

Redefining susceptibility testing categories **S**, **I** and **R**.

Gunnar Kahlmeter and the EUCAST Steering Committee

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The presentation describes changes in the definitions of susceptibility test categories S, I and R and the consequences thereof. The changes take effect with EUCAST breakpoint table v 9.0 (2019).

The EUCAST Steering Committee (SC) has decided to change the definitions of susceptibility testing categories but to retain the abbreviations S, I and R.

This decision was taken in June, 2018, following three general consultations (2015, 2017 and 2018). The results of the consultations are available on the EUCAST website (see Consultations)

New definitions are valid from 2019-01-01 (EUCAST breakpoint table v.9.0)

The 2002 – 2018 definitions of S, I and R

“The old definition”.

Since 2002, EUCAST has used the following definitions to categorise the microorganisms as treatable or not treatable with the agent in question.

Breakpoints in breakpoint tables are clinical, i.e. are meant to predict the clinical outcome in the infected patient.

S = Susceptible

I = Intermediate

R = Resistant

EUCAST definitions of clinical breakpoints and epidemiological cut-off values

Clinical resistance and clinical breakpoints

Clinically Susceptible (S)

- a micro-organism is defined as susceptible by a level of antimicrobial activity associated with a high likelihood of therapeutic success
- a micro-organism is categorized as susceptible (S) by applying the appropriate breakpoint in a defined phenotypic test system
- this breakpoint may be altered with legitimate changes in circumstances

Clinically Intermediate (I)

- a micro-organism 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 physically 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.
- a micro-organism is categorized as intermediate (I) by applying the appropriate breakpoints in a defined phenotypic test system
- these breakpoints may be altered with legitimate changes in circumstances

Clinically Resistant (R)

- a micro-organism is defined as resistant by a level of antimicrobial activity associated with a high likelihood of therapeutic failure.
- a micro-organism is categorized as resistant (R) by applying the appropriate breakpoint in a defined phenotypic test system
- this breakpoint may be altered with legitimate changes in circumstances

Clinical breakpoints are presented as $S \leq x$ mg/L; $I > x, \leq y$ mg/L; $R > y$ mg/L

In the old definition it is unclear which part is valid in the individual AST report.

“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.”

The old definition of intermediate has four definitions rolled into one.

1. **uncertain therapeutic effect** (pharmacology/microbiology)
2. **where the drugs are physiologically concentrated**
(pharmacokinetics)
3. **when a high dosage of drug can be used**
(pharmacology/toxicology)
4. **a buffer zone to prevent technical errors ...**
(methodology)

Intermediate results thus encompass both...

- **Uncertainty**
 - uncertain therapeutic effect
 - uncertain laboratory result
- **Exposure**
 - agent physiologically concentrated
 - Dosing strategy (dose, frequency, mode of administration)

Uncertainty and Exposure

- **Uncertainty**

- responsibility of breakpoint committees

- Breakpoints should avoid dividing wild type MIC distributions of important species; otherwise reproducibility in AST cannot be achieved

- responsibility of the laboratory

- Laboratories are responsible for using appropriate methods and interpretative criteria and for the quality control (QC) of test results.

Uncertainty and **Exposure**

- **Exposure**
 - responsibility of breakpoint committees
 - breakpoint committees should inform users of dosing strategies relevant to the breakpoints and under what other conditions breakpoints are valid.
 - responsibility of the clinician
 - It is possible to adjust the level of exposure by changing the dosing strategy; individual dose, frequency of dosing, from oral to intravenous, from intermittent to continuous infusion.

All clinical breakpoints are related to the achievable level of exposure* of the microorganism.

The achievable level of exposure* depends on many factors.

Individual differences in pharmacokinetics are allowed for in the calculations leading up to pharmacodynamic indices following population simulation. Others factors as follows are determined by the the site of infection or can be varied during therapy:

1. Site of infection

- concentration in certain tissues and body fluids may be high (urine, bile, lymphatic tissues).

2. Dose and dosing frequency

3. Mode of administration (Oral, Intravenous, IV infusion etc)

*Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution, metabolism and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

Dosing and mode of administration are in the EUCAST breakpoint table.

EUCAST breakpoints are related to the doses and modes of administration listed by EUCAST in rationale documents and in the breakpoint table, "Dosing" tab.

With regimens other than those listed in the EUCAST tables, breakpoints may be invalid.

For this reason EUCAST has made every effort to consult with all countries to ascertain that the doses and modes of administration listed in EUCAST documents are representative of international practices.

New definitions of S, I and R

- The changes in the definitions of **S** and **R** categories are minor. They mostly emphasise the relationship between the susceptibility category and the level of exposure.
- The changes in the **I** category will have major clinical and technical impact and will affect antimicrobial resistance surveillance. They have also required a change in some breakpoints.

The new definitions of S, I and R

The new definitions reflect the need for correct exposure and for laboratories to take responsibility for technical difficulties and solve them prior to finalising AST reports.

The dosing strategies relevant to EUCAST breakpoints are available in the breakpoint table, “Dosing” tab.

These are the new definitions:

Susceptible, standard dosing regimen (S)

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.

* Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution, metabolism and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

Susceptible, increased exposure (I)

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.

* Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution, metabolism and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

Resistant (R)

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

* Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution, metabolism and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

