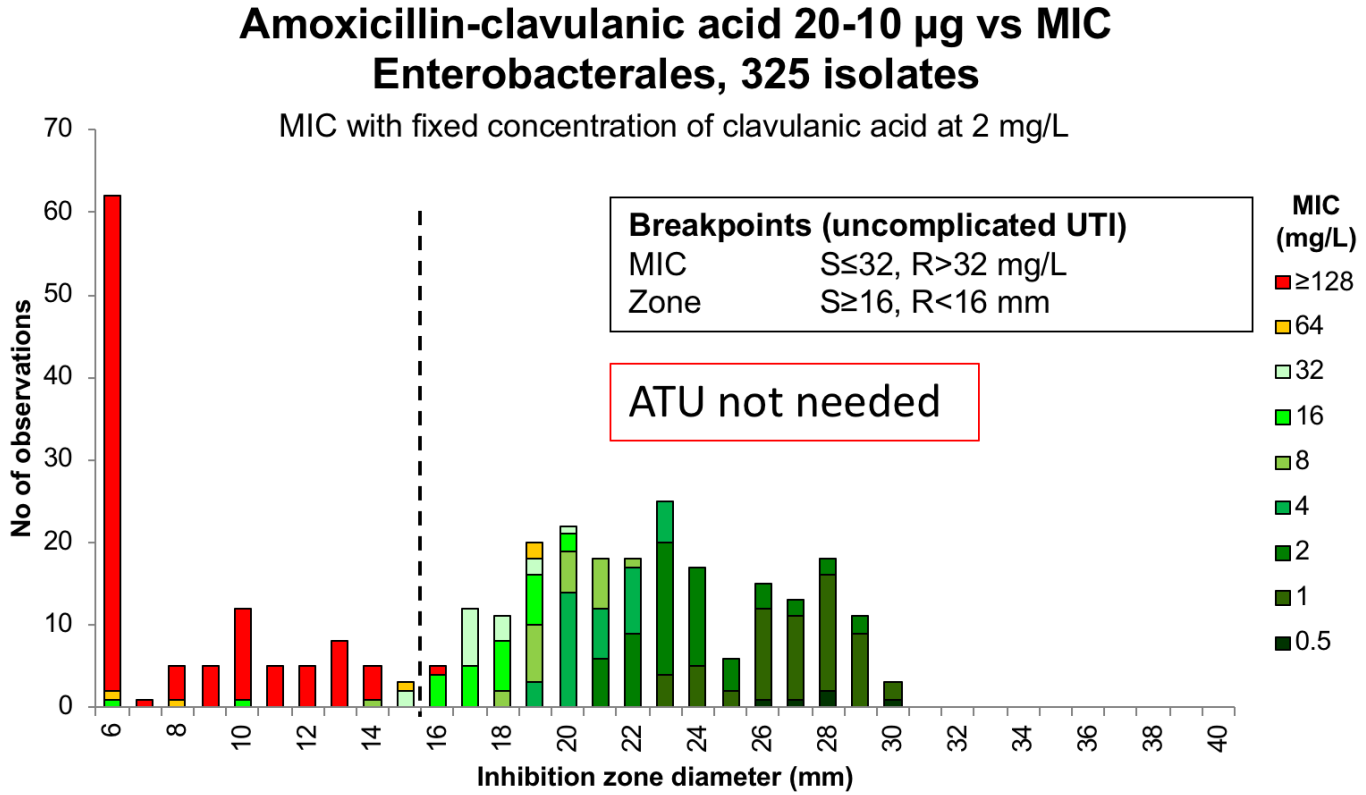


Breakpoints
Zone diameter (screen) S≥22, R<22 mm

Meropenem and Enterobacterales – one of many examples where an ATU is not needed.



