22nd ECCMID London, United Kingdom 31 March – 3 April 2012

Educational Workshop

EW03: Antimicrobial susceptibility testing with EUCAST breakpoints and methods

Arranged with the European Committee on Antimicrobial Susceptibility Testing (EUCAST)

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ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

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

EUCAST – what is new?

A summary of activities over the past 12 months and of planned activities over the next year.

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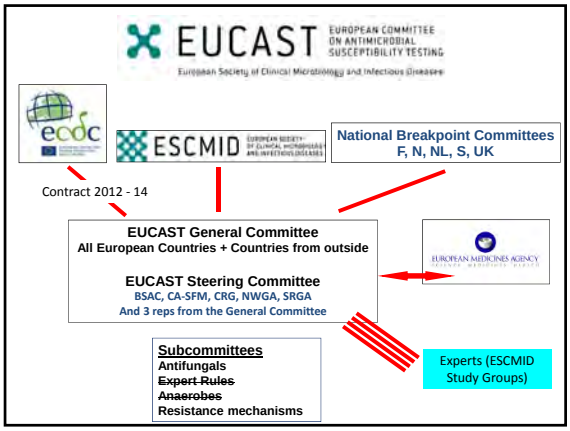
ESCMID

ecdc



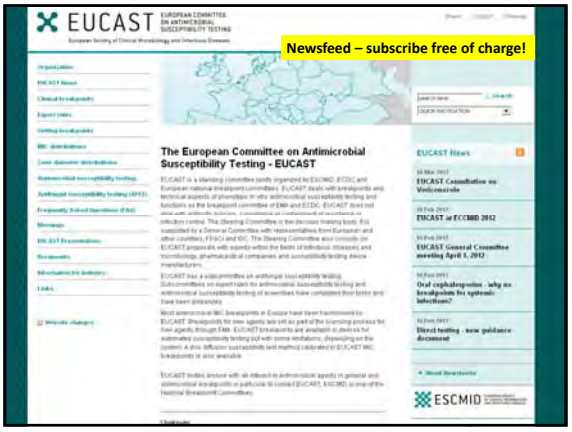
EUCAST

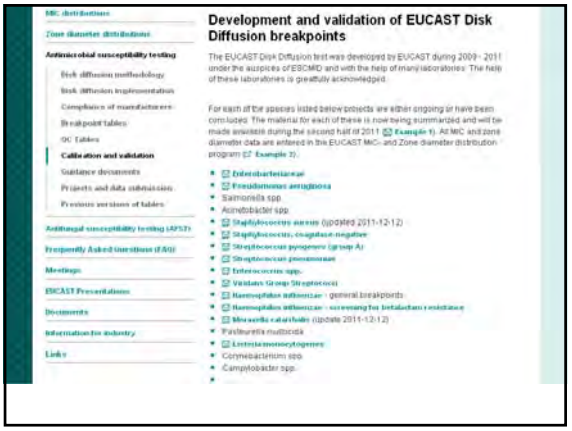
The European Committee on Antimicrobial Susceptibility Testing

- **EUCAST - ECDC**
  - contract for the period 2012 – 14
- **EUCAST - EMA**
  - SOP for the determination of breakpoints as part of the process for registration of new compounds.
  - currently new antibacterial and antimycobacterial agents
- **National breakpoint committees** provide expertise in breakpoint setting.
- **ESCMID**
  - commitment to the development and upkeep of the European disk diffusion test
  - ESCMID Study Groups groups provide expertise in special areas (C. difficile, H.pylori, Legionella etc)

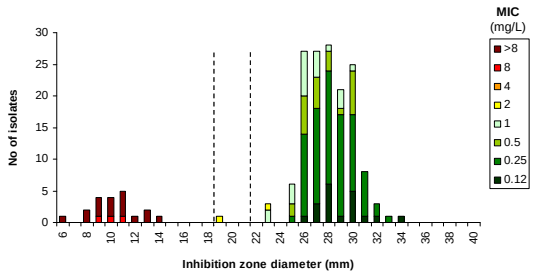
Summary of activities completed 2011 - 2012

- Breakpoint table v 2.0 released 1 Jan 2012 (see website).
- ”Expert Rules v 2.0” published in CMI (see website).
- ”Pk/Pd in EUCAST” published in CMI (see website).
- New subcommittee ”on detection of resistance mechanisms of clinical and/or epidemiological importance”.
- Several guidance and position documents , SOPs and RDs published.
- EUCAST AFST - breakpoints and RDs for antifungal agents published (Candidae and Aspergillus).
- New agents with EMA including a betalactam with anti-MRSA activity and anti-mycobacterial agents.
- EUCAST website – several new developments and functions.
- MIC/Zone diameter distribution website moved and secured.
- Validation of zone diameter breakpoints – files available on website.





Tetracycline 30 µg vs. MIC
***S. aureus*, 172 clinical isolates**



Breakpoints		ECOFF
MIC	S≤1, R>2 mg/L	WT≤1 mg/L
Zone diameter	S≥22, R<19 mm	

EUCAST breakpoint decisions 2011/12

- *Moraxella catarrhalis* breakpoints for all relevant agents.
- *C.difficile* breakpoints for metronidazole (2/2 mg/L) and vancomycin (2/2 mg/L).
- *H.pylori* breakpoints (based on ECOFFs) for several agents.
- *L. monocytogenes* breakpoints for several agents.
- Fosfomycin breakpoints **removed** from Pseudomonas.
- Nitrofurantoin breakpoints **removed** from *E. fecium.*
- Vancomycin breakpoints for Coag neg staphs **increased** from 2/2 to 4/4 mg/L.
- Trimethoprim breakpoints 2/2 mg/L included for *S. agalactiae* in UTI.
- Amoxicillin & amox/clav breakpoints in *H. influenzae* **increased** to 2/2 mg/L.
- Chloramphenicol breakpoint in *H. influenzae* **increased** to 2/2 mg/L.
- Rifampicin breakpoint in *H. influenzae* **increased** to 1/1 mg/L.
- Ceftibuten – **removed** "uncomplicated" in the caveat "uncomplicated UTI".
- Replace "IE" with "dash" for ceftibuten for *S. pneumoniae.*
- *Stenotrophomonas maltophilia* – guidance document.
- Oral cephalosporins – no systemic breakpoints for Enterobacteriaceae (position document on website).

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Stenotrophomonas maltophilia

The organism
Stenotrophomonas maltophilia is a ubiquitous environmental organism. In patients it is most often associated with colonization, but is an occasional cause of infection, particularly in immunocompromised patients and patients with cystic fibrosis.

Antimicrobial resistance
Intrinsic antimicrobial resistance of this organism is a major problem, particularly to aminoglycosides and carbapenems. Multiple efflux pumps and modifications to outer membrane proteins confer variable resistance to a wide range of agents. Chromosomal genes for beta-lactamases affect all beta-lactams including carbapenems. Aminoglycoside acetyl transferase and SmQnr genes (conferring reduced susceptibility to fluoroquinolones) are almost always present (3). In addition, acquired genes may be present conferring resistance to a wide range of agents, including trimethoprim-sulfamethoxazole (co-trimoxazole) (17). Moreover, the formation of biofilms reduces antimicrobial effectiveness.

EUCAST

Why do EUCAST have no systemic breakpoints for Enterobacteriaceae with oral cephalosporins?

There have been multiple questions from clinicians, particularly those working in orthopaedics, who have "successfully used oral cephalosporins for prophylaxis and to treat Enterobacteriaceae infections for many years". They ask what has changed and why these agents are now considered inappropriate.

In EUCAST rationale documents it is stated that Enterobacteriaceae are inappropriate targets in sites other than uncomplicated urinary tract infection, but there is no further explanation. In early EUCAST discussions oral cephalosporins were originally considered inappropriate for treatment of infections in other sites than the urinary tract infection for several reasons:

1. Comparison of free drug pharmacokinetics with MICs alone indicates that inadequate concentrations are achieved for most agents and are borderline at best (see table).

2. The relevant pharmacodynamic relationship indicative of activity of cephalosporins is T>MIC and the target %fT>MIC is 40-50%. Approximate calculations based on common dosages indicate that activity is inadequate for all agents (see table). It should be emphasized that the figures in the table are based on pharmacokinetic parameter values for the mean of the population. Monte Carlo simulations would show that the %fT>MIC values are even less than those in the table for half the population treated.

EUCAST

Direct antimicrobial susceptibility testing

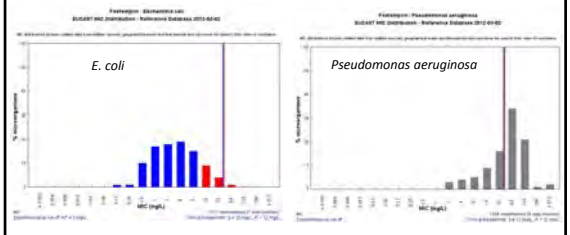
In direct antimicrobial susceptibility testing the specimen (commonly urines) is used as the source of the inoculum. Tests where positive blood cultures are used as the source of the inoculum are also included as direct tests, although they do not use the specimen directly.

The advantage of direct testing is that results may be available earlier than when the organism is isolated in pure culture before testing and this may have direct patient benefit in terms of early appropriate chemotherapy. There may be additional benefits from the ability to narrow the spectrum of therapy at an early stage.

