Antimicrobial susceptibility testing – facts and challenges

Gunnar Kahlmeter

EUCAST Technical Data Coordinator

Paul Ehrlich Society, Frankfurt, 2017

The development of AST

- Beijerinck in 1889 used agar diffusion to study the effect of plant growth hormones on bacterial growth.
- Fleming in 1924 used a “ditch plate” technique for evaluating antimicrobial qualities of antiseptic solutions and later developed the broth dilution technique with turbidity as an endpoint.
- The WHO commissioned the International Collaborative Study (ICS), published in 1971 (Ericsson and Sherris).
- The 1970ies - the formation of national breakpoint committees (DIN, NCCLS, and others) and national disk diffusion AST systems.
- In 2001 national committees were convinced to take responsibility for European harmonisation, finalised in 2008.
- ISO 20776-1 (2006) – International reference for broth microdilution MIC determination in non-fastidious bacteria.

Hans Ericsson (Sweden)

John Sherris (USA)

Antibiotic Sensitivity Testing

Report of an International Collaborative Study

BY

HANS M. ERICSSON and JOHN C. SHERRIS

MUNKSGAARD. COPENHAGEN

Courtesy Ron Jones

WHO, Ericsson and Sherris were critizised for recommending rigorous standardisation

- Balows, head of CDC 1972, commenting on the ICS approach, Balows deemed it impractical and too demanding. It also implied a level of standardisation that might result in violation of property rights: 'I doubt seriously that commercial concerns would willingly or should even be expected to describe or reveal their procedures for impregnation and drying [of discs]. In the USA this might well be regarded as an infringement of their proprietary procedures …

- Garrod: "I must explain that although I took some part in the International Collaborative Study I have for several years disagreed with the direction it was leading.
" The ICS demands a degree of standardisation of the culture medium and of other features of the test, which I believe to be impractical".

- Germany: A national committee on sensitivity testing had voiced concerns in September 1963 that some of Ericsson approaches were 'too complicated given conditions in German laboratories; it seems possible to implement simplifications without compromising precision'.

….similar arguments are reiterated throughout the following 50 years!

- "…different breakpoints for different species….??"
- "…are we to speciate gramnegatives in UTI?"
- "…we cannot put our recommendations on the internet (1996) – only few laboratories will have access…"
- "…distinguish between E. faecalis and E. faecium – recommendations will have to be the same!"
- "…very few laboratories will ever afford a masspec..."
- "…laboratories are not staffed to cope with the extra workload of measuring zone diameters…"

It used to be so simple….

In the beginning there was one table for everything - one MIC breakpoint and one zone diameter breakpoint to fit all.

TABLE 2. ***Zone Diameter Interpretive Standards and Approximate Minimum Inhibitory Concentration (MIC) Correlates***