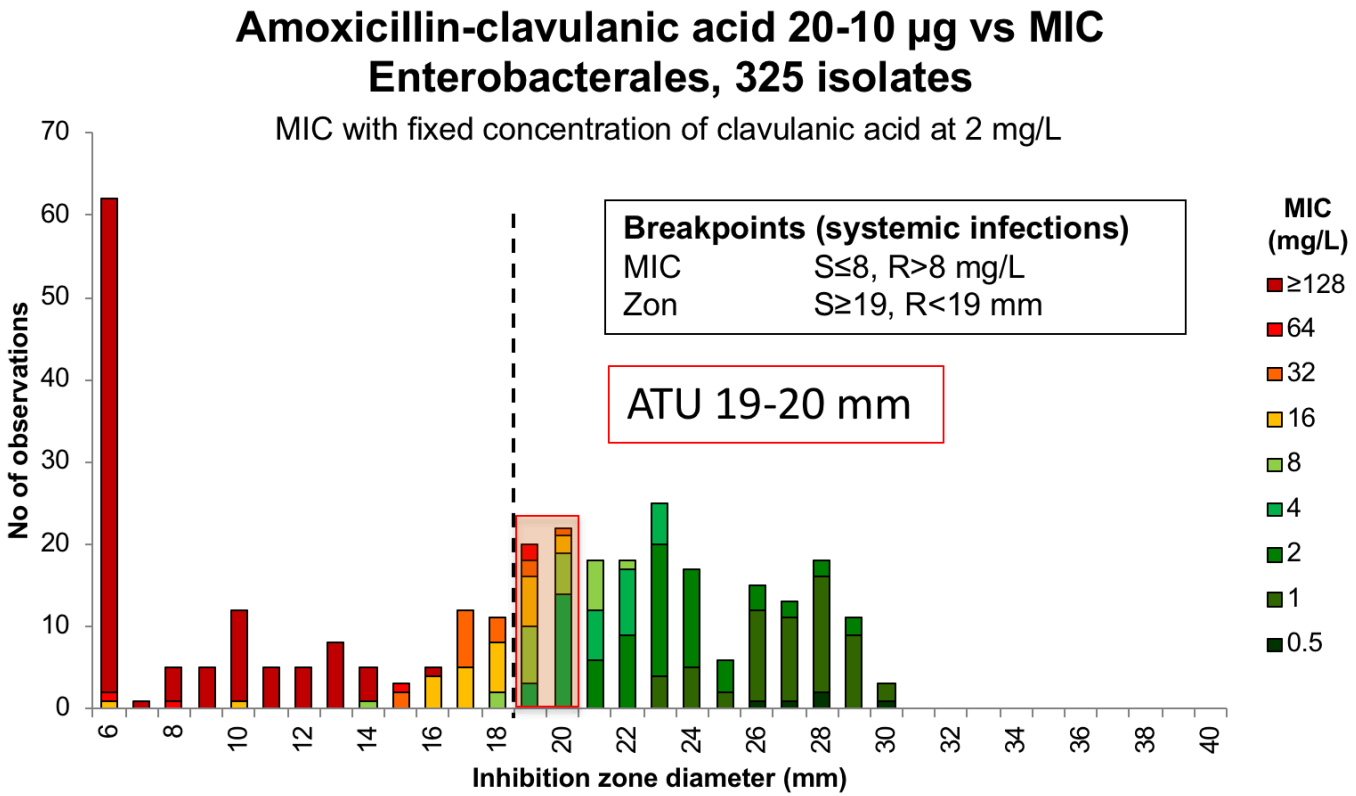
Amoxicillin-clavulanic acid vs. Enterobacterales with breakpoints for uncomplicated UTI