SIR – the old definitions

Susceptible

Intermediate

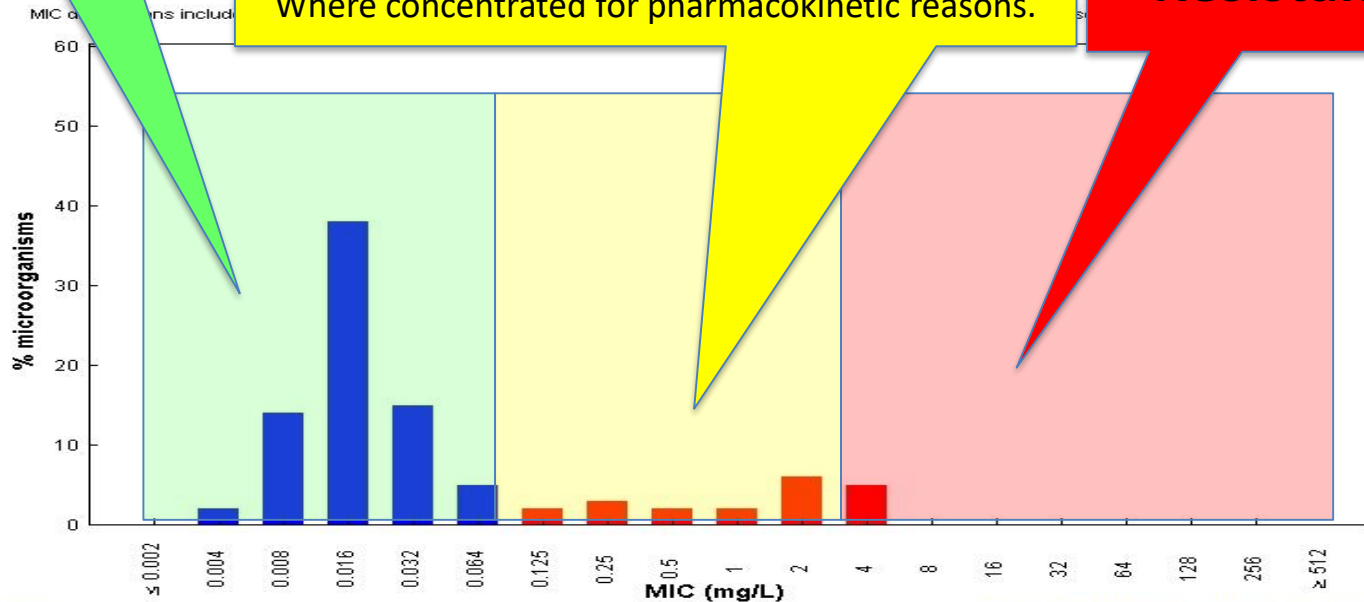
Uncertain effect.

Buffer zone for technical variation.

For a high dose.

Where concentrated for pharmacokinetic reasons.

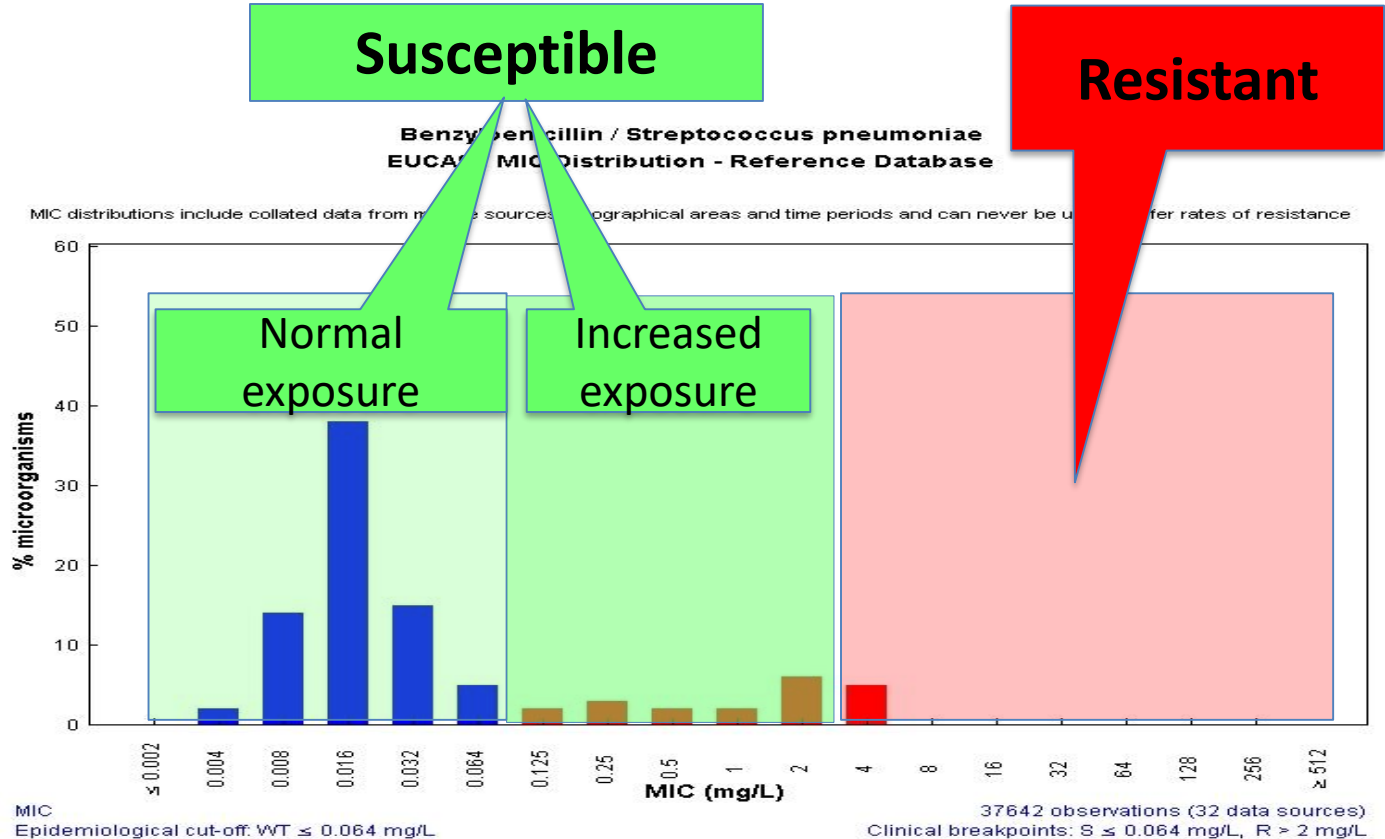
Resistant



MIC
Epidemiological cut-off: WT ≤ 0.064 mg/L

37642 observations (32 data sources)
Clinical breakpoints: S ≤ 0.064 mg/L, R ≥ 2 mg/L

SIR - new definitions 2019



EUCAST decision 2018

- To change the definition of **S**, **I** and **R**.
- To retain the abbreviations **S**, **I** and **R**.
- To emphasise the relationship between the exposure of the microorganism at the site of infections and the breakpoint and to task National AST Committees (NAC) with informing colleagues about the relationship between dosing practices and breakpoints.
- To task laboratories with taking the responsibility for and deal with "technical variation and errors".

With the modified definition of the "I-category"

....the only difference between "S" and "I" is the amount of drug at the site of the infection necessary to achieve an adequate clinical response.

The term "intermediate" is replaced by the term "Susceptible, increased exposure" but the abbreviation in reports is still "I".