The main disadvantage is that the inoculum cannot be effectively controlled. Also there may be mixed cultures and there may be pH variations or substances in the specimens that affect results (e.g. antimicrobial agents in urine, antimicrobial absorption materials in blood cultures). These problems may result in less reliable results than with pure cultures. EUCAST does not recommend primary susceptibility testing and any laboratory using this approach must take responsibility for ensuring that results are reliable. The following should be noted:

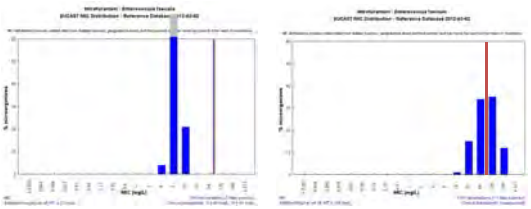
1. There are currently no validated methods for processing specimens to ensure that the correct inoculum is achieved.
2. Tests should be repeated on pure cultures as needed and the correlation of direct and secondary tests should be monitored so that the reliability of direct tests can be assessed.
3. In disk diffusion tests, if the inoculum is visibly light, do not report susceptible results as zone diameters may be increased leading to resistant isolates appearing susceptible.

Fosfomycin breakpoints for *Ps. aeruginosa* removed



Decision: fosfomycin breakpoints for *Ps. aeruginosa* removed

Enterococci and nitrofurantoin



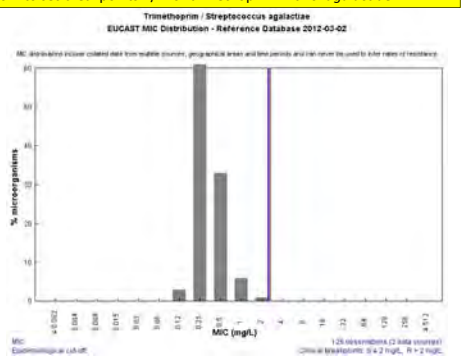
Decision: nitrofurantoin breakpoints are only valid for *E. faecalis*

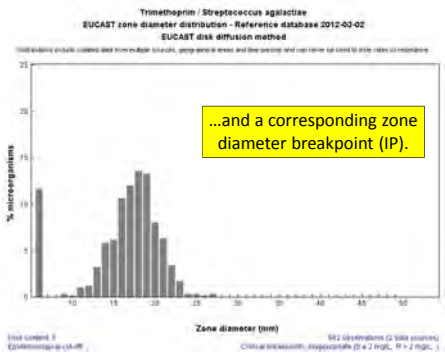
Decision: To increase the vancomycin breakpoint for coagulase negative staphylococci from 2/2 to 4/4 mg/L.

Organism	MIC (mg/L)										
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
Staphylococcus coagulase-negative	0	12	69	595	5469	5765	190	6	2	0	0
Staphylococcus capitis	0	0	9	98	331	82	2	0	0	0	0
Staphylococcus cohnii	0	0	0	0	18	2	0	0	0	0	0
Staphylococcus epidermidis	0	3	27	402	5416	8920	327	2	0	0	0
Staphylococcus haemolyticus	0	0	11	178	1059	859	60	0	1	0	0
Staphylococcus hominis	0	0	10	148	734	243	6	0	0	0	0
Staphylococcus lyticus	0	0	0	0	13	116	0	0	0	0	0
Staphylococcus intermedius	0	1	0	10	108	10	0	0	0	0	0
Staphylococcus lugdunensis	0	0	2	16	44	7	0	0	0	0	0
Staphylococcus saprophyticus	0	0	0	45	233	96	5	0	0	0	0
Staphylococcus simulans	0	0	0	9	53	24	0	0	0	0	0
Staphylococcus warneri	0	0	1	43	151	87	4	1	0	0	0
Staphylococcus xylosus	0	0	0	1	14	8	0	0	0	0	0

…to avoid categorising CoNS resistant to vancomycin due to breakpoint cutting into wild type distribution

EUCAST had not determined breakpoints for *S. agalactiae* and trimethoprim.
Decision: to set breakpoints 2/2 for trimethoprim with *S. agalactiae*





…and a corresponding zone diameter breakpoint (IP).

Proposal 5: Replace the "IE" designation with "-" for ceftibuten with *S. pneumoniae*

The use of "IE" suggests that there is a reasonable possibility that ceftibuten might be useful for treating pneumococcal infection. However, ceftibuten MICs for *S. pneumoniae* for the wild type are 1-16 mg/L.

The current EUCAST MIC distribution for *S. pneumoniae* is as follows:

Agent	MIC (mg/L)												
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
Ceftibuten	0	0	0	0	0	0	70	99	101	13	11	0	14

It is unlikely that with wild type MICs up to 16 mg/L there will ever be clinical data supporting the use of ceftibuten.

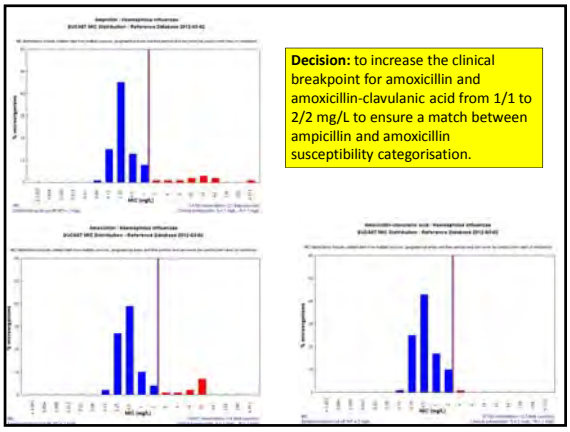
It is proposed that for *S. pneumoniae* the designation "IE" is replaced with "-"

Decision: For *S. pneumoniae* - replace "IE" in breakpoint table with "—" for ceftibuten.

Proposal 9: Change breakpoints for amoxicillin and amoxicillin-clavulanate with *Haemophilus influenzae* to S ≤2 mg/L, R >2 mg/L

Ampicillin MICs are generally one dilution lower than amoxicillin but ampicillin is used to report susceptibility to both agents. With current breakpoints (ampicillin, amoxicillin and amoxicillin-clavulanic acid all S ≤1 mg/L, R >1 mg/L) some isolates with no resistance mechanism appear amoxicillin susceptible, ampicillin resistant when tested separately. There is no difference in clinical outcome for ampicillin and amoxicillin so the reported susceptibility should be the same. Currently the listed ECOFFs are 1 mg/L for ampicillin, amoxicillin and amoxicillin-clavulanic acid whereas the median MICs are 0.25, 0.5 and 0.5 mg/L respectively, and the ECOFFs should be 1mg/L, 2mg/L and 2 mg/L respectively.

There is no difference in clinical outcome for ampicillin and amoxicillin so the reported susceptibility should be the same.



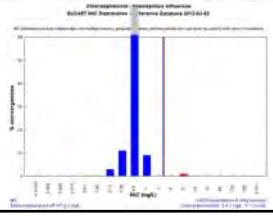
Decision: to increase the clinical breakpoint for amoxicillin and amoxicillin-clavulanic acid from 1/1 to 2/2 mg/L to ensure a match between ampicillin and amoxicillin susceptibility categorisation.

Proposal 10: Change breakpoints for chloramphenicol with *Haemophilus influenzae* to S ≤2 mg/L, R >2 mg/L

The current breakpoints are S ≤1 mg/L, R >2 mg/L. The intention of the susceptible breakpoint was to distinguish the wild type whereas the resistant breakpoint was set to distinguish chloramphenicol acetyl transferase-producers from isolates that do not produce the enzyme. The current EUCAST MIC distribution with additional data shows that 1 mg/L cuts the shoulder of the wild type and that the ECOFF should be 2 mg/L.

The current EUCAST MIC distribution for *H. influenzae* is as follows:

Agent	MIC (mg/L)												
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
Chloramphenicol	0	0	0	806	2067	13655	1818	39	47	320	88	0	0



Decision: to change breakpoints from 1/2 mg/L to 2/2 mg/L to avoid dividing the wild type distribution.

EUCAST projects in the near future

- Continue facilitating implementation of breakpoints and methods in Europe through country visits, workshops and lectures.
- Organise meeting(s) with NACs.
- Expert advisory committee to ECDC and EMA and EFSA.
- Complete, with EMA, setting breakpoints for several new agents.
- Continue development and upkeep of the disk diffusion method.
- Publish remaining RDs (currently 45 published).
- Review colistin breakpoints (possibly together with CLSI).
- Breakpoints and methods for several organisms and agents (see next slide).
- Recently formed new subcommittee on the detection of antimicrobial resistance mechanisms of clinical and/or epidemiological (public health) importance.

Miscellaneous organisms

Breakpoints and/or Methods

- Neisseria meningitidis (review of brpts) - 2012
- Campylobacter (B/M) - 2012
- Pasteurella multocida (B/M) - 2012
- Corynebacteria (B/M) - 2012
- Yersinia (B/M) - 2012
- FQ resistance in Salmonellae (screen test) (M) - 2012
- Betalactam resistance in viridans group strepts (screen-M) - 2012
- Breakpoints for topicals based on ECOFFs (B) - 2012

- Burkholderia cepacia (B/M) - 2013
- Legionella (B/M) - 2013
- Neisseria gonorrhoeae (M) - 2013
- Actinomyces, Nocardia (B/M) - 2013
- Anaerobe bacteria (M) - 2013

EUCAST in Europe 2012

Summary of Questionnaire

A summary of the Questionnaire will be presented

Compliance of manufacturers

see www.eucast.org for comprehensive review

- Almost all manufacturers of disks can now provide all ”EUCAST strength disks”:
  - Abtek
  - BD
  - Bio-Rad
  - I2A
  - Liofilchem
  - Mast group
  - Thermo Fisher Scientific (Oxoid)
  - Rosco (tablets)

Each manufacturer is responsible for the quality of their products used for AST.