Antimicrobial Agent	Disc Content	Zone Diameter, nearest whole mm			Approximate MIC Correlates[a]	
		Resistant	Intermediate[q]	Susceptible	Resistant	Susceptible
Amikacin[b]	30 μg	≤ 14	15–16	≥ 17	≥ 32 μg/mL	≤ 16 μg/mL
Ampicillin[c] when testing gram-negative enteric organisms and enterococci	10 μg	≤ 11	12–13	≥ 14	≥ 32 μg/mL	≤ 8 μg/mL
Ampicillin[c] when testing staphylococci[d] and penicillin G-susceptible microorganisms	10 μg	≤ 20	21–28	≥ 29	β-lactamase[d]	≤ 0.25 μg/mL
Ampicillin[c] when testing *Haemophilus* species[e]	10 μg	≤ 19	—	≥ 20	≥ 4 μg/mL	≤ 2 μg/mL
Bacitracin	10 units	≤ 8	9–12	≥ 13	—	—
Carbenicillin when testing the ***Enterobacteriaceae***	100 μg	≤ 17	18–22	≥ 23	≥ 32 μg/mL	≤ 16 μg/mL
Carbenicillin when testing *Pseudomonas aeruginosa*	100 μg	≤ 13	14–16	≥ 17	≥ 256 μg/mL	≤ 128 μg/mL
Cefamandole[f]	30 μg	≤ 14	15–17	≥ 18	≥ 32 μg/mL	≤ 8 μg/mL
Cefotaxime[f]	**30 μg**	**≤ 14**	**15–22[q]**	**≥ 23**	**≥ 64 μg/mL**	**≤ 8 μg/mL**
Cefoxitin[f]	30 μg	≤ 14	15–17	≥ 18	≥ 32 μg/mL	≤ 8 μg/mL
Cephalothin[g]	30 μg	≤ 14	15–17	≥ 18	≥ 32 μg/mL	≤ 8 μg/mL
Chloramphenicol	30 μg	≤ 12	13–17	≥ 18	≥ 25 μg/mL	≤ 12.5 μg/mL
Clindamycin[h]	2 μg	≤ 14	15–16	≥ 17	≥ 2 μg/mL	≤ 1 μg/mL
Colistin[i]	10 μg	≤ 8	9–10	≥ 11	≥ 4 μg/mL	j
Erythromycin	15 μg	≤ 13	14–17	≥ 18	≥ 8 μg/mL	≤ 2 μg/mL
Gentamicin[b]	10 μg	≤ 12	13–14	≥ 15	≥ 8 μg/mL	≤ 4 μg/mL
Kanamycin	30 μg	≤ 13	14–17	≥ 18	≥ 25 μg/mL	≤ 6 μg/mL
Methicillin[k]	5 μg	≤ 9	10–13	≥ 14	≥ 16 μg/mL	≤ 4 μg/mL
Nafcillin[k]	**1 μg**	**≤ 10**	**11–12**	**≥ 13**	**≥ 8 μg/mL**	**≤ 2 μg/mL**
Nalidixic Acid[l]	30 μg	≤ 13	14–18	≥ 19	≥ 32 μg/mL	≤ 12 μg/mL
Neomycin	30 μg	≤ 12	13–16	≥ 17	—	—
Nitrofurantoin[l]	300 μg	≤ 14	15–16	≥ 17	≥ 100 μg/mL	≤ 25 μg/mL
Oxacillin[k]	**1 μg**	**≤ 10**	**11–12**	**≥ 13**	**≥ 8 μg/mL**	**≤ 2 μg/mL**
Penicillin G when testing ***staphylococci[m]***	10 units	≤ 20	21–28	≥ 29	β-lactamase[d]	≤ 0.1 μg/mL
Penicillin G when testing other microorganisms[n]	10 units	≤ 11	12–21	≥ 22		

NCCLS First Supplement, 1981

- “useful for anything that would grow”

It is now 40 years later and much more complicated than anything suggested by the ICS and Ericsson and Sherris.

National breakpoint committees

DIN (G Linzenmeier)	Germany	1973?
NCCLS (later CLSI) (A Barry)	USA	1975
NWGA (K Mellby)	Norway	1978
SRGA (RAF) (LO Kallings)	Sweden	1979
CA-SFM (Y Chabbert)	France	1980
WRG (later CRG) (P Mouton)	The NL	1981
BSAC WP on AST (I Phillips)	The UK	1988

Enterobacteriaceae 1975 – 2001

Committee	Amoxicillin	Cefotaxime	Piperacillin-tazob.
BSAC (UK)	8 / 16	2 / 2	16 / 16
CA-SFM (F)	4 / 16	4 / 32	8 / 64
CRG (NL)	2 / 16	4 / 8	0.25 / 4
DIN (D)	2 / 8	2 / 8	0.12 / 1
NCCLS (USA)	8 / 16	8 / 32	16 / 64
NWGA (N)	0.5 / 8	1 / 2	8 / 16
SRGA (S)	1 / 8	0.5 / 1	16 / 16

All of us managed to come up with different breakpoints.

The breakpoint committees did not agree…

- …not because we disagreed
- …but we were out of sync
- …and did not communicate with each other
- …and we all knew best

EUCAST was formed by ESCMID in 1997 and restructured in 2001…..

I was asked to chair EUCAST and realised that Ian Phillips´ mistake was to have ignored the national committees.

Within 12 months, all national committees agreed to take joint responsibility for harmonising European breakpoints.

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

ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

ESCMID
EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

National Breakpoint Committees
D, F, N, NL, S, UK

EUCAST General Committee

All European Countries + many countries outside Europe

EUCAST Steering Committee

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Subcommittees

Antifungals
Anaerobes
Mycobacteria
Expert Rules and intrinsic resistance
Detection of resistance mechanisms of clinical or public health interest
The relationship between phenotypic susceptibility testing and WGS
MIC distributions and ECOFFs

Expert groups

EUCAST leadership

Chair

- Ian Philips 1997 – 2001
- Gunnar Kahlmeter 2001 – 2012
- Rafael Canton 2012 – 2016
- Christian Giske 2016 –

Scientific secretary

- Derek Brown 1997 – 2016
- John Turnidge 2017 –

Webmaster

- Gunnar Kahlmeter 2001 -

EUCAST Subcommittees

- AFST - Antifungal susceptibility testing
- Anaerobes
- Mycobacteria
- Intrinsic resistance and expert rules
- Detection of resistance mechanisms of clinical or public health importance
- Relationship between WGS and Phenotypic AST
- MIC distributions and the setting of ECOFFs

The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee.