Retaining abbreviations S, I and R

- There are good arguments both for and against changing the abbreviations. However, during the consultation process a clear majority advised against a change at this point in time.

However, EUCAST has not ruled out a future change.

LIS systems and manufacturers of AST devices are urged to look into how a change of the abbreviation used to designate the I category will affect their systems and to inform EUCAST.

A few breakpoints will be revised to fit with the new definitions of S, I and R

Species group	Agent	Breakpoint 2018 mg/L	Breakpoint 2019 mg/L
<i>Pseudomonas</i>	Aztreonam	1 / 16	16 / 16
<i>Enterococcus</i>	Trimethoprim	WT I-category	Note+ECOFF
<i>Enterococcus</i>	Trimethoprim-sulfamethoxazole	WT I-category	Note+ECOFF
<i>N. meningitidis</i>	Chloramphenicol	2 / 4	2 / 2
<i>H. influenzae</i>	Cefpodoxime	0.25 / 0.5	0.25 / 0.25
<i>Proteus</i> <i>Morganella</i> <i>Providencia</i>	Imipenem	2 / 4	0.12 / 4
<i>Acinetobacter</i>	Ciprofloxacin	1 / 1	0.06 / 1

Inconsistencies in breakpoints 2019

There are a few inconsistencies with the new system – these need to be corrected, most probably already 2020.

- The treatment of infections with *Pseudomonas* spp require increased exposure for almost all active agents (including imipenem but possibly excepting meropenem) – therefore wild type *Pseudomonas* should have been categorized "Susceptible, increased exposure" for all relevant antimicrobials. The committee decided that more time was needed to explain that meropenem should not because of this be preferred over other available antimicrobials.
- The treatment of *Enterobacterales* with aminopenicillins and cefuroxime, of *S. aureus* with ciprofloxacin and *S. pneumoniae* with levofloxacin require increased exposure and should have been categorized "Susceptible, increased exposure".
- A general consultation on these issues will be needed before a final decision can be taken during 2019. Until then, these are reported "Susceptible" with a note to emphasise the need for "increased exposure".

Inconsistencies in breakpoints 2019, continued

For these situations laboratories should consider adding a note about the need for high exposure, particularly with...

- *Pseudomonas* and piperacillin-tazobactam, ceftazidime, cefepime, imipenem, aztreonam, fluroquinolones, aminoglycosides.
- *Enterobacterales* and aminopenicillins (with or without inhibitor) and cefuroxime.

New terminology

- An organism can still be reported "Susceptible (**S**)" and "Resistant (**R**)" but can no longer be reported using the word "intermediate" to an agent. It should instead be reported using the words "Susceptible, increased exposure" but still with the abbreviation "I".

EUCAST suggests that during 2019 one of the following wordings (one longer, one shorter) are included in laboratory reports:

- A microorganism is categorised as *Susceptible, increased exposure* (abbreviated "I") when there is a high likelihood of therapeutic success because exposure to the agent can be increased at the site of infection by adjusting the dosing regimen, mode of administration or because the concentration is naturally high at the site of infection (see http://www.eucast.org/clinical_breakpoints/).
- An isolate may be categorized as *Susceptible, increased exposure* (abbreviated "I") to the agent provided higher exposure of the microorganism can be achieved (dose, frequency, mode of administration).

New terminology

– the following language is appropriate following the change in definitions:

- The isolate belongs to the **S**, **I** or **R** category.
- The isolate belongs to the susceptibility category **S**, **I** or **R**.
- The isolate is susceptible (which includes **S** and **I**).
- The isolate is susceptible at standard dosing (which includes **S**).
- The isolate is susceptible only at increased exposure (which includes **I**).
- The isolate is resistant (which includes **R**).
- Susceptibility test reports - report isolates **S**, **I** or **R**.

Surveillance of antimicrobial resistance

It has been common practice to combine susceptibility categories 'Resistant' and 'Intermediate', as non-susceptible, when reporting antimicrobial resistance rates. From 2019, this is no longer appropriate.

- For surveillance purposes, avoid combining categories – present **S**, **I** and **R** separately.
- If there is a need to combine, then combine **S** and **I** and present **R** separately.

Laboratory technical variation and uncertain results

- The old definition of I encompasses a degree of uncertainty and/or uncontrolled technical variation. Where and to what degree was not defined.
- This part of the definition has been removed and EUCAST has identified obvious situations where laboratories must take specific action to avoid reporting highly uncertain results.
- There are situations where poor reproducibility of results is predictable.

Breakpoint committees and laboratories are tasked with minimising technical problems in AST.

Technical problems typically appear when

1. a breakpoint **bisects the wild type**.
2. a breakpoint **bisects a resistant population**.
3. there is **uncontrolled testing variation**.
 - Poor quality of AST material (broth, agar, disks, devices etc).
 - Poor calibration/validation of AST procedures.
 - Poor QC practices in the laboratory.

A few examples of where a warning against uncertain and poorly reproducible results is warranted

- **Amoxicillin-clavulanic acid vs. Enterobacterales.**
 - The wild type distribution of most Enterobacterales end at 8 mg/L. PK/PD of the mother agent indicates a breakpoint of maximum 8 mg/L and then only if high exposure is achieved. For UTI the standard dose will tolerate a breakpoint of 32 mg/L. Unfortunately, when determining MICs or disk diffusion test results, there is poor reproducibility in the critical area 16 mg/L.
- **Piperacillin-tazobactam vs. Enterobacterales.**
 - The wild type distribution of most Enterobacterales end at 8 mg/L. PK/PD of the mother agent indicates a breakpoint of 8/16 mg/L with the highest possible exposure for organisms in the I-category. Unfortunately, when determining MICs or disk diffusion test results, there is poor reproducibility in the critical area 16 mg/L.

EUCAST will advise laboratories on how to handle uncertain AST results.

The following slides are primarily for staff in microbiological laboratories.

Redefining susceptibility testing categories S, I and R - Consequences for laboratories.



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Area of Technical Uncertainty (ATU)

- EUCAST's ability to detect areas where the technical uncertainty is such that it seriously affect the predictive value of antimicrobial susceptibility testing (AST) has improved.
- In 2019 we introduce the term "ATU" in susceptibility testing where a warning is needed to alert the laboratory to the uncertainty of the AST result.
- The warning affects the laboratory, not the clinician, and the laboratory needs a strategy to (1) ascertain the correctness or (2) to report the uncertainty of the result.