EUCAST will not be able to provide comprehensive QC on all disks from all manufacturers but will, on intitative from EUCAST, users or manufacturers provide important information on products used for AST on the website.

Compliance of manufacturers

see www.eucast.org for comprehensive review

- Manufacturers with commercial MH-F plates:
  - Thermo Fisher Scientific (Oxoid)
  - bioMérieux
  - Bio-Rad
  - Liofilchem
- Manufacturers not yet ready to supply MH-F plates:
  - BD

Each manufacturer is responsible for the quality of their products used for AST.

EUCAST will not be able to provide comprehensive QC on all disks from all manufacturers but will, on intitative from EUCAST, users or manufacturers provide important information on products used for AST on the website.

The preparedness of manufacturers of AST materials 26 January, 2012

3. Automated systems (I)

Phoenix/EpiCenter (BD)

EUCAST terminology	S ≤	R >	-	IE
Computer Report	Yes Yes	No (R ≥) Yes (converted to R >)	Yes Yes*	Yes Yes*
EUCAST Expert Rules	Yes			
Organisms with no EUCAST test	*H. influenzae* *M. catarrhalis* *N. gonorrhoeae* *N. meningitidis* Gram-positive anaerobes Gram-negative anaerobes			
Antibiotics with no EUCAST test	**Breakpoints Not Available**		**Antibiotics Not Available**	
	Rifampicin (Staphylococci) Trimethoprim (Enterococci) Cotrimoxazole (Enterococci)		None	

* Only MIC values are reported for drugs with no EUCAST breakpoints.

The preparedness of manufacturers of AST materials 26 January, 2012

Automated systems (II)

Microscan (Siemens Healthcare Diagnostics)

EUCAST terminology	S ≤	R >	-	IE
Computer Report	Yes Yes	Yes Yes	No Most "-" do not have interpretations reported	No No
EUCAST Expert Rules	Yes			
Organisms with no EUCAST test	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			
Antibiotics with no EUCAST test	**Breakpoints Not Available**		**Antibiotics Not Available**	
	Trimethoprim Chloramphenicol Fusidic acid Rifampicin		Tigecycline/Gram-positive organisms Roxithromycin Telithromycin Doxycycline	

The preparedness of manufacturers of AST materials 26 January, 2012

Automated systems (III)

ATB Expression mini API (bioMérieux)

EUCAST terminology	S ≤	R >	-	IE
Computer	Yes	Yes	No*	No
Report	Yes	Yes	No*	No
			* most are not reported or reported R	

EUCAST Expert Rules	Yes	
Organisms with no EUCAST test	*N. gonorrhoeae* *N. meningitidis*	
Antibiotics with no EUCAST test	**Breakpoints Not Available**	**Antibiotics Not Available**
	Cefotaxime (*M. catarrhalis*) Ceftaroline and/or Metronidazole (*C. difficile*) Rifampicin Teicoplanin Trimethoprim-sulfa (Enterococci) Vancomycin	Ampicillin-sulbactam Cefadroxil Cefalexin Cefpodoxime Ceftibuten Doripenem Ertapenem Aztreonam Netilmicin Tigecycline Trimethoprim Azithromycin Clarithromycin Roxithromycin Doxycycline Daptomycin Cefaclor Cefazolin

The preparedness of manufacturers of AST materials 26 January, 2012

Automated systems (IV)

Vitek 2 (bioMérieux)

EUCAST terminology	S ≤	R >	-	IE
Computer	Yes	No	No*	No**
Report	Yes	No	No*	No**
			* not reported or reported R	** not reported

EUCAST Expert Rules	Yes	
Organisms with no EUCAST test	*H. influenzae* *N. gonorrhoeae* Gram-positive anaerobes	*M. catarrhalis* *N. meningitidis* Gram-negative anaerobes
Antibiotics with no EUCAST test	**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)

The last slides are just to point out the terminology used in EUCAST breakpoint tables

Terminology in EUCAST tables

–

dash

Susceptibility testing not recommended – do not report or report “R” without testing.

Intrinsic resistance (or intrinsic insufficient activity).

Terminology in EUCAST tables

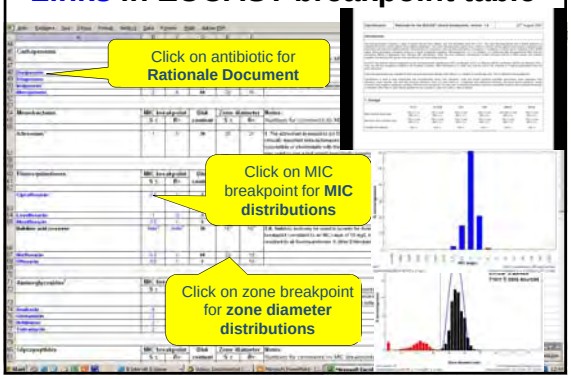
IE

(insufficient evidence)

The susceptibility category (S, I or R) of organisms without resistance mechanisms cannot be determined.

Do not report or report “IE with an MIC” - categorical interpretation not possible.

Links in EUCAST breakpoint table



Issues in implementation of EUCAST breakpoints and methods

Petra Apfalter
Elisabethinen Hospital Linz, Austria

Where is Austria?

How we started

- **11/ 2009 Gunnar Kahlmeter visits us in Linz**
  - Rationale why EUCAST
  - NAC decides to switch to EUCAST
  - Time frame: start in October 2010 with the intention to finish the process in 2011
  - Discussion on prerequisites
    - German EUCAST files would be helpful
    - National ÖGACH recommendations (AB panels) needed update
      - Agree to update for a last time to CLSI (2010) but with respect to upcoming EUCAST rules
    - Organize EUCAST kick-off workshop (summer 2010) at the MOH for all labs

AMR in Austria

- **EUCAST General Committee Member(s) should represent EUCAST issues in their countries, the Austrian GCM is:**
  - **AMR focal point** and ISCM (ECDC) nominated by MOH
  - Runs national reference laboratory for AMR and NI (**NRZ**) nominated by MOH
    - EARS-Net and ESAC
  - Thus, is supported by MOH
  - Close contact to national scientific societies
- **NAC-AT**
  - Formed in 2008 - member meet at least once a year
  - Since 2010 NRZ organizes workshops (at the MOH) for all microbiology laboratories
  - Close contact to scientific societies
    - EUCAST issues
    - Other AMR and NI related topics as far as laboratory testing and infection control are affected

www.analyse.eu Seite ‹Nr.›

The time before EUCAST

ÖSTERREICHISCHE GESELLSCHAFT FÜR ANTIMIKROBIELLE CHEMOTHERAPIE

Kick-off workshop to reach all

www.analyse.eu Seite ‹Nr.›

First questions in our lab

- Who is responsible?
- Which testing systems are affected ?
- Which tools are affected?
- Are all materials available?
- Who needs to be informed?

Make someone responsible

- Our „2 champions“
  - Accompany implementation process
  - Know all EUCAST files und rules
  - Prepare tools
  - Prepare crew
  - Remain informed
    - Pro futoro check for news
  - Develop - together with crew - a program for all technicians: **in our lab everyone should know everything**
    - structured ppt
    - exercise
    - establish QC in daily routine
    - Trouble-shooting

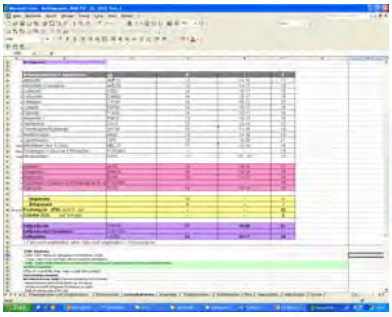
Which testing systems are affected?

- Disc diffusion test / CLSI breakpoints
  - diverse AB-panels
    - Enterobacteria, Staphylococci, Enterococci, Nonfermenters, Strep and Pneumococci, Haemophilus, …
    - We had 25 various panels
- VITEK2 – cartridges
  - For Gramnegatives in blood cultures (GN27)
- MIC and gradient tests
  - Diverse strip tests for
    - Some AB-panels (z.B. Pen G with viridans Strep, anaerobs, …)
    - some resistance mechanisms (Pneumococci Oxa res., ect.)
    - Selected specimens and patients

Which „tools“ are affected?

- excel file „antibiograms“
  - Check breakpoint tables
    - http://eucast.www137.server1.mensemedia.net/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/EUCAST_breakpoints_v1.1.xls
      -> interactive table online?
    - Are BP for all AB available?
    - Show discrepancies and find solutions
- ppt file with dispenser content
  - Needed to be adapted
- Poster – reading guide
- LIS, KIS
  - LIS
    - antibiograms
      - » Raw data
      - » Data entry and report (optimal: MIC and/or mm inhibition zone with interpretative category)
      - » Interpretative category automatically after entry of inhibition zone in mm
  - Reporting
    - Which AB?
    - diverse commentaries („Expert rules“; upcoming)
  - Data export

Rewrite the laboratory manual



Sources and literature

- www.eucast.org
- EUCAST – disc diffusion test
  - manual
  - Slide-show
  - Reading guide
  - Preparation of media
  - *Checklist for the implementation phase*

Is everything available?