Review article

Ellington MJ, et al. Clin Microbiol Infect. 2017.

Authors

Ellington MJ[1], Ekelund O[2], Aarestrup FM[3], Canton R[4], Doumith M[1], Giske C[5], Grundman H[6], Hasman H[7], Holden MT[8], Hopkins KL[1], Iredell J[9], Kahlmeter G[2], Köser CU[10], MacGowan A[11], Mevius D[12], Mulvey M[13], Naas T[14], Peto T[15], Rolain JM[16], Samuelsen Ø[17], Woodford N[18].

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

search term

- Organization
- EUCAST News
- Clinical breakpoints
- Expert rules and intrinsic resistance
- Resistance mechanisms
- Guidance documents
- MIC distributions and ECOFFs
- Zone distributions and ECOFFs
- AST of bacteria
- AST of mycobacteria
- AST of fungi
- AST of veterinary pathogens
- Frequently Asked Questions (FAQ)
- Meetings
- Presentations and statistics
- Warnings!
- Documents
- Videos from EUCAST

>50 000 hits per month

QUICK NAVIGATION

10 May 2016

The European Committee on Antimicrobial Susceptibility Testing - EUCAST

EUCAST is a standing committee jointly organized by ESCMID, ECDC and European national breakpoint committees. EUCAST was formed in 1997. It has been chaired by Ian Phillips (1997 - 2001), Gunnar Kahlmeter (2001 - 2012), Rafael Canton 2012 - 2016) and Christian Giske (2016 -). Its scientific secretary is Derek Brown (1997 -). Its webmaster is Gunnar Kahlmeter (2001 -). From 2016, Rafael Canton is the Clinical Data Co-ordinator and Gunnar Kahlmeter the Technical Data Co-ordinator.

EUCAST deals with breakpoints and technical aspects of phenotypic in vitro antimicrobial susceptibility testing and functions as the breakpoint committee of EMA and ECDC. EUCAST does not deal with antibiotic policies, surveillance or containment of resistance or infection control. The Steering Committee is the decision making body. It is supported by a General Committee with representatives from European and other countries, FESCI and ISC. The Steering Committee also

www.eucast.org

EUCAST News

10 Sep 2016
A new version of the EUCAST Expert Rules document is published.

09 Sep 2016
Legionella pneumophila - EUCAST guidance document on AST

09 Sep 2016
Splitting wild type MIC distributions with breakpoints - or not!

01 Sep 2016
Instructions videos from EUCAST - 5 published

31 Aug 2016
ECDC documents on European Lab Capacity and on whole genome sequencing as an epidemiological tool.

EUCAST News
Clinical breakpoints
Expert rules and intrinsic resistance
Resistance mechanisms
Guidance documents
Consultations
MIC distributions and ECOFFs
Zone distributions and ECOFFs
AST of bacteria
AST of mycobacteria
AST of fungi
AST of veterinary pathogens
Frequently Asked Questions (FAQ)
Meetings
Presentations and statistics
Warnings!
Documents
Videos from EUCAST
Translations
Information for industry

Instruction videos from EUCAST

In collaboration with the World Health Organisation (WHO), EUCAST publishes instruction videos on how to perform antimicrobial susceptibility testing (AST) using EUCAST recommended methods and interpretation. During 2016, five videos have been completed and 5 more are under construction in 2017.

The videos are published on Youtube™ and have an English speaker voice and English subtitles. There is a mechanism by which subtitles can be translated to other languages.

1. **Preparation of inoculum (English).**
2. **Inoculation of agar plates for disk diffusion (English).**
3. **Application of antibiotic disks and incubation of plates (English).**
4. **Reading of inhibition zone diameters (English).**
5. **Guidance on the use of the breakpoint table (English).**

Instruction videos on EUCAST susceptibility testing with subtitles in other languages than English:

Instruction videos in German.

Instruction videos in Russian.

Instruction videos in Turkish.

Instruction videos in French.

Instruction videos in Spanish.

Instruction videos in Portuguese.

Instruction videos in ...(more to follow shortly)

What is new in EUCAST 2016/17?