To ascertain correctness or uncertainty of AST results.

The warnings are typically in the form of a defined **MIC or inhibition zone interval** (overlap between susceptible and resistant organisms) where interpretation is uncertain. The warning is between the AST system and the laboratory and the laboratory needs to decide how to react to the warning.

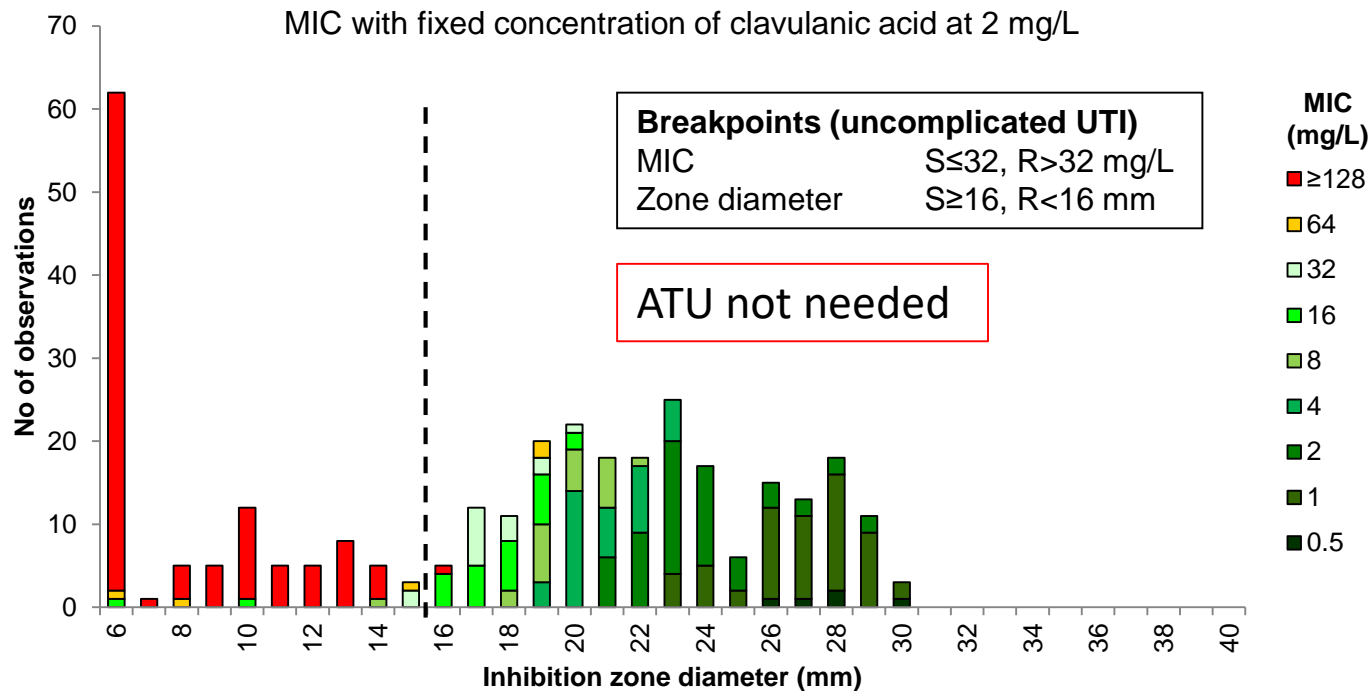
In the following graphs we present a few typical examples of where a warning to the lab is warranted.

A few examples of where a warning against uncertain and poorly reproducible results is warranted

- **Amoxicillin-clavulanic acid vs. Enterobacterales.**
 - The wild type distribution of most Enterobacterales end at 8 mg/L. PK/PD of the mother agent indicates a breakpoint of maximum 8 mg/L and then only if high exposure is achieved. For UTI the standard dose will tolerate a breakpoint of 32 mg/L. Unfortunately, when determining MICs or disk diffusion test results, there is poor reproducibility in the critical area 16 mg/L.
- **Piperacillin-tazobactam vs. Enterobacterales.**
 - The wild type distribution of most Enterobacterales end at 8 mg/L. PK/PD of the active agent indicates a breakpoint of 8/16 mg/L with the highest possible exposure for organisms in the I-category. Unfortunately, when determining MICs or disk diffusion test results, there is poor reproducibility in the critical area 16 mg/L.

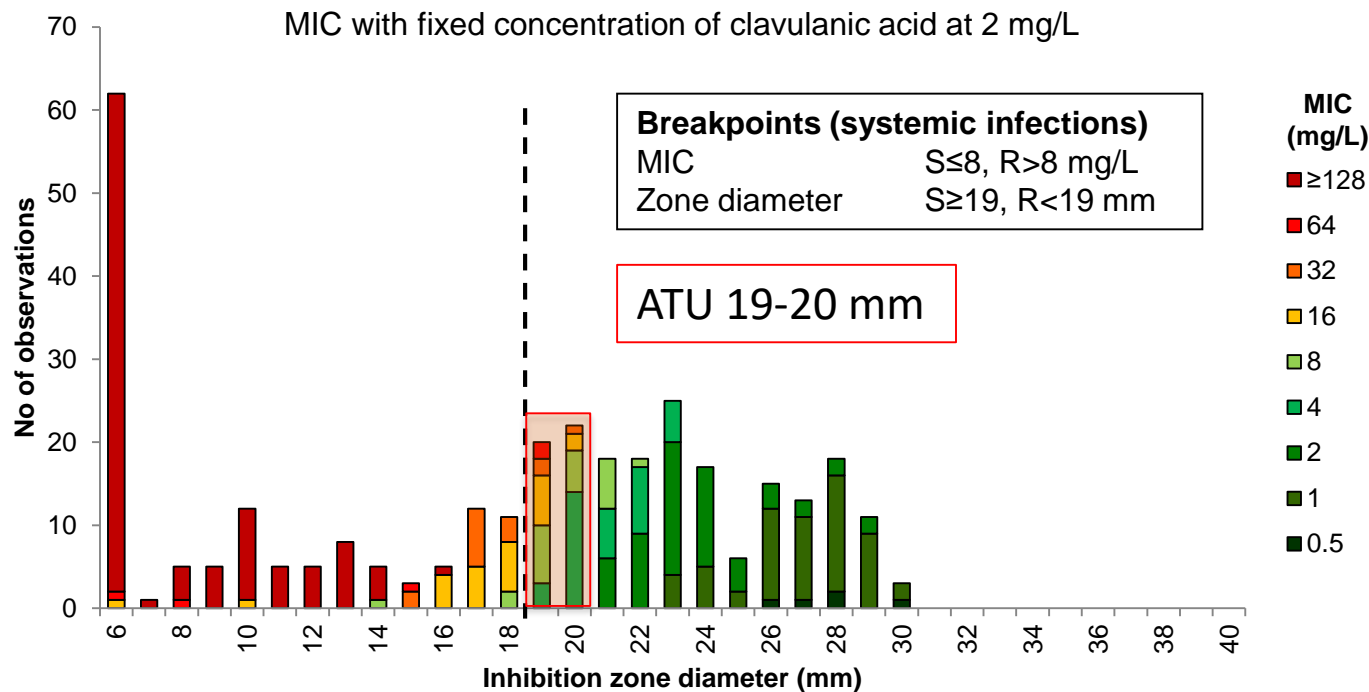
Amoxicillin-clavulanic acid vs. Enterobacterales with breakpoints for uncomplicated UTI