- **Media**
  - buy or cook?
  - preparedness of manufacturers of AST materials
- **Discs**
  - check agents and concentration
- **„hard-ware“**
  - densidometer, rotators, ruler & Co
- **QC strains**
  - Organize missing ones early enough
  - QC-panels for diverse working stations
  - How to store QC-results (excel, LIS)
  - …

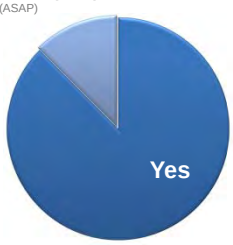
Storage of quantitative data

Reload and feed-back 9 months later

Apfalter - Issues in implementation of EUCAST breakpoints and methods

EUCAST in Austria

5 Labs (12 %)
(ASAP)



35 Labs (88 %)

- How many labs switched to EUCAST?
- Telephone interviews using a structered questionnaire in all 40 EARS-Net Labs
- as of Sept 15 2011
- as of March 2012
  - 2 missing

www.analyse.eu

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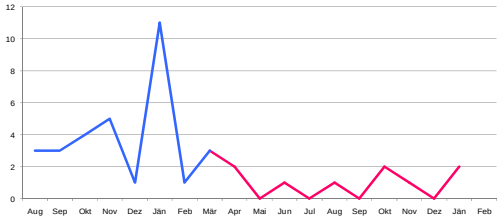
When did the 40 labs switch?

2010 2011 ->



Phone interviews as of February and September 2011

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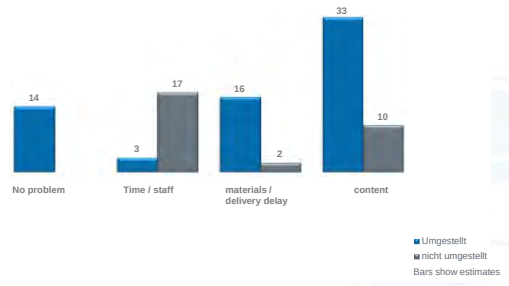
EUCAST online tools (www.eucast.org)

- Did you use the checklist?
  - Yes = 22 No = 3
- Are the online tools sufficient?
  - Yes = 20 No = 4
- May we use your information?
  - Yes = 26

Missing labs after 6 Mo

- Methods: 9 x discs, 3 x Vitek, 1 x no answer
- All knew about the background of EUCAST
- All knew the checklist
- 9 (out of 13) knew the online-tools

EUCAST moves Austria



...

Who are you?

- **Individual micobiology lab**
  - Simply go to www.eucast.org and download checklist and tools and go ahead
  - Calculate at least 3 Mo
- **Opinion lead lab**
  - NAC already formed?
  - Know your network
  - Try to get official support
  - Win opinion leaders and agree on a deadline
  - Involve national scientific societies
  - Consider preparation of tools in your language
  - Plan a kick-off
    - Inform all labs about the change process
    - Follow up 6 Mo later
    - Stay in contact

Networking is the key issue

- **Get official institutions involved**
  - Ministry of Health (MOH)
- **Scientific societies**
  - ÖGACH
  - ÖGIT
  - ÖGHMP
- **Universities – opinion leaders**
  - Vienna, Graz, Innsbruck
- **Win informal leaders**
  - Engaged people
- **Get the net of laboratories involved and make EUCAST to THEIR project**
  - > 40 EARS-Net Labs

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Austrian reference centre for nosocomial infections and antibiotic resistance

+43 (0)732 7676-3654
www.referenzzentrum.at
office@referenzzentrum.at

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

Quality assurance of antimicrobial susceptibility testing

Derek Brown

EUCAST Educational Workshop: 31 March 2012

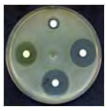
Quality Assurance in the clinical diagnostic laboratory

The total process by which the quality of laboratory reports can be guaranteed

Not just routine quality control
(repeated testing of controls in parallel with tests to ensure that the test system is performing reproducibly within defined limits)

Components of quality assurance

QC of disk diffusion tests



- Specified routine quality control strains are used to monitor test performance
- Quality control strains must be from a reliable source (culture collections or from commercial sources)
- Store control strains correctly to maintain characteristics (see EUCAST website for guidance)

EUCAST routine quality control strains

Use the recommended routine quality control strains daily to monitor test performance with agents in routine test panels

Organism	Culture collection numbers	Characteristics
E. coli	ATCC 25922; NCTC 12241; CIP 7624 DSM 1103; CCUG 17620	Susceptible, wild-type
P. aeruginosa	ATCC 27853; NCTC 12903; CIP 76110 DSM 1117; CCUG 17619	Susceptible, wild-type
S. aureus	ATCC 29213; NCTC 12973; CIP 103429 DSM 2569; CCUG 15915	Weak β-lactamase producer
E. faecalis	ATCC 29212; NCTC 12697; CIP 103214 DSM 2570; CCUG 9997	Susceptible, wild-type
S. pneumoniae	ATCC 49619; NCTC 12977; CIP 104340 DSM 11967; CCUG 33638	Penicillin intermediate
H. influenzae	NCTC 8468; CIP5494, CCUG 23946	Susceptible, wild-type

ATCC, American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA.
NCTC, National Collection of Type Cultures, Health Protection Agency Centre for Infections, 61 Colindale Avenue, London NW9 5HT, UK.
CIP, Collection de Institut Pasteur, 25–28 Rue du Docteur Roux, 75724 Paris Cedex 15 France.
DSMZ, Deutsche Stammsammlung für Mikroorganismen und Zellkulturen, Mascheroder Weg 16, D-38124 Braunschweig, Germany.
CCUG, The Culture Collection University of Gothenburg http://www.ccug.se/

Quality control limits

***Escherichia coli* ATCC 25922**

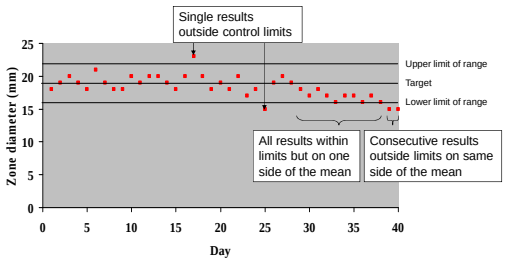
(NCTC 12241, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)

Mueller-Hinton agar, McFarland 0.5, air, 35±2
no growth from the back of the plate against

Target 8 Range 4-16

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone size (mm)	
	Target[1]	Range[2]		Target[1]	Range[3]
Amikacin	1-2	0.5-4	30	23	19-26
Amoxicillin	8	4-16	10	***17***	***14-20***[4]
Amoxicillin-clavulanic acid	4/2	2/2-8/2	20-10	21	18-24[4]
Ampicillin	4	2-8	10	19	16-22[4]
Ampicillin-sulbactam	4/2	2/2-8/2	10-10	22	19-24[4]
Aztreonam	0.12	0.06-0.25	30	32	28-36
Cefadroxil	-	-	30	***17***	***14-20***

Monitoring disk diffusion test performance



Response to disk diffusion QC results out of range

- Single test out of range – report susceptibility if no obvious problem.
- Each day that tests are set up, examine the results of the last 20 consecutive tests. If two non-consecutive control zone diameters of 20 tests are out of range – then report results if no obvious problem but investigate.
- If two consecutive control zone diameters are outside the acceptable range – then investigate before reporting results. The tests may have to be repeated.
- If multiple antibiotics (>2) are out of range on one day – then investigate before reporting results. The tests may have to be repeated.

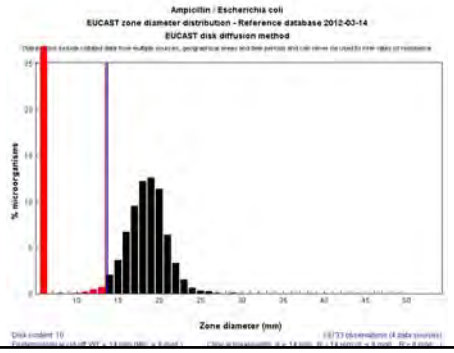
EUCAST strains for detection of resistance mechanisms (in progress)

Quality control strains with defined resistance mechanisms may be used to confirm the ability to detect resistance.

Organism	Characteristics
E. coli	TEM-1 β-lactamase producer
S. aureus	Oxacillin hetero-resistant, *mecA* positive
E. faecalis	VanA (low teicoplanin MIC) and VanB (low vancomycin MIC)
S. pneumoniae	Penicillin MIC 4 mg/L
H. influenzae	β-lactamase negative, ampicillin-resistant (BLNAR)
E. coli	ESBL, cefotaxime S, ceftazidime R
E. coli	ESBL, ceftazidime R, cefotaxime S
E. coli	Plasmid AmpC
E. coli	Carbapenemase producer
K. pneumoniae	KPC producer

If resistance in a resistant control strain is not recognised suppress test results, retest and investigate.

Quality control by comparison of wild type with reference distributions from EUCAST website



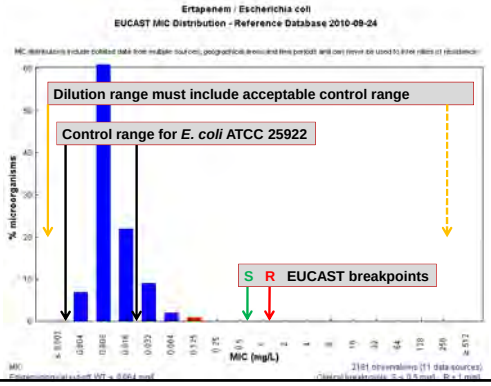
Sources of error in disk diffusion tests

- Medium
- Disks
- Test conditions
- Control strains

Quality control of MIC testing

- Use the recommended routine quality control strains to monitor test performance (see EUCAST QC tables).
- Test range must include the MIC of the control strain.