- New organisms – breakpoints 2016/17
  - Aerococcus spp, Kingella kingae, Aeromonas, Plesiomonas.
- Review of breakpoints
  - Revised: Colistin, fluoroquinolones - finalised
  - Review: Carbapenems, ceftaroline (aminoglycosides, tigecycline)
- Disk diffusion methods for existing agents
  - Nitroxoline, fosfomycin, methicillin resistance in Coag,neg staphylococci.
  - Aerococcus spp, Kingellla kingae, (Anaerobes)
- The relationship between WGS and phenotypic AST (2016)
- What to do when there are no breakpoints? (SOP 2016)
- Redefining the intermediate category!? (2015 & 2017)
- Instruction videos (commissioned by WHO) 5 + 5
- Intrinsic resistance and Expert Rules revised.
- Methods for the detection of resistance mechanisms of clinical and/or public health importance (revised).

The EUCAST decision process

The EUCAST decision process

- EUCAST, EMA, ECDC, EFSA, Colleagues, Laboratories, Industry may all suggest areas in need of decision.
- Suggestions screened, prioritized and developed by the Steering Committee (SC) or a subcommittee. A decision is suggested.

Consultation process

- Major decisions go to a 6 week open general consultation published on the website.
- Comments (from NACs, institutions, companies, colleagues, etc) are discussed and a response to each prepared. Anonymous comments are not accepted.

The final decision with comments and responses are published on the website.

(Decisions on new agents are between EMA, EUCAST and the pharma company. Confidentiality issues prevent open consultation).

Recent general consultations (2016)

1. Redefining the INTERMEDIATE category.
2. Suggested breakpoints for Aerococcus spp. and Kingella kingae.
3. Revision of the colistin breakpoint for Pseudomonas aeruginosa
   – EUCAST suggested to lower it from 4/4 to 2/2 mg/L to match new PK/PD data.
4. Revision of fluoroquinolone breakpoints.
5. 1st report from The subcommittee on the relationship between WGS and phenotypic AST.

Guidance documents
Consultations
MIC distributions and ECOFFs
Zone distributions and ECOFFs
AST of bacteria
AST of mycobacteria
AST of fungi
AST of veterinary pathogens
Frequently Asked Questions (FAQ)
Meetings
Presentations and statistics
Warnings!
Documents
Rationale Documents
Standard Operation Procedures
Discussion documents
Consultations
Publications in journals
Technical notes
Posters
Other Documents
External documents
Reports
Videos from EUCAST
Translations
Information for industry
Links
Contacts

EUCAST Consultations

Current consultations

- **Consultation - letter of invitation** 3 March, 2017 - 14 April, 2017: Revision of **"EUCAST guidelines for detection of resistance mechanisms and specific resistances of Clinical and/or epidemiological importance"**.
  Form to be used for comments (no later than 14 April, 2017)

- **Consultation - letter of invitation** 9 March, 2017 - 14 May, 2017:
  "EUCAST discussion document (v 3) on MIC distributions and the determination of epidemiological cut-off values (ECOFFs)"
  - from the EUCAST Subcommittee on MIC distributions and ECOFFs.
  Form to be used for comments (no later than 14 May, 2017)

Consultations with comments and responses:

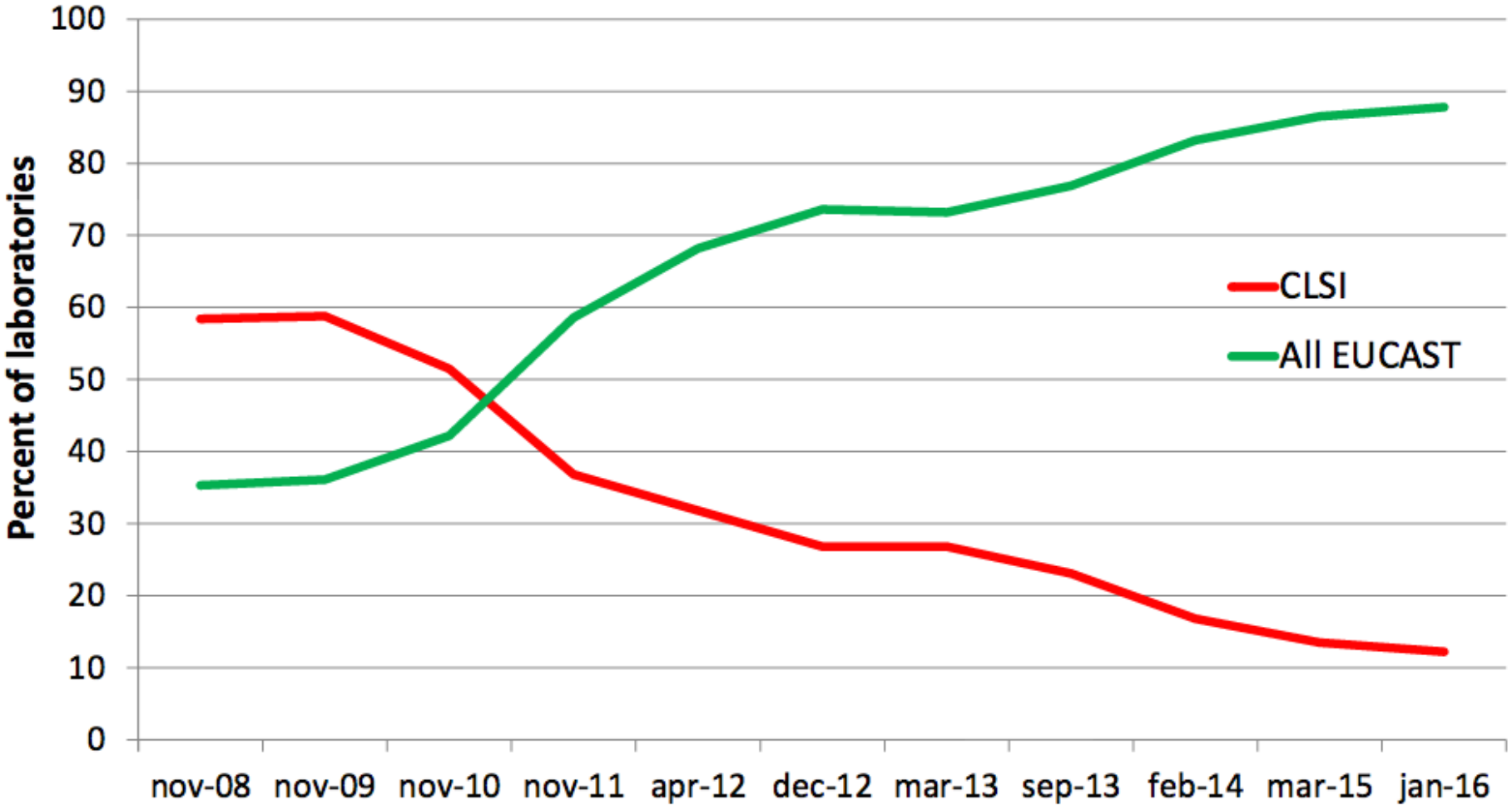
- **Proposed breakpoints for Aerococcus spp and Kingella kingae - comments and responses.**
- **Proposed revision of fluoroquinolone breakpoints. - Comments and responses.**
- **Proposed revision of the colistin breakpoint for *Pseudomonas aeruginosa*. - Comments and responses.**
- **Report from the EUCAST Subcommittee on the role of whole genome sequencing (WGS) in antimicrobial susceptibility testing. - Comments and responses.**
- **Wide consultation the EUCAST proposed changes in the definition of the intermediate category. - Comments and responses.**
- **Nitroxoline breakpoints - Comments and responses.**
- **The Intermediate category - the need for a modified definition.**
  Document, comments and responses Sept 2016
  The first consultation will be followed by a second consultation 2017.
- Revision of **Expert rules (v 3.0).**
  Wide consultation 2016: **External comments and Steering Committee and Subcommittee responses.**

Comments not entered into the designated document (Document for comments) will not be considered.

Implementation of EUCAST breakpoints, April 2017

AST guidelines used in UK NEQAS External Quality Assurance

(630-750 participants per year from a total of 40 countries)



UK NEQAS

Warnings on the EUCAST website

- The EUCAST Development Laboratories evaluate AST material (spontaneously or because of problems detected by user or company)
- Disks, media, gradient tests have been investigated
- Warnings are issued on the website
- Currently there are warnings against
  - Disks from several manufacturers
  - Gradient tests for piperacillintazobactam from two manufacturers
  - Colistin gradient tests from two manufacturers and against colistin disk diffusion testing in general.