Amoxicillin-clavulanic acid 20-10 µg vs MIC Enterobacterales, 325 isolates



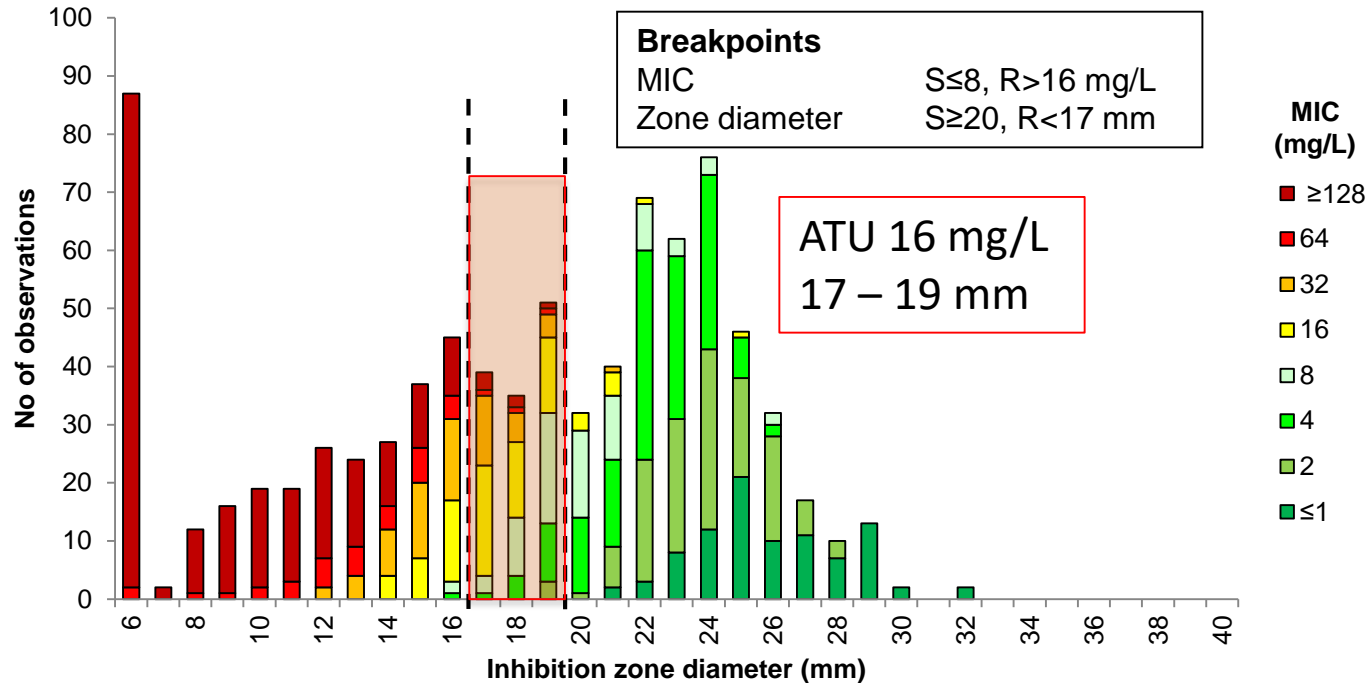
Amoxicillin-clavulanic acid vs. Enterobacterales with breakpoints for systemic infections

Amoxicillin-clavulanic acid 20-10 µg vs MIC Enterobacterales, 325 isolates



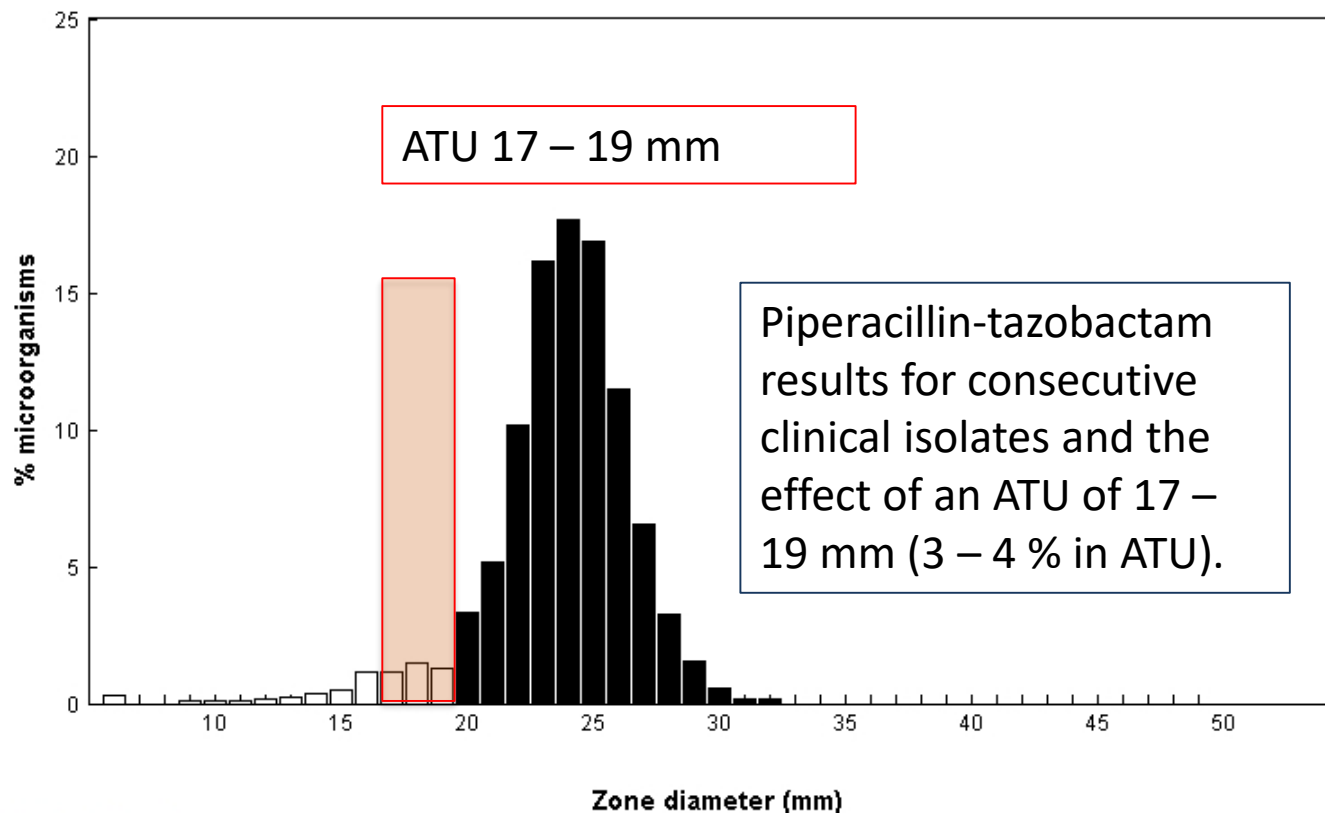
Piperacillin-tazobactam vs. Enterobacterales