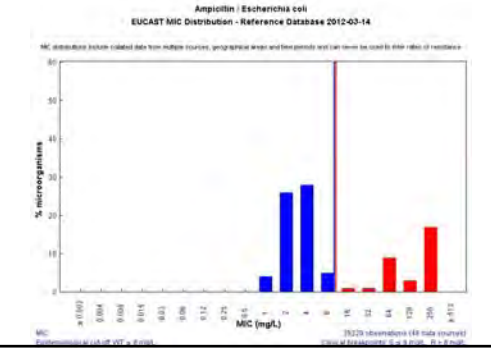
Quality control of MIC testing



Quality control of MIC testing

- Include a control without antibiotic to ensure that the test strain grows adequately.
- Test the purity of inoculum by culture on solid medium to obtain isolated colonies.
- If MIC of control is out of range the source of error must be sought and the test repeated.
- Check wild type distribution against EUCAST distribution on website.

Quality control by comparison of wild type with reference distributions from EUCAST website



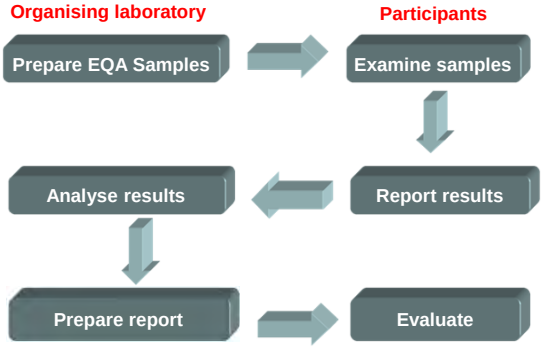
QC of automated systems

- Use the recommended routine quality control strains to monitor test performance (see manufacturer's instructions).
- Restricted range of test concentrations mean that the range may not include the MIC of the control strain.
- Purity of inoculum tested by culture on solid medium to obtain isolated colonies.
- If control is out of range the source of error must be sought and the test repeated.

External Quality Assessment

The challenge of laboratory procedures with specimens of known but undisclosed content

The EQA process (UKNEQAS)



Benefits of EQA in antimicrobial susceptibility testing

- Independent assessment of performance
- Assessment of performance over time
- Comparison with other laboratories
- Highlights problem areas
- Performance related to guidelines and methods
- International differences highlighted
- Gives practical experience of difficult tests (especially if resistance is uncommon)
- Provides background information and guidance on appropriate methods
- Performance indicator for accreditation

"Limitations" of EQA in antimicrobial susceptibility testing

- Number of specimens distributed is small
- May be considered inappropriate to send some organisms
- Specimens do not reflect routine isolates
- Laboratories may not treat specimens as routine

Performance may be affected by breakpoint guidelines used

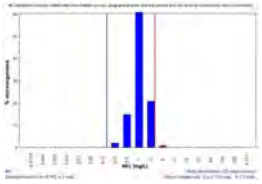
E. faecalis
vancomycin MIC 8-16 mg/L (VanB)

Guideline	Breakpoints (mg/L)		Percent reporting		
	S≤	R>	S	I	R
EUCAST (n=316)	4	4	5.1	1.9	93.0
CLSI (n=314)	4	16	10.2	35.0	54.8

Guidelines are not always followed

S. pneumoniae ciprofloxacin MIC 0.5-1 mg/L

Guideline	Breakpoints (mg/L)		Percent reporting		
	S≤	R>	S	I	R
EUCAST (n=202)	0.12	2	30.2	67.8	2.0
CLSI (n=181)	-	-	92.9	14.9	2.2



Performance may be affected by the method used

E. faecalis vancomycin MIC 8-16 mg/L

VanB EUCAST resistant, CLSI intermediate

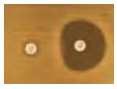
Method	Percent reporting		
	S	I	R
Automated (n=333)	3.9	6.2	89.9
MIC (n=71)	2.8	13.9	83.3
Disk diffusion (n=262)	15.5	11.6	72.9

Borderline susceptibility leads to variable reporting

Organism	Agent	Expected result	% reporting		
			S	I	R
E faecalis 0138	Vancomycin	I/R	7.3	18.4	74.3
E coli 0270	Piperacillin-tazobactam	S/I	32.7	23.6	43.6

Uncertainty in reporting
e.g. *S. aureus* with dissociated (MLSB-inducible) resistance to clindamycin

clindamycin MIC 0.12-0.5 mg/L, resistance induced by erythromycin



Percent reporting (n=775)		
S	I	R
24.0	1.8	74.2

- EUCAST expert rules recommend reporting resistant, or susceptible with warning of possible failure due to selection of resistant mutants. Avoid use in serious infections
- CLSI – report resistant with note that some may respond

Internal Quality Assessment (IQA) -specimen reprocessing

The challenge of laboratory procedures by repeat testing of specimens of unknown content

Internal quality assessment (IQA) process

- Specimens split and both processed on same day, or same specimen processed twice on the same day, with identification of repeat test blinded
- For susceptibility testing the same organism could be processed twice on same day or repeated on different days
- Reports compared and discrepancies investigated
- Feedback

Antimicrobial susceptibility testing problems highlighted by IQA

- Different organisms picked from mixture on primary plates
- Wrong disk contents used
- Borderline susceptibility leads to variation
- Discrepancies with “difficult” tests
- Typographical errors

Quality assurance of antimicrobial susceptibility testing

- Quality assurance is essential to ensure reliable results
- Multiple components contribute to maintaining the quality of antimicrobial susceptibility testing

Antifungal susceptibility testing: the EUCAST approach

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Disclosures:
Research grants & Speaker: Astellas, Gilead, MSD & Pfizer;
Advisory board: MSD, Pcovery, Pfizer; Acted as consultant for: Alcimed, Astellas, Gilead & Pfizer
Chair(wo)man for EUCAST-AFST
Advisor for CLSI-AFST

Temperature check

Which method do you use when testing *Candida?*

1. EUCAST microdilution
2. CLSI microdilution
3. Commercially available method (e.g. Etest, Vitek, Sensititre)
4. We refer to a reference laboratory
5. Susceptibility testing of *Candida* is not necessary

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Temperature check

Which method do you use when testing *Aspergillus* ?

1. EUCAST microdilution
2. CLSI microdilution
3. Commercially available method (e.g. Etest, Vitek, Sensititre)
4. We refer to reference laboratory
5. Susceptibility testing of *Aspergillus* is not necessary

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EUCAST Susceptibility Testing

Reference Methods

- Yeast
  - **E.DEF 7.2 (2012)**
  - **TN- E.DEF 7.2 (Submitted)**
  - E.DEF 7.1 (2008)
  - TN- E.DEF 7.1 (2008)
- Conidia forming moulds
  - E.DEF 9.1 (2008)
  - TN-E.DEF 9.1 (2008)

Breakpoints

Compound	*Candida*		*Aspergillus*	
	Rationale Doc	Techn. Note CMI	Rationale Doc	Techn. Note CMI
Amphotericin	2010	**2011**	**2012**	**Submitted ***
Anidulafungin	2010	**2011**		
Fluconazole	2007	2008	-	-
Itraconazole			**2012**	**Submitted ***
Posaconazole	2010	**2011**	**2012**	**Submitted ***
Voriconazole	2008	2008	**Discussion Doc.**	

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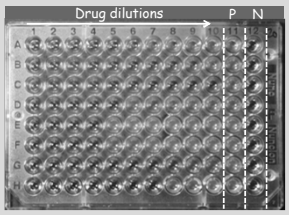
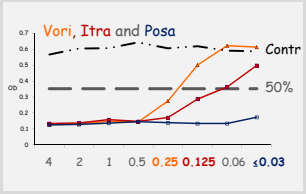
Testing Yeast: EUCAST E.DEF 7.2

- 2 fold drug dilutions (0.1 ml/well)
- 1:10 dilution of a McFarland 1
  - 1-5 x 10^5 CFU/ml; 0.1 ml /well
- 24 h incubation
- Spectrophotometer
  - Output in excel-format
  - Automated calculation of 50% endpoint
  - Objective MIC reading





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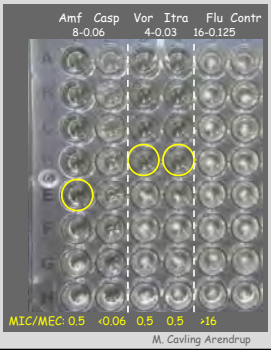
Testing Moulds: EUCAST E.DEF 9.1

	EUCAST E.DEF 9.1
Glucose	2%
Inoculum size	0.5-1.25 x 10^5 (counted)
Plates & Reading	Flat bottom & Visual
Incubation time	48 (24-72) h
Endpoint	No growth (echinocandins MEC, macroscopic endpoint)



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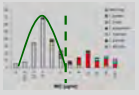
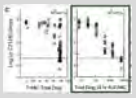
EUCAST BP establishing procedure

- MIC distributions
  - Per species
  - Several data sets
  - Epidemiological Cut Off Value (ECOFF)
- MIC-clinical outcome relationships
  - Per species
  - For wild type and non-wild type isolates
- PK/PD
  - BP never higher than ECOFF unless supported by data





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Q 3: Breakpoints for *Candida*

Which set of breakpoints have you adopted for *Candida* interpretation?