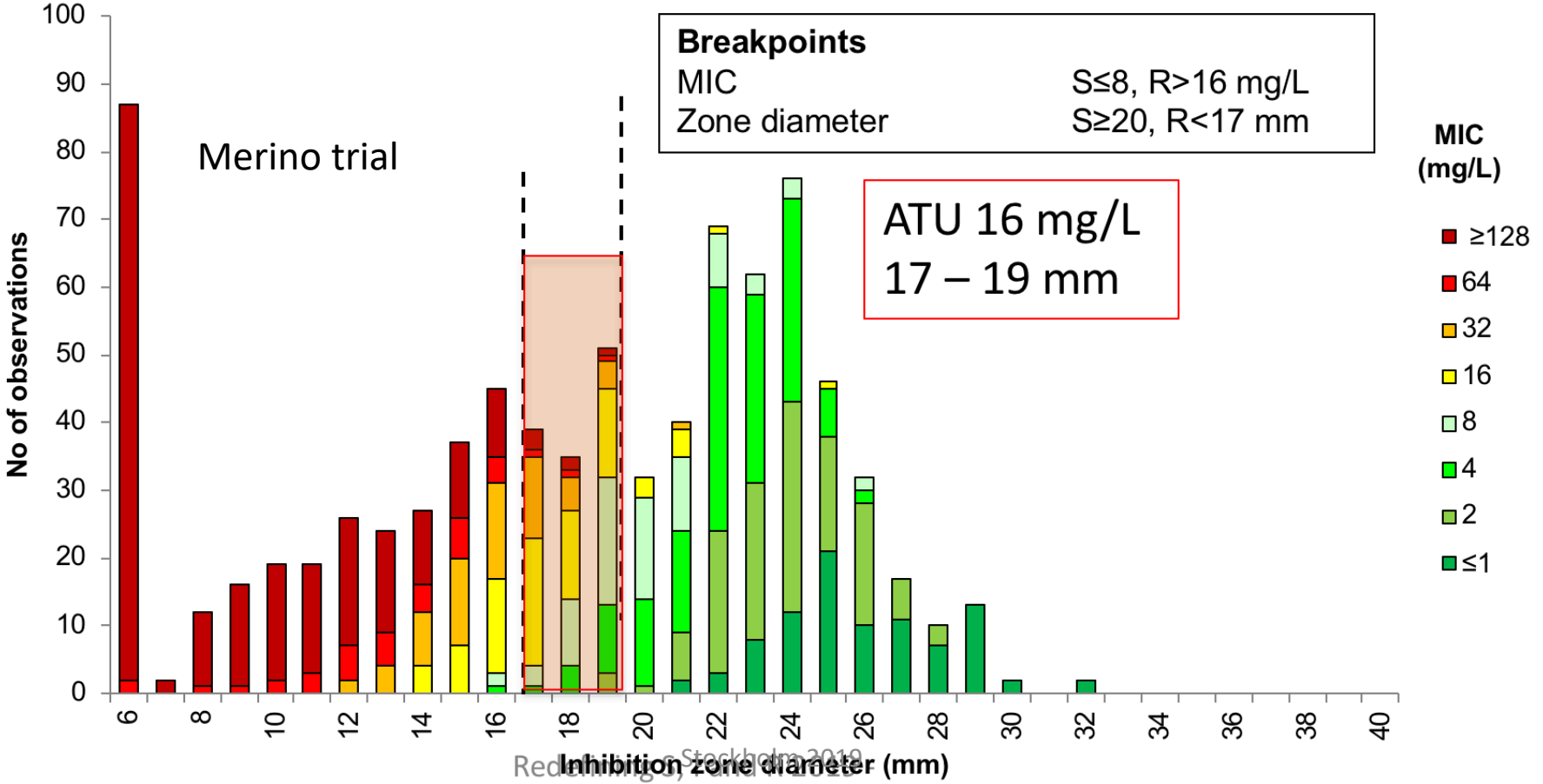
BUT, sometimes there is a need to “warn” laboratory staff!