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



Piperacillin-tazobactam / *Escherichia coli*
International wild type zone diameter distribution - Reference database 2017-04-21
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: 36

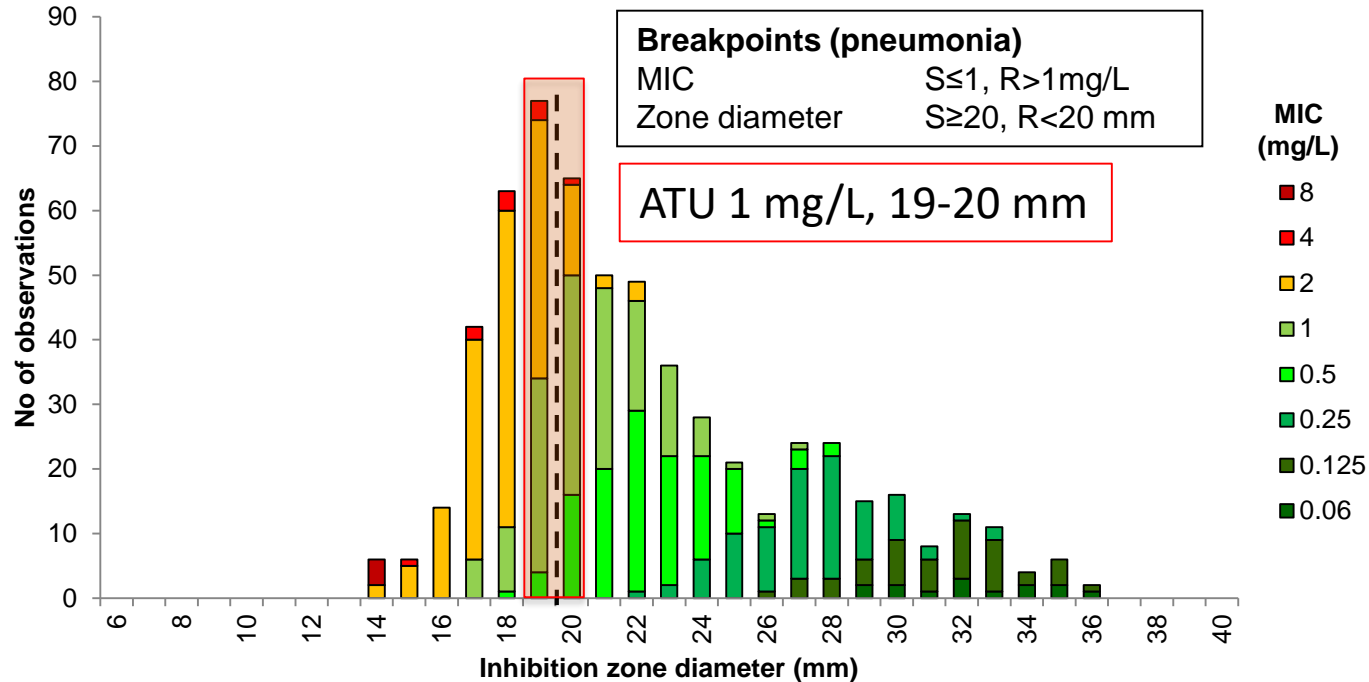
Epidemiological cut-off (ECOFF): 20 mm (MIC = 8 mg/L)

Wildtype (WT) organisms: ≥ 20 mm (MIC = 8 mg/L)

6033 observations (9 data sources)