1. EUCAST
2. CLSI M27S-3
3. Revised CLSI breakpoints as published in the scientific literature
4. National breakpoints different from the above
5. In house breakpoints

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AMB & Azole breakpoints for *Candida* spp

Breakpoints (BPs): S: ≤X; R: >Y **Revised BPs**

	CLSI M27-S3	CLSI Revised 2010/11	EUCAST
AMB	≤1	≤1	≤1; >1
Fluco	≤8; >32	≤2; >4 (*alb, para, trop*) SDD ≤32; R >32 (*glab*) (*krus* poor target)	≤2; >4 (*albi, trop, para*) (*glab* IE) (*krus* poor target)
Itra	≤0.125; >0.5	≤0.125; >0.5	-
Posa	-	-	≤0.06; >0.06 (*alb, trop, para*) (*glab/krus* IE)
Vori	≤1; >2	≤0.125; >0.5 (*alb, para, trop*) ≤0.5; >1 (*krus*) (*glab* IE)	≤0.125; >0.125 (*alb, trop, para*) (*glab/krus* IE)

www.eucast.org; Pfaller Drug Resist Updat. 2010 & 2011; www.clsi.org

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EUCAST Fluconazole MIC & outcome

Fluconazole 258 cases
(128 candidaemia 58% *C. albicans*; 133 OPC all *C. albicans*)

MIC in mg/L	Candidaemia		OPC ≥ 100 mg/d		All	
	No. cure/Total	% response	No. cure/Total	% response	**% response**	
‹ 0.5	98/107	92	26/26	100	**93**	
1	6/6	100	4/4	100	**100**	S
2	1/1	100	1/1	100	**100**	
4	3/3	100	5/9	56	**67**	I/SDD
8	2/5	40	7/32	22	**24**	
≥16	3/4	75	0/60	0	**5**	R

Rodriguez-Tudela AAC 2007

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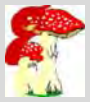
Outcome Triangle



FUNGUS
- Virulence
- Susceptibility

HOST
- Immune status
- Severity/duration of infection at the start of therapy

DRUG PK/PD & Timing
- C_{max}, $T_{\frac{1}{2}}$, AUC
- Concentration at site

S → higher likelihood of success
Some pts will die despite appropriate treatment

R → higher likelihood for failure
Some pts will get well despite inappropriate treatment

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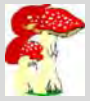
Outcome Triangle



FUNGUS
- Virulence
- Susceptibility

HOST
- Immune status
- Severity/duration of infection at the start of therapy

DRUG PK/PD & Timing
- C_{max}, $T_{\frac{1}{2}}$, AUC
- Concentration at site

S → Similar as in other pts

IR → Increased Risk Intelligence Required !!!

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Q 4: echinocandin susceptibility

My *C. glabrata* is classified as "S" (susceptible) if

1. The anidulafungin MIC is ≤0.06 mg/L
2. The caspofungin MIC is ≤2 mg/L
3. The micafungin MIC is ≤0.25 mg/L
4. 4. None of these

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Echinocandin breakpoints for *Candida* spp

Breakpoints (BPs): S: ≤X; R: >Y **Revised BPs**

	CLSI M27-S3	CLSI Revised 2011	EUCAST
ANF	≤2	≤0.25; >0.5 (*alb, krus, trop*) ≤0.125; >0.25 (*glab*) ≤2; >4 (*para, guillier*)	≤0.032; >0.032 (*alb*) ≤0.06; >0.06 (*glab, krus, trop*) (*para* not a good target) (*guillier* IE)
CSF	≤2		-
MFG	≤2	≤0.25; >0.5 (*alb, krus, trop*) ≤0.06; >0.125 (*glab*) ≤2; >4 (*para, guillier*)	-

www.eucast.org; Pfaller Drug Resist Updat. 2010 & 2011; www.clsi.org

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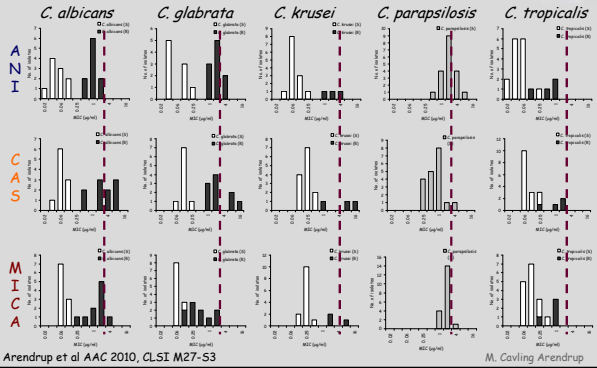
CLSI echinocandin MICs "M27-S3 BP"



Arendrup et al AAC 2010, CLSI M27-S3

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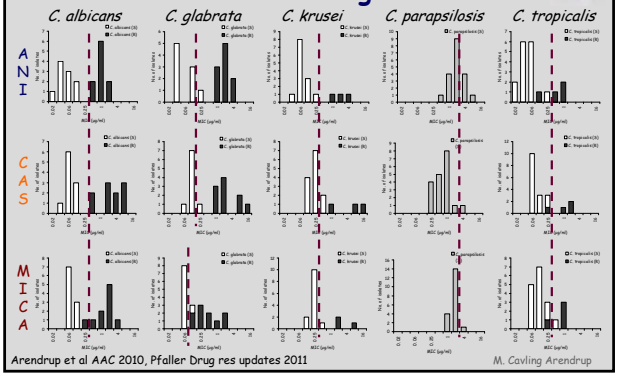
CLSI echinocandin testing – revised BP



Arendrup et al AAC 2010, Pfaller Drug res updates 2011

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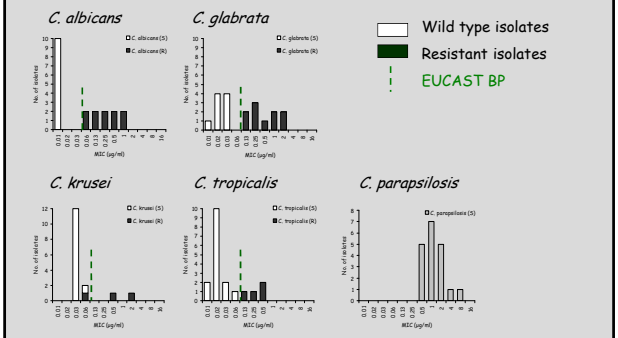
EUCAST Anidulafungin WT vs. resistant



Arendrup et al AAC 2010 and EUCAST-AFST

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EUCAST *Aspergillus* BPs 2012

BPs indicated as S ≤x / R >y

AF compound	*Aspergillus*				
	flavus	*fumigatus*	*nidulans*	*niger*	*terreus*
Amphotericin	IE*	1/2	Note	1/2	Poor Target
Itraconazole	1/2	1/2	1/2	IE*	1/2
Posaconazole	IE*	0.125/0.25**	IE*	IE*	Note
Voriconazole	Note	1/2	Note	Note	Note

* MICs are higher than for *A. fumigatus*

** provided sufficient levels can be achieved

Note: the MICs are similar to *A. fumigatus* but insufficient clinical data for BP setting

Rationale documents available at www.eucast.org

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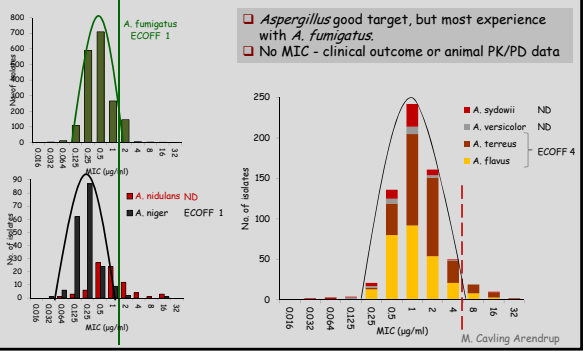
Aspergillus and Amphotericin B



- *Aspergillus* good target, but most experience with *A. fumigatus*.
- No MIC - clinical outcome or animal PK/PD data

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EUCAST *Aspergillus* BPs 2012

BPs indicated as S ≤x / R >y

AF compound	*Aspergillus*				
	flavus	*fumigatus*	*nidulans*	*niger*	*terreus*
Amphotericin	IE*	1/2	Note	1/2	Poor Target
Itraconazole	1/2	1/2	1/2	IE*	1/2
Posaconazole	IE*	0.125/0.25**	IE*	IE*	Note
Voriconazole	Note	1/2	Note	Note	Note

* MICs are higher than for *A. fumigatus*

** provided sufficient levels can be achieved

Note: the MICs are similar to *A. fumigatus* but insufficient clinical data for BP setting

Rationale documents available at www.eucast.org

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Posaconazole ECOOF: Overlap WT & non-WT

8 Individual dataset for *A. fumigatus*: MIC_{50} ~0.1 mg/L; ECOFF 0.25 mg/L

	MIC (mg/L)												
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
EUCAST		7	70	**384**	**205**	55	12	3	3	3	4		
EUCAST			5	**28**	**50**	11	4		1		1		
EUCAST	3	1	7	5	6	8	3	1			3		
EUCAST			113	**116**	**128**	100	46	16	2		5		
EUCAST			6	49	**163**	57	7	6	2		1	14	
EUCAST		5	12	**98**	**77**		48	44	69	18	1	2	1	6
EUCAST 24h						1	3	15	1				
CLSI			21	1	73	87	1	1					

2095 MICs

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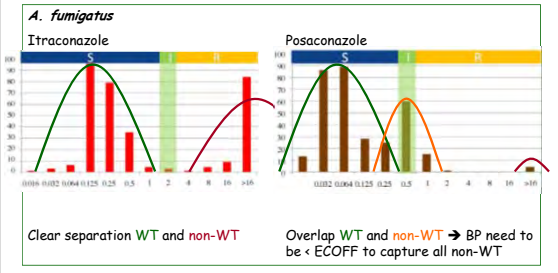
Overlap between WT and non-WT