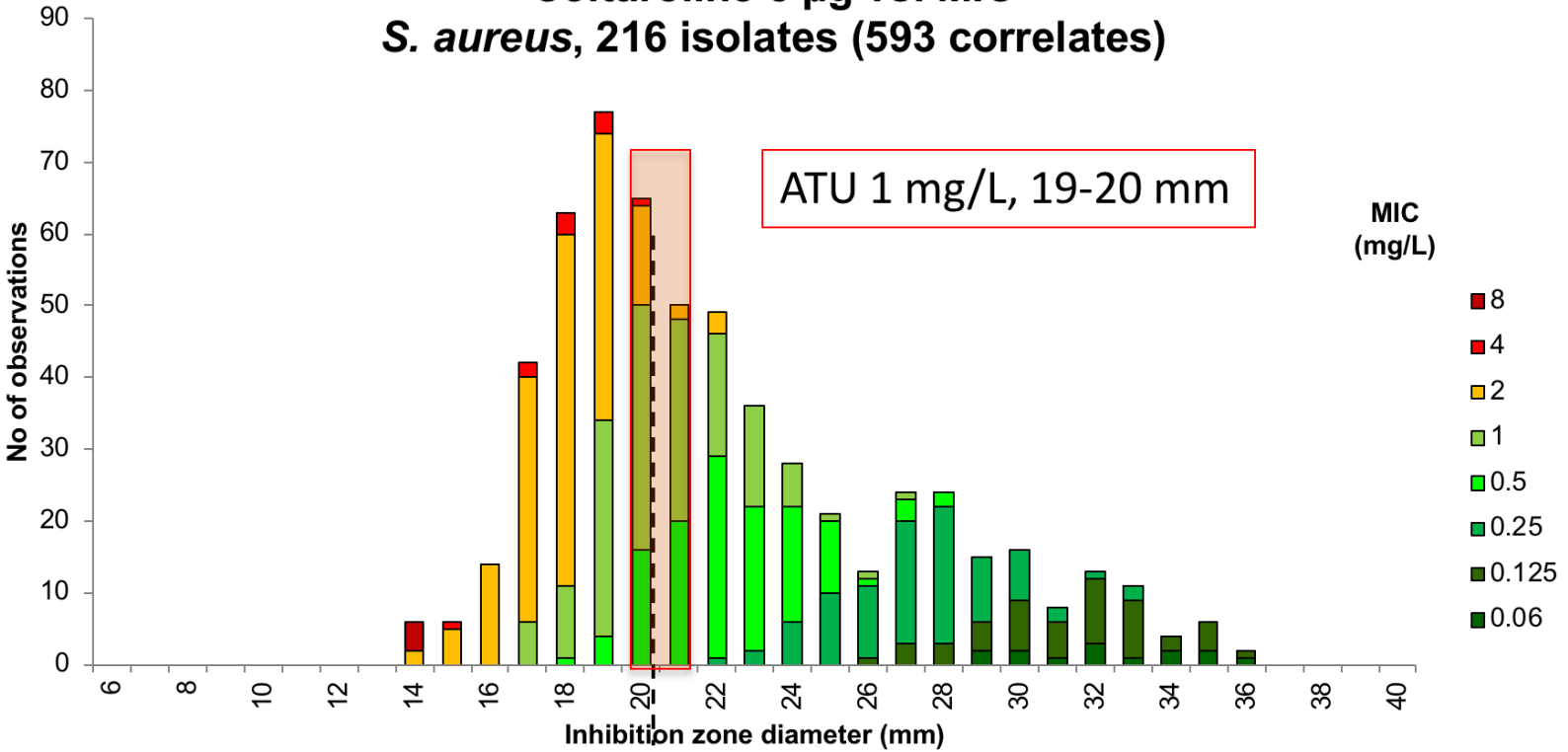
- variation in the method
- variation in the interpretation
  - Breakpoint splits wild type (mostly avoided by EUCAST)
  - Breakpoint splits an important resistant population (piperacillintazobactam in Enterobacterales and Pseudomonas ;ceftaroline and ceftobiprole in MRSA).
- **ATUs are to warn staff about problems which are not due to poor quality of AST material.**

Amoxicillin-clavulanic acid vs. Enterobacterales with breakpoints for systemic infections



Piperacillin-tazobactam 30-6 µg vs. MIC
Enterobacterales, 531 isolates (840 correlates)





Breakpoints (pneumonia)