Checking on manufacturers

Jenny Åhman et al, Poster 0824, ECCMID 2016

Table 1. Evaluation of disks from nine manufacturers vs. EUCAST QC targets and ranges.**
1 = First Study, 2 = Follow-up Study

	Bio-Rad		Liofilchem		BD		Abtek		SirScan		Oxoid		HiMedia		Bioanalyse		Mast	
Antimicrobial disk	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Benzylpenicillin 1 unit					L				H	H			NA	NA	H	H		
Amoxicillin-clav. 30 µg	H	H*					L						H	H		L		
Piperacillin-tazo. 36 µg							L	L	H				NA	NA				
Oxacillin 1 µg			L		L				L				H	H	L			
Mecillinam 10 µg							L		H				H		H			
Cefotaxime 5 µg							NA		L				NA	NA				
Cefoxitin 30 µg	H*	H*	H	H*			NA	L					L*	L*		L		
Ceftazidime 10 µg							L	L					L	H				
Meropenem 10 µg	H		H*				L	L			H		H					
Ciprofloxacin 5 µg	L				L		L	L					H	H*		L	L	
Norfloxacin 10 µg							L		L				H*	H				
Pefloxacin 5 µg			L	L	L		NA	NA	NA				H					
Gentamicin 10 µg					H		L		NA				H	H				
Tobramycin 10 µg	NA	NA	H										H*	H*				
Erythromycin 15 µg			L		L		L		L				H	H	L*	L		
Tetracycline 30 µg			L	L*	L*		L		L*					L	L		L	

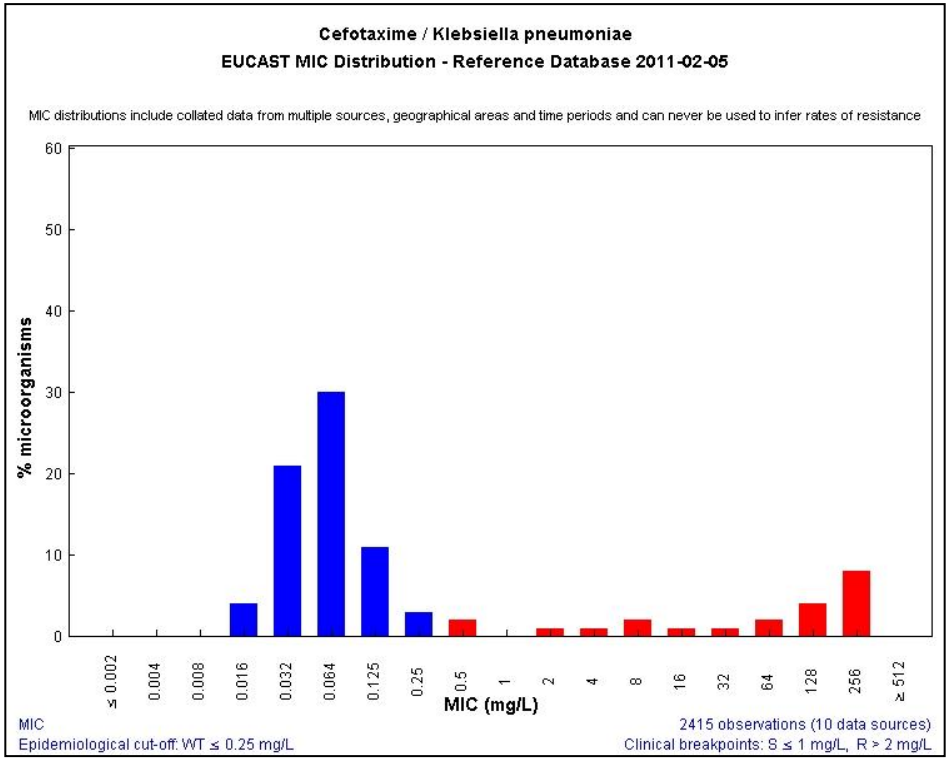
**Data from the first study has been reanalyzed due to changes in QC criteria between 2015 and 2016.
These data, including information on disk lot numbers, are published on www.eucast.org.