Verweij Drug Res Updates 2009

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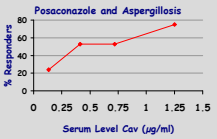
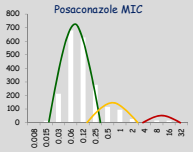
Clinical data & animal PK/PD

- Borderline exposure with the current formulation
  - C_{av} of 1.25 mg/L → better outcome
  - C_{av} of 1.25 mg/L ~ AUC of ~30 mg.h/L



- Mouse models
  - AUC:MIC of 167 → ED_{50} (include 0.125)
    - GM as endpoint, neutropenic model
  - AUC:MIC of 498 → ED_{50} (include 0.06)
    - Survival as endpoint, non-neutropenic model
  - Poorer outcome for *cyp51A* mutants
    - some of which may have an MIC of 0.25 mg/L



Walsh CID 2007; Howard JID 2011; Mavridou AAC 2010; Verweij DRU 2009

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Conservative Posaconazole BP

- *Aspergillus fumigatus*
  - S ≤0.125, R >0.25 mg/L
    - Provided adequate drug exposure has been confirmed using therapeutic drug monitoring
  - TDM targets:
    - >1 mg/L at steady state for salvage treatment
    - >0.7 mg/L for prophylaxis
- App. 15% WT may be classified as non-S
- Itraconazole "S" → probably also posaconazole "S"

Jang Clin Pharmacol Ther. 2010; FDA; soon at www.EUCAST.org

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Commercial testing methods

- Interpretation of endpoints
  - Caveats applying CLSI or EUCAST breakpoints
  - Echinocandins
    - Etest
    - Vitek

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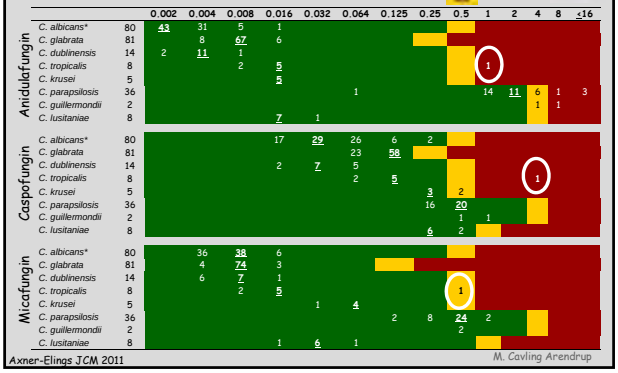
Echinocandin Etest & CLSI BP



Axner-Elings JCM 2011

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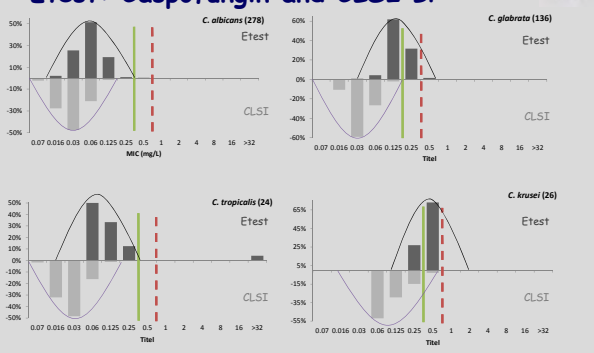
Etest: Caspofungin and CLSI BP



Arendrup & Pfaller submitted

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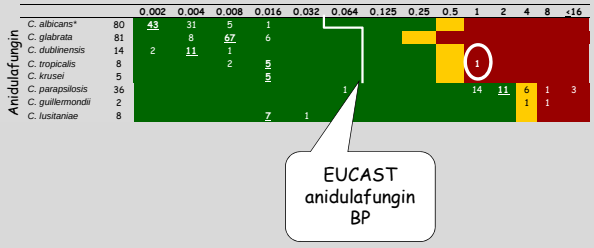
Anidulafungin Etest: EUCAST vs CLSI BP



Axner-Elings JCM 2011

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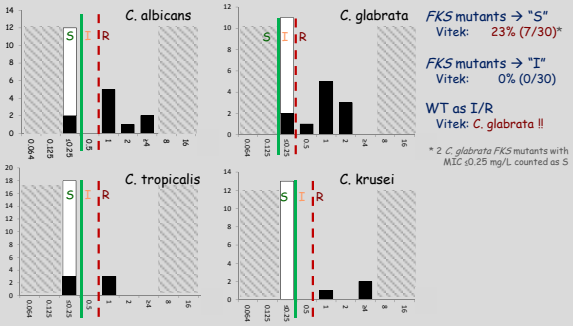
Vitek Caspofungin



FKS mutants → "S"
Vitek: 23% (7/30)*

FKS mutants → "I"
Vitek: 0% (0/30)

WT as I/R
Vitek: *C. glabrata* !!

* 2 *C. glabrata FKS* mutants with MIC ≤0.25 mg/L counted as S

Astvad, Johansen & Arendrup, in preparation

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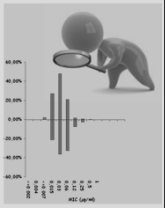
Take home messages Commercial Tests



- You have to check for each drug-bug combination
- BPs can only be adopted if MICs matches the reference method!
- Recommendations for echinocandins
  - Echinocandins:
    - Anidulafungin Etest with EUCAST BPs
    - VITEK2 problematic: MIC range doesn't cover the BP for *C. glabrata*

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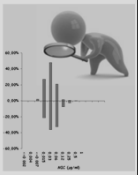
Conclusion: Susceptibility testing

- Antifungal Susceptibility testing
  - Requires training
  - Precise within ± 2 dilutions
  - The MIC_{50} reflects the susceptibility of the species
  - Correlates with the likelihood of success
  - BPs should be carefully selected
    - CLSI M27-S3 ≠ revised CLSI ≠ EUCAST
    - check your "commercial" MIC mirrors those of the ref method
    - incorrect breakpoints → random/incorrect categorization S, I, R
  - Available BPs
    - *Candida*: amphotericin, anidulafungin, flu-, vori- and posaconazole
    - *Aspergillus*: amphotericin, itraconazole, posaconazole and voriconazole



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Available documents

- CLSI

M27-A3	Yeast broth dilution	120 $	880 $
M27-S3	QC and BPs	35 $	
M38-A2	Mould broth dilution	120 $	
M44-A2	Yeast disk diffusion	200 $	
M44-S3	QC and BPs	35 $	
M51-A	Mould disk diffusion	170 $	
M51-S1	QC and ECVs	200 $	

- EUCAST
  - Methods EDef 7.2 (yeast) and EDef 9.1 (mould)
  - Rationale documents (breakpoints) — Free
    - *Candida*: amphotericin, anidulafungin, flu-, vori- and posaconazole
    - *Aspergillus*: amphotericin, itraconazole, posaconazole
  - Technical notes in Clin Microbiol Infect

www.clsi.org, www.eucast.org

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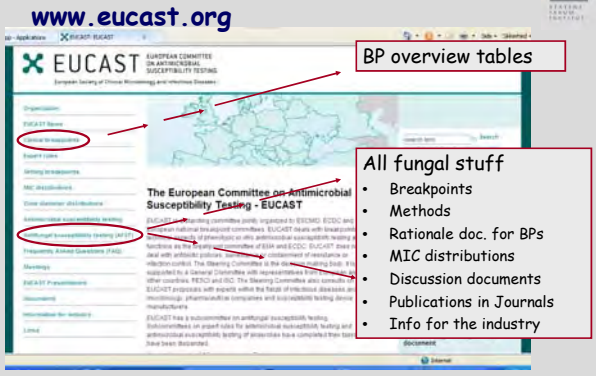
www.eucast.org



BP overview tables

All fungal stuff
- Breakpoints
- Methods
- Rationale doc. for BPs
- MIC distributions
- Discussion documents
- Publications in Journals
- Info for the industry

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Acknowledgements
(in alphabetic order):
The EUCAST Steering Committee
M Cuenca-Estrella
W Hope
C Lass-Flörl
The EUCAST General Committee
Collaborators on MIC studies
SJ Howard
DS Perlin
M Pfaller
P Verweij
The Danish Fungaemia Study Group
Thank you for your attention

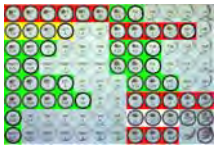
EUCAST and fastidious micro-organisms

G Kahlmeter

Update ECCMID 2012

EUCAST AST of fastidious organisms

MH-F -
Mueller-Hinton agar (or broth) with 5% horse blood and 20 mg/L β-NAD.