MIC	S≤1, R>1mg/L
Zone diameter	S≥20, R<20 mm

ATU = Warning in the laboratory

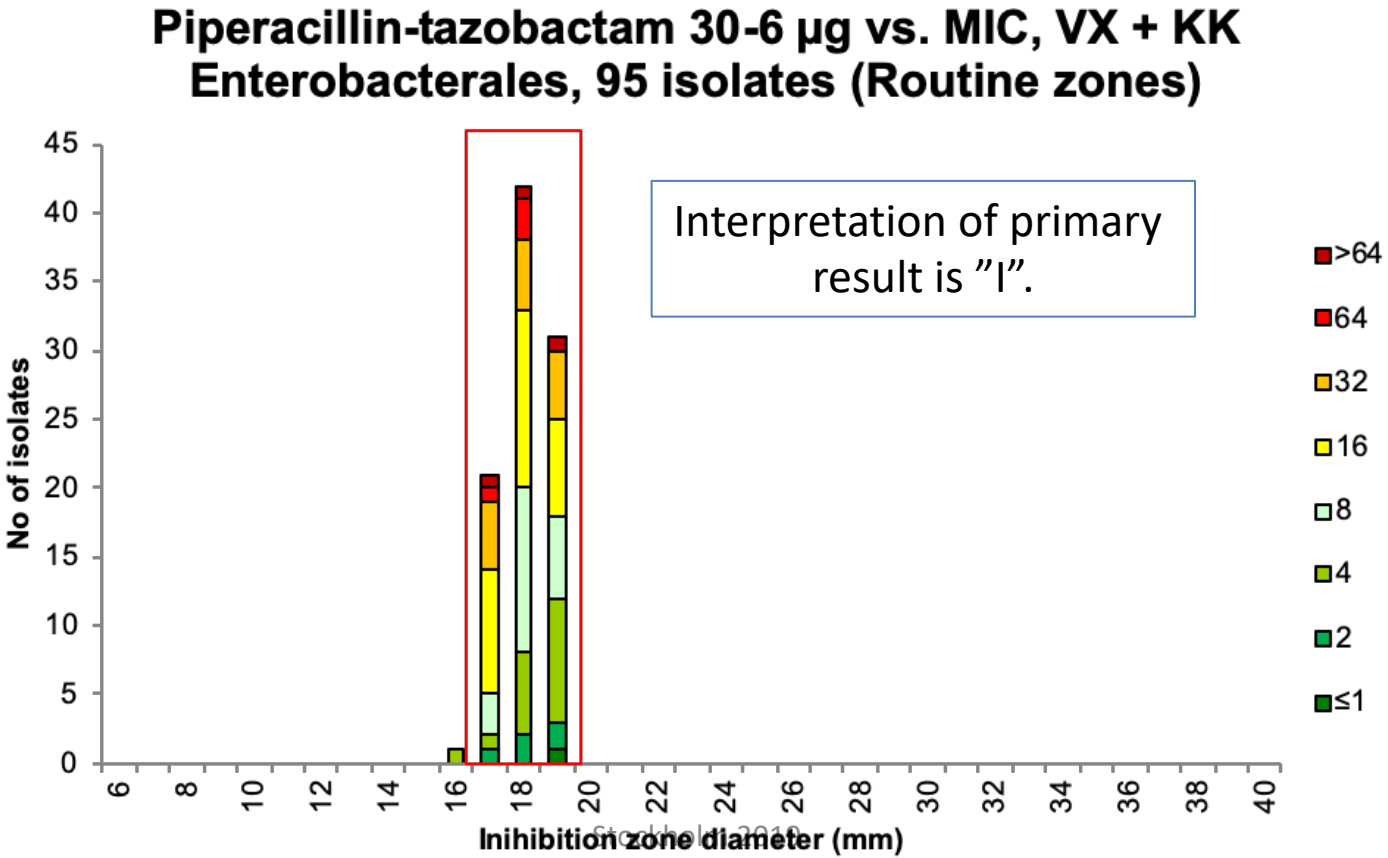
Current ATUs (2019)

- Enterobacterales
  - amoxicillin-clavulanic acid (systemic)
  - piperacillin-tazobactam
  - ciprofloxacin
- *Ps. aeruginosa*
  - piperacillin-tazobactam
  - ceftazidime-avibactam
- *S. aureus*
  - ceftaroline, ceftobiprole
- *S. epidermidis*
  - MRSE cefoxitin screen test on some media
- *H. influenzae* with PBP3-mutations (betalactams)

ATU i Kronoberg/Blekinge 2019

Art	Piperacillintazobaktam (ATU)	Ciprofloxacin (ATU)
E. coli	2.9 %	2.9 %
K.pneumoniae	6 %	6.6 %
Citrobacter freundi	4 %	2.3 %
Proteus mirabilis	<1 %	3.2 %
Morganella morganii	<1 %	Ca 5%

EUCAST determined MIC (by BMD) on all zone diameters in the ATU on consecutive clinical isolates