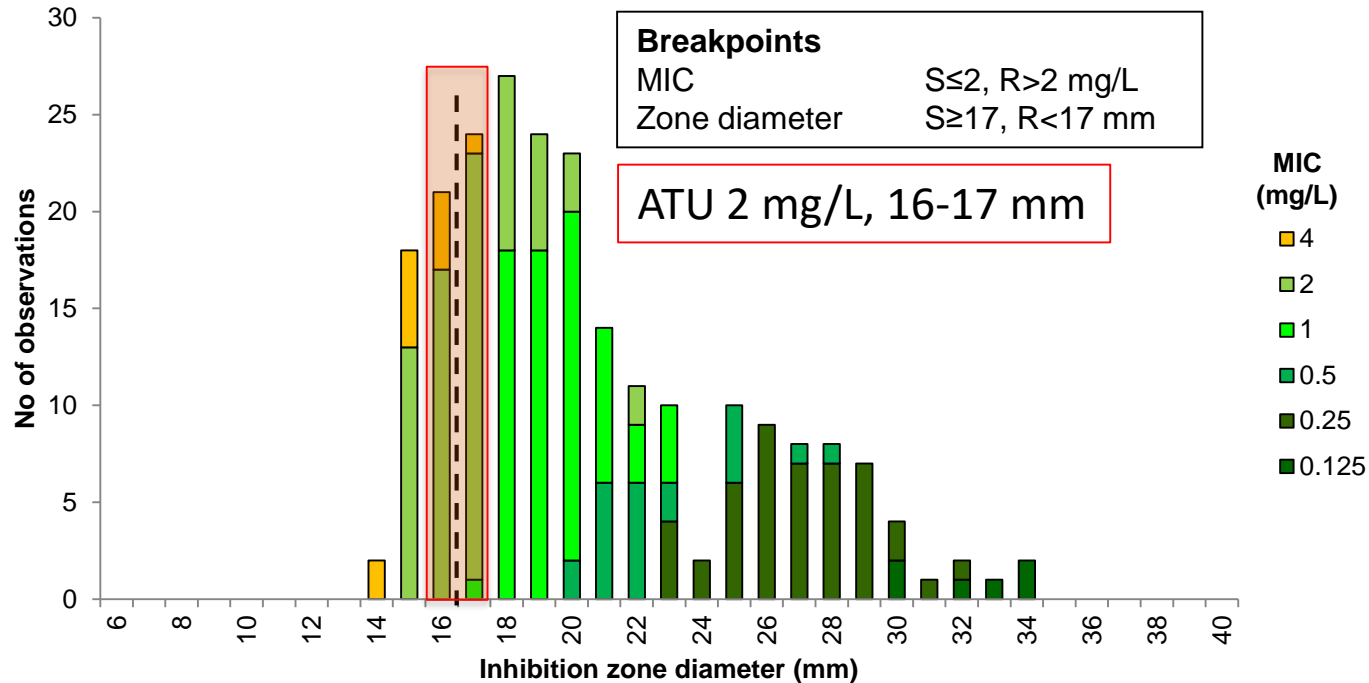
Ceftaroline vs. *S. aureus*

Ceftaroline 5 µg vs. MIC *S. aureus*, 216 isolates (593 correlates)

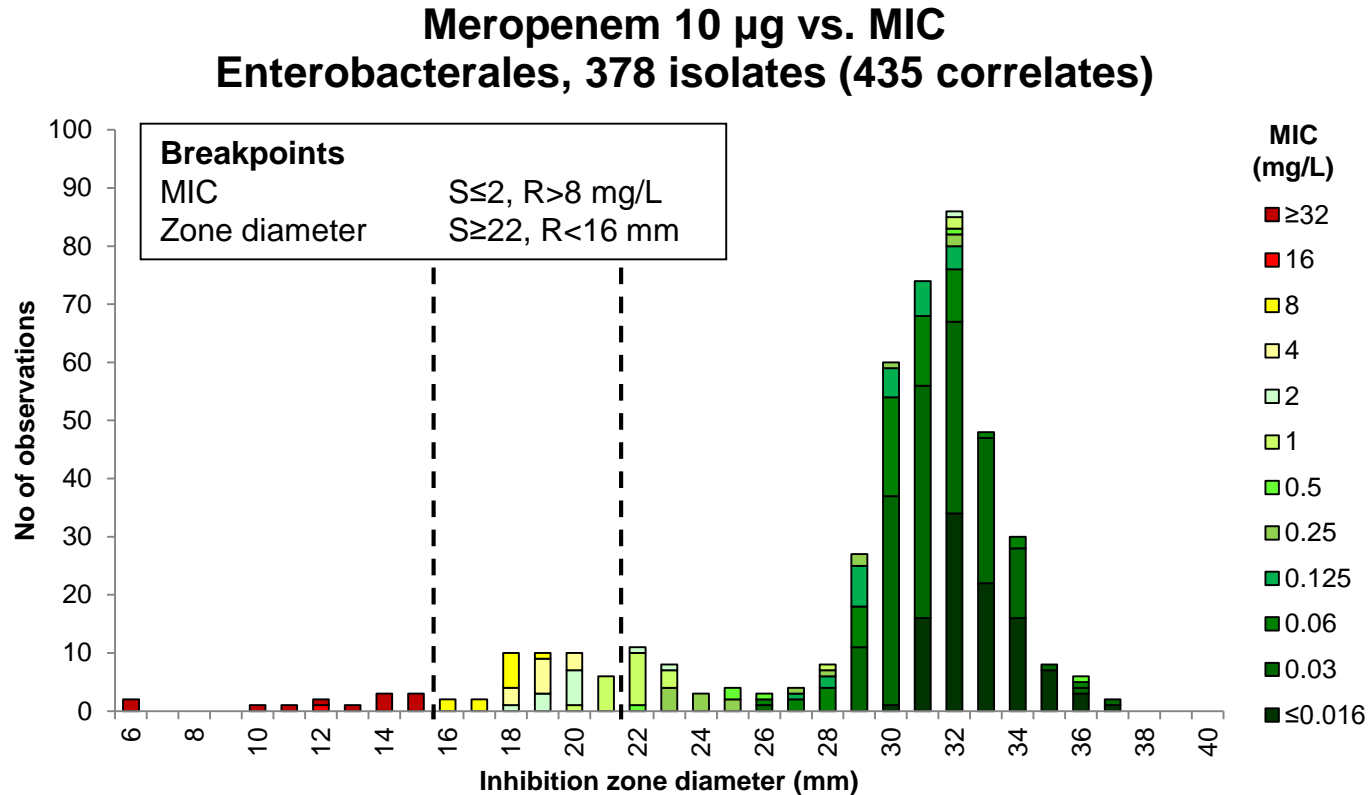


Ceftobiprole vs. *S. aureus*

Ceftobiprole 5 µg vs. MIC *S. aureus*, 114 isolates (228 correlates)



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



EUCAST breakpoint table v.9.0 (2019) with columns for ATU warnings for MIC and/or disk diffusion testing