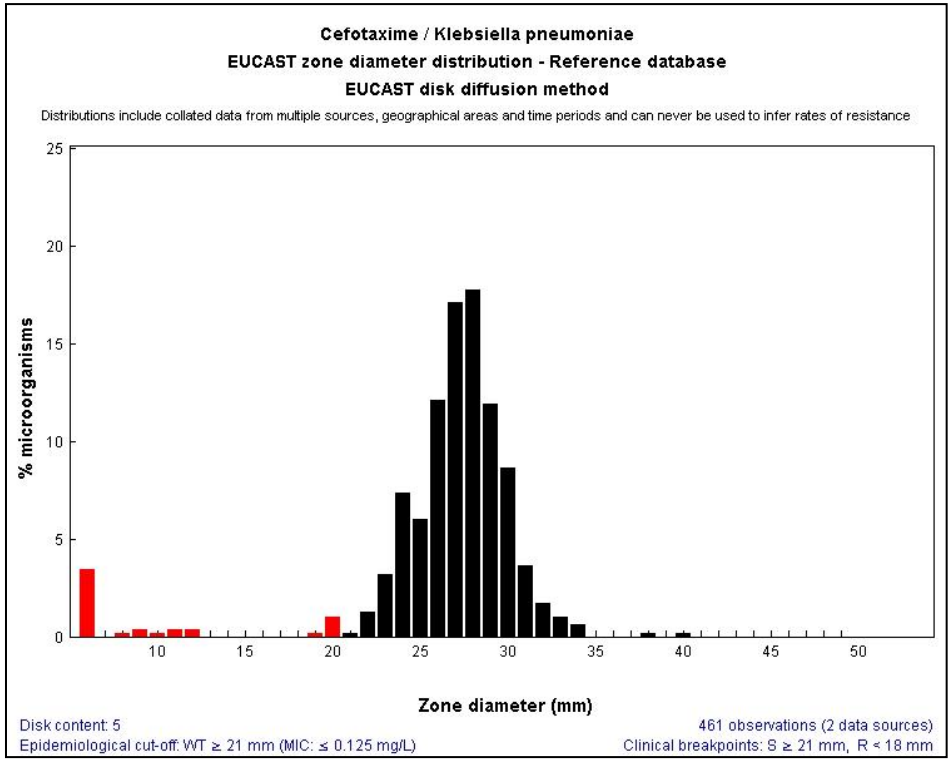
- Mean value within ± 1 mm of the target value
- Mean value >1 mm but within ± 2 mm of the target value
- Mean value >2 mm from target value but still within the QC range
- **Mean value out of the QC range**
- Disk included in first study, but not supplied for follow-up study

NA = Not Available
H = High, mean value > 1 mm above target
L = Low, mean value > 1 mm below target
* One or more readings out of QC range

Determining breakpoints and ECOFFs

Determining breakpoints





Tools for determining clinical breakpoints

- Clinical targets (indications)
- Target organisms (indications), MIC distributions and ECOFFs of these.
- Resistance mechanisms of clinical relevance in target organisms
- Dose and mode of administration
- Pharmacokinetics of agent in target patients
- Pharmacodynamics of agent in relation to dose, infection and target organism

- Clinical outcome data for target infections
  - Clinical outcome initially pertain to organisms with wild type MIC-values.

"X-ithromycin"		Bacteriological outcome		Clinical outcome
MIC (mg/L) S.pneumoniae	N	% Eradicated or Presumed Eradicated	Recurrence	% Cure
0.004	4	4 (100)	0	4 (100)
0.008	125	123 (98.4)	0	121 (96.8)
0.015	32	30 (93.8)	0	29 (90.6)
0.016	81	79 (97.5)	0	77 (95.1)
0.03	23	21 (91.3)	0	21 (91.3)
0.06	6	6 (100)	0	6 (100)
0.12	6	4 (66.7)	0	4 (66.7)
0.25	1	1 (100)	0	1 (100)
0.5	3	3 (100)	0	3 (100)
1	5	5 (100)	0	5 (100)
Total	286	276 (96.5)	0	271 (94.8)

Breakpoints may vary with target microorganism, disease, dosage and resistance mechanism.

- Penicillin breakpoints for *S.pneumoniae* (0.06/2 mg/L) and Streptococci (0.25/0.25 mg/L) are different (**microorganism**)
- Penicillin breakpoints for *S.pneumoniae* are different in pneumonia and meningitis (0.06/2 vs. 0.06/0.06 mg/L) (**disease**)
- Penicillin breakpoints may vary with **dosage**:

EUCAST breakpoint	Dosage in pneumonia
S ≤0.5 mg/L	1.2 g x 4 or more
S ≤1 mg/L	2.4 g x 4 or 1.2 g x 6 or more
S ≤2.0 mg/L	2.4 g x 6 or more

- "Betalactam breakpoints in *S.aureus* are only valid in the absence of a *mecA*-gene" (**resistance mechanism**).

Breakpoints can fail in several ways!

- Fail to predict failure (**undercall resistance**)
  - CLSI piperacillintazobactam breakpoints in *Pseudomonas*
- Fail to predict success (**overcall resistance**)
  - Penicillin breakpoints in *S.* pneumoniae in pneumonia
- Generally fail to be useful (lack of correlation with either success or failure)
  - Erythromycin breakpoints in *H. influenzae* (dividing a WT population in three SIR-categories)