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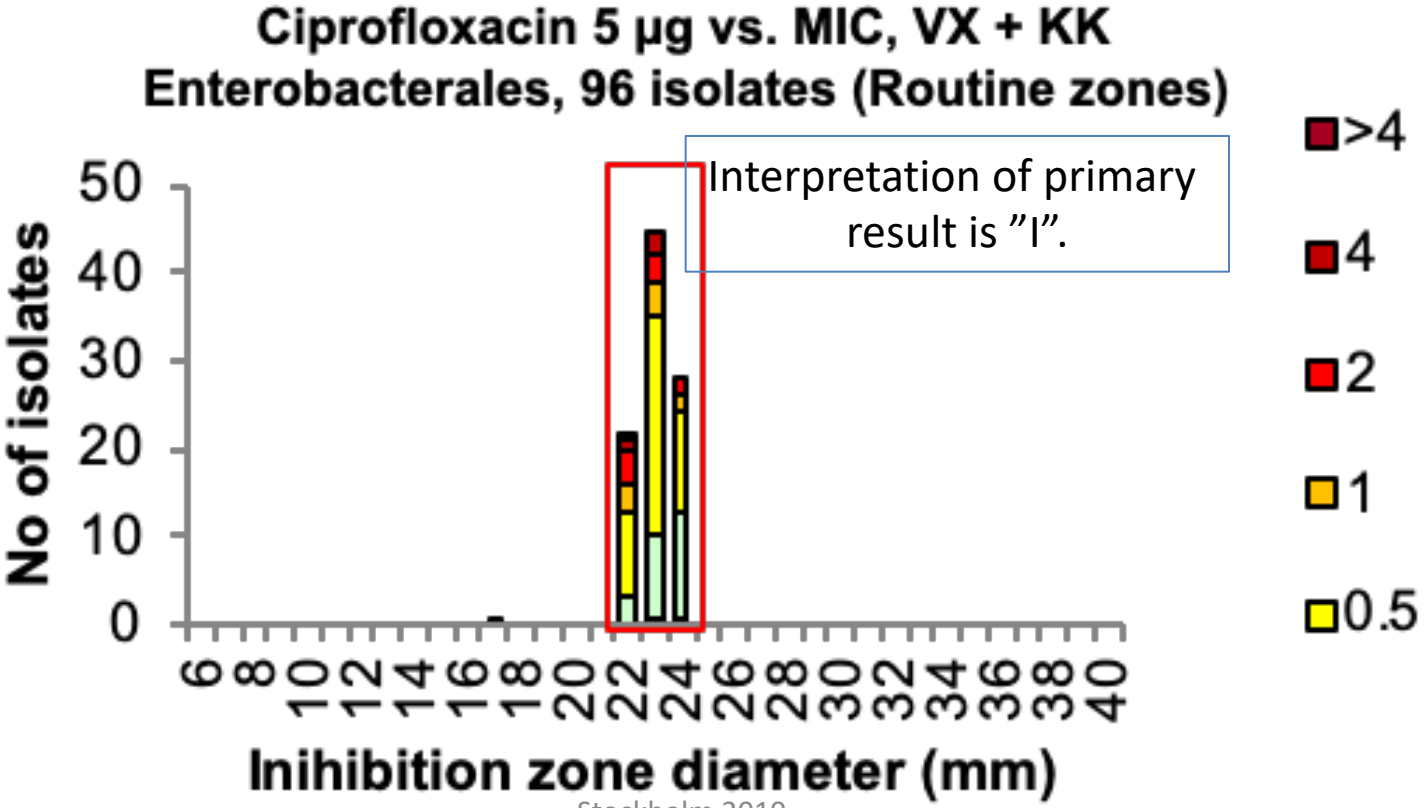

Area of Technical Uncertainty (ATU)

- ATU är inte en “fjärde” resistensbestämningskategori – det är endast en teknisk varning och måste hanteras av laboratoriet.
- ATU interfererar inte med S, I and R kategorisering.
- ATU kompenserar inte för bristande kunnande inom området resistensbestämning.
- ATU definieras av ett enda MIC-värde och motsvarande zon-interval (vanligen 2 – 3 mm)
- ATU kan inte hanteras med en enda regel – hur man agerar måste bestämmas av situationen (provtyp, art och antibiotikum.)

Warning (ATU) – alternativa åtgärder

- **Upprepa testen** – om tekniska problem (inokulat, fel lapp, lapp ramlat på sniskan etc).
- **Upprepa testen och konfirmera med en alternativ test** (MIC, PCR, PBP-agglutination…).
  Två tester med samma resultat styrker tolkningen.
- **Rapportera blankt MED en kommentar:**
  "Resultatet av resistensbestämningen kunde inte tolkas till S, I eller R.
- **Rapportera ett "nedtolkat" resultat"**
  "För Piperacillin 17-19 mm (eller MIC 16 mg/L) svara "R".
- **Diskutera och förklara – ring kollegerna.**

Try hard to solve IF.....

- easy to solve.
- only few alternative antibiotics for therapy.
- in a positive blood culture (or other serious infection).
- a frequently recurring problem

Tack!

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