Penicillins ¹	MIC breakpoint (mg/L)			Disk content (µg)	Zone diameter breakpoint (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	
Benzylpenicillin	-	-			-	-		Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ampicillin	8 ¹	8		10	14 ^{A,B}	14 ^B		
Ampicillin-sulbactam	8 ^{1,2}	8 ²		10-10	14 ^{A,B}	14 ^B		1/A. Wild type Enterobacterales are categorised as susceptible to aminopenicillins. Some countries prefer to categorise wild type isolates of <i>E. coli</i> and <i>P. mirabilis</i> as "Susceptible, increased exposure". When this is the case, use the MIC breakpoint S ≤ 0.5 mg/L and the corresponding zone diameter breakpoint S ≥ 50 mm.
Amoxicillin	8 ¹	8		-	Note ^C	Note ^C		
Amoxicillin-clavulanic acid	8 ^{1,3}	8 ³		20-10	19 ^{A,B}	19 ^B	19-20	2. For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
Amoxicillin-clavulanic acid (uncomplicated UTI only)	32 ^{1,3}	32 ³		20-10	16 ^{A,B}	16 ^B		3. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Piperacillin	8	16		30	20	17		4. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
Piperacillin-tazobactam	8 ⁴	16 ⁴	16	30-6	20	17	17-19	5. Breakpoints still under consideration.
Ticarcillin	8	16		75	23	20		6. Agar dilution is the reference method for mecillinam MIC determination.
Ticarcillin-clavulanic acid	8 ³	16 ³		75-10	23	20		B. Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agars. C. Susceptibility inferred from ampicillin.
Temocillin	Note ⁵	Note ⁵			Note ⁵	Note ⁵		
								D. Ignore isolated colonies within the inhibition zone for <i>E. coli</i> .
Phenoxymethylpenicillin	-	-			-	-		
Oxacillin	-	-			-	-		
Cloxacillin	-	-			-	-		
Dicloxacillin	-	-			-	-		
Flucloxacillin	-	-			-	-		
Mecillinam (uncomplicated UTI only) <i>E. coli</i> , <i>Klebsiella</i> spp. (except <i>K. aerogenes</i>), <i>Raoultella</i> spp. and <i>P. mirabilis</i>	8 ⁶	8 ⁶		10	15 ^D	15 ^D		

There are only few proposed ATUs

All will be listed in EUCAST breakpoint tables 2019.

- | | |
|-----------------------------|----------|
| • <i>Enterobacterales</i> | 4 agents |
| • <i>Pseudomonas</i> spp | 3 agents |
| • <i>Staphylococcus</i> spp | 3 agents |
| • <i>H. influenzae</i> | 8 agents |
| • Other species | 0 agents |

Preliminary ATUs in Enterobacterales, Pseudomonas and Staphylococcus

Species	Agent	MIC (mg/L, ATU)	Zone diameter (mm, ATU)
<i>Enterobacterales</i>	Amoxicillin-clavulanic acid	-	19-20
	Piperacillin-tazobactam	16	17-19
	Ceftaroline	-	22-23
	Ciprofloxacin	0.5	22-24
<i>Ps. aeruginosa</i>	Piperacillin-tazobactam	-	18-19
	Ceftazidime-avibactam	-	16-17
	Colistin	4	-
<i>St. aureus</i>	Ceftaroline	1	19-20
	Ceftobiprole	2	16-17
	Amikacin	16	15-19
<i>St. epidermidis</i>	Cefepime	16	25-27

Preliminary ATUs

Preliminary ATUs in *H. influenzae*

Species	Agent	MIC (mg/L, ATU)	Zone diameter (mm, ATU)
<i>H. influenzae</i>	Ampicillin		16-19
	Amoxicillin-clavulanic acid		14-16
	Piperacillin-tazobactam	0.5	24-27
	Cefotaxime		25-27
	Ceftriaxone		31-33
	Cefuroxime (iv and oral)	2	25-27
	Cefepime, Cefpodoxime and Imipenem		See flow chart

Preliminary ATUs

How can the ATU be implemented in laboratory practices?

- Laboratories **without IT support** (manual **S**, **I** and **R** categorisation on MIC or disk diffusion results)
 - List manually species/agents with ATUs and proposals on how to handle each.
- Laboratories **with IT support** (where **S**, **I** and **R** categorisation is performed automatically on entering MIC or disk diffusion results)
 - Develop the software to include IF/THEN algorithms such as:
IF *S. aureus* and ceftaroline and MIC 1 mg/L (or zone 19-20 mm), THEN take ACTION*...”
IF *E. coli* and piperacillintazobactam MIC 16 mg/L (or zone 18 – 19), THEN take ACTION*...”

The basic principle is the same irrespective of which methods are used, but there may be an ATU in only one system.

- Disk diffusion
- MIC determination
- Semi-automated AST devices

*Action may vary – see next few slides for proposed actions!

Disk diffusion (ATU)

- If computerised interface where zone diameters are (manually or automatically) registered for categorical interpretation:
 - Introduce ATU (species, agent, interval) to generate
 - "Warning signal" (sound, light, asterisk in report protocol,)
 - Block automatic interpretation and force manual decisions.
- If manual interface, print a manual list of ATUs or use EUCAST breakpoint table printout.

MIC determination

- Automatic reading with computerized interpretation of full scale MIC determination.
 - Introduce ATU (species, agent, interval) to generate:
 - "Warning signal" (sound, light, asterisk in report protocol,)
 - Block automatic interpretation and force manual decisions.
- Manual reading of full scale MIC determination
 - print a manual list of ATUs or use EUCAST breakpoint table printout

Semi-automated AST devices

- Start by checking which ATUs can be detected in relation to the often short dilution series (2 – 4 dilutions)
- If ATUs are outside dilution series, control will be impossible
- If ATUs are inside dilutions series, use ATUs as for MIC determination (previous slide)

ATU – alternative actions for the laboratory

- **repeat the test** – this is only if there is reason to suspect a technical error.
- **perform an alternative test** (perform an MIC, a PCR, a test to determine the resistance mechanism) – this is relevant when the alternative test is conclusive (PCR to detect a *vanA* or *vanB* gene in enterococci, a *Bla* test in *H. influenzae*).
- **report results in the ATU as “uncertain”** – this can be achieved by leaving the interpretation blank + comment. Or by developing the LIS to deliver an asterix (instead of an S, I or R) which refers to a comment explaining the uncertainty.
- **report results in the ATU as “R”**. If there are several good alternatives in the AST report this may be the easiest and safest option.
- take the opportunity to **discuss the results with the clinician**.

ATU – the appropriate action may vary with circumstances

- IF few antibiotics available to the clinician, THEN try to achieve trustworthy categorisation.
- IF in a blood culture, THEN try to achieve trustworthy categorisation.
- IF can be solved with an alternative method without delay, THEN try to achieve trustworthy categorisation.
- IF many alternative antibiotics available, THEN report R (with or without a comment).
- IF the result must be reported, THEN include a comment to discuss uncertainty.

The End

...of the beginning....

Questions and comments can be addressed to
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