Developed for:

Haemophilus influenzae
Moraxella catarrhalis
Streptococci A, B, C, G and viridans
Streptococcus pneumoniae
Campylobacter jejuni and *C. coli*
Listeria monocytogenes
Corynebacterium spp.
Pasteurella multocida

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

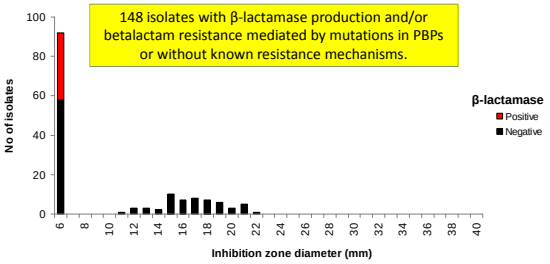
Haemophilus influenzae

- Susceptibility testing on
  - MH-F
  - inoculum McF 0.5
  - 5% CO_2
  - incubation 16 – 20h
- Disk diffusion for all relevant agents and screen method for betalactam resistance (β-lactamase, PBP-mediated resistance)

H. influenzae
– algorithm for betalactam resistance

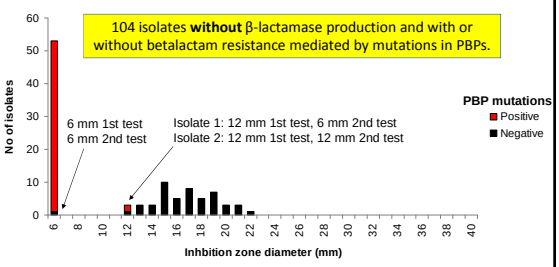
- Test all isolates with Benzylpenicillin 1 unit disk
- Isolates **≥12 mm** can be reported susceptible to penicillins and cephalosporins categorised as therapeutic alternatives for *H. influenzae*
- Isolates **<12 mm** are suspected of
  - β-lactamase: ampicillin, amoxicillin
    AND/OR
  - PBP mutations: all betalactam antibiotics at risk, including aminopenicillins with inhibitors, cephalosporins and carbapenems. Determine susceptibility to relevant agent.

Benzylpenicillin 1 unit vs. β-lactamase
***H. influenzae*, 148 clinical isolates**



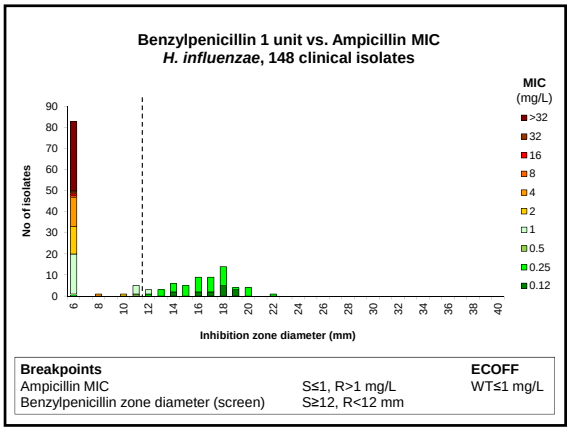
Breakpoints	
Benzylpenicillin zone diameter (screen)	S≥12, R<12 mm

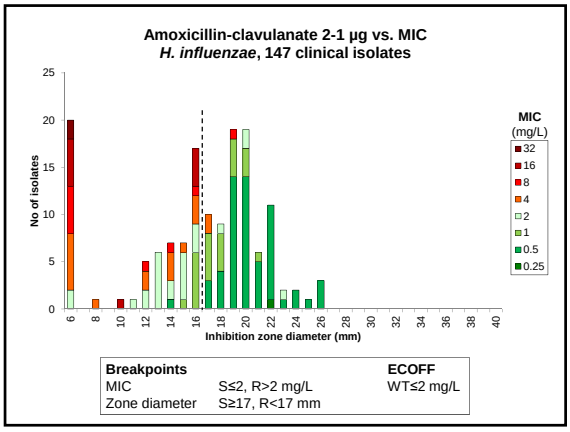
Benzylpenicillin 1 unit vs. PBP mutations
***H. influenzae*, 104 β-lactamase negative clinical isolates**

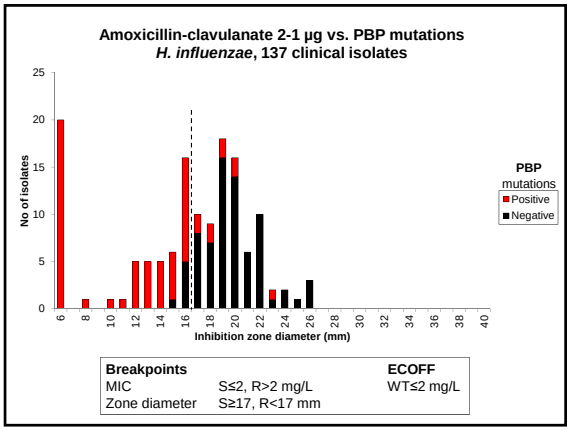


Breakpoints	
Benzylpenicillin zone diameter (screen)	S≥12, R<12 mm

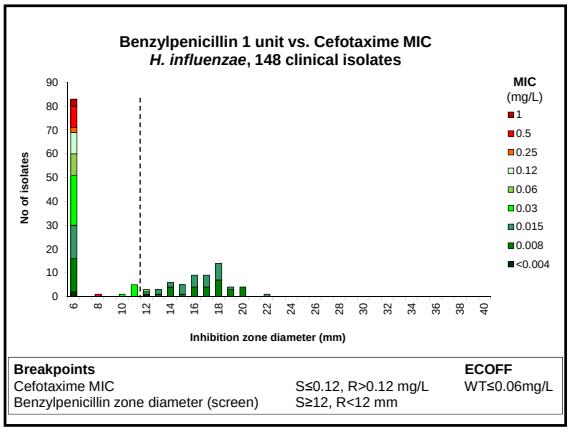
Kahlmeter – EUCAST and fastidious micro-organisms



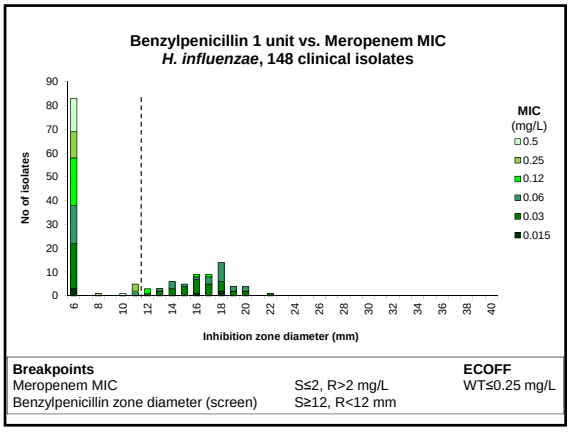




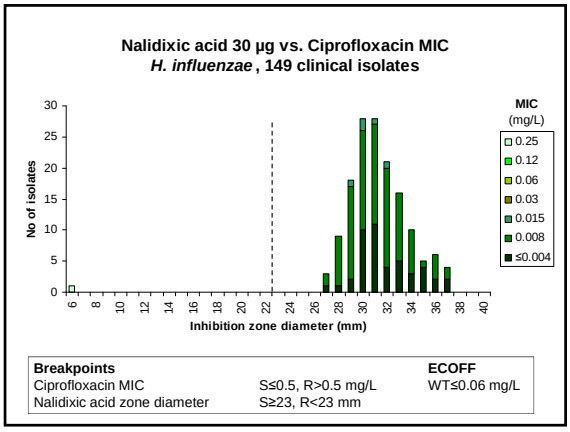
Kahlmeter – EUCAST and fastidious micro-organisms



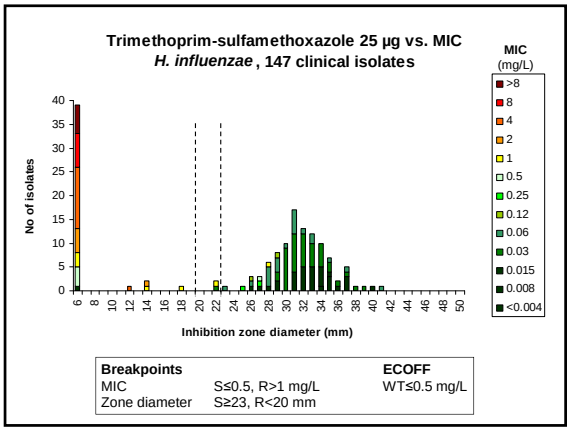
Breakpoints		ECOFF
Cefotaxime MIC	S≤0.12, R>0.12 mg/L	WT≤0.06mg/L
Benzylpenicillin zone diameter (screen)	S≥12, R<12 mm	



Breakpoints		ECOFF
Meropenem MIC	S≤2, R>2 mg/L	WT≤0.25 mg/L
Benzylpenicillin zone diameter (screen)	S≥12, R<12 mm	



Breakpoints		ECOFF
Ciprofloxacin MIC	S≤0.5, R>0.5 mg/L	WT≤0.06 mg/L
Nalidixic acid zone diameter	S≥23, R<23 mm	



EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

Disk diffusion testing of *Campylobacter jejuni* & *Campylobacter coli*

Erika Matuschek
Dik Mevius, Kees Veldman
Antti Hakanen, Mirva Lehtopolku
Gunnar Kahlmeter
2012

EUCAST methodology

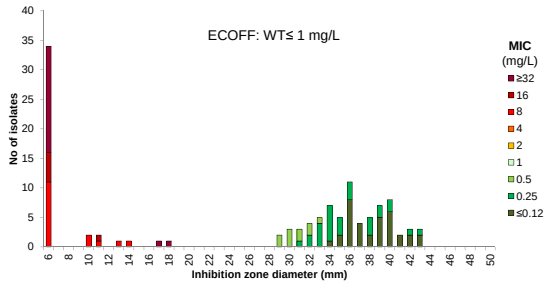
Media	MH-F (pre-dried plates)
Inoculum	McFarland 0.5
Incubation	Microaerobic environment 41°C 24 h ± 30 min*
Reading	EUCAST standard reading

* *Campylobacter coli* isolates with insufficient growth are reincubated immediately and read after a total of 40-48 h incubation

Validation of methodology