AST methods

Methods for susceptibility testing

- **Phenotypic test methods**
  based on **antimicrobial activity (MIC)** and **breakpoints**
  - MIC, disk diffusion, automated systems like Phoenix, Vitek2, Microscan
  - **Predict susceptibility and resistance**
  - **Quantifiable**
- **Genotypic test methods**
  based on the detection of a **resistance gene** or its **product**
  - mecA, vanA, vanB, ….PBP2, … betalactamase detection (enzyme detection, Maldi Tof)
  - **Predict resistance, not sensitivity**
  - **Not quantifiable**
  - **Useful for epidemiological purposes**
- **By deduction** – ”expert rules”
  - If MRSA then report all betalactam antibiotics R – or soon not?
    If ESBL-positive, then report betalactam antibiotics R – but not any longer!
    If erythromycin-resistant, then report all macrolide antibiotics as R;
  - **Some rules predict susceptibility, others resistance.**
  - **Not quantifiable.**
  - **Unreliable !**

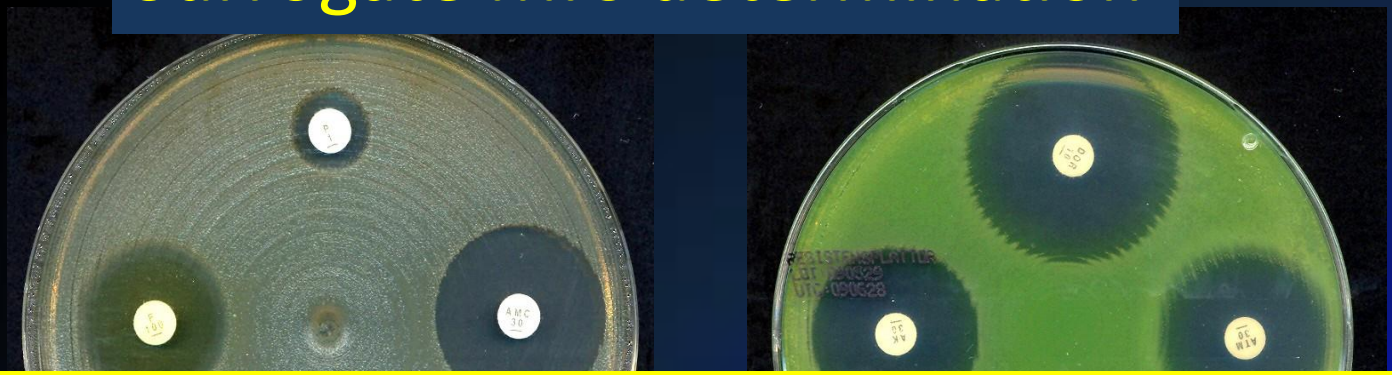
Issues in AST methods

- Daily QC testing mandatory
  - Accreditation authorities being adviced
- Development delays in semi-automated AST (Microscan, Phoenix, Vitek2)
- Colistin – broth micro dilution. EUCAST warns against disk diffusion and gradient tests.
- Poor quality of disks from some manufacturers

MIC

- Assessed primarily by essential agreement.
- Delivers a quantitative measure in 16h – 44h.
- Flexible - redevelopment is fast.
- Problems: contamination goes undetected, skipped wells and trailing endpoints; cumbersome and/or expensive.

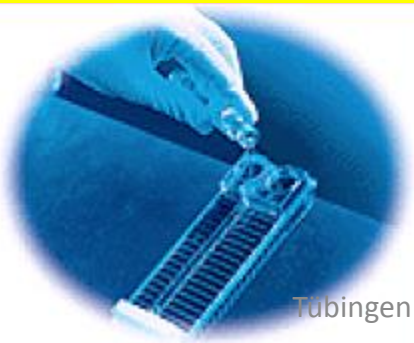
Surrogate MIC determination

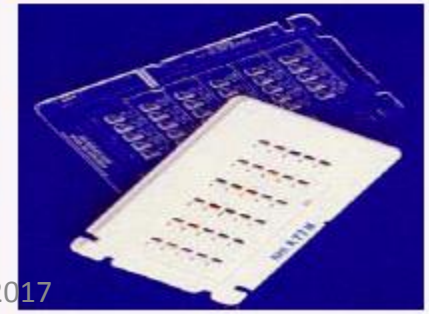


- Assessed primarily by categorical and essential agreement.
- Easy daily QC
- Delivers a quantitative measure in 4 – 16h.
- Flexible - redevelopment is fast.
- Contaminations can be handled.
- Correlation between MIC and zone diameter is good when species specific

Semiautomated AST machines!

- Report S, I or R in 8 – 20 h.
- Do not deliver acceptable MICs (many ≤ or >).
- Assessed by categorical (S, I, R) agreement
- (Re-)development is time consuming.
- Almost impossible to QC.
- Capacity limited.
- Expensive consumables.





Thank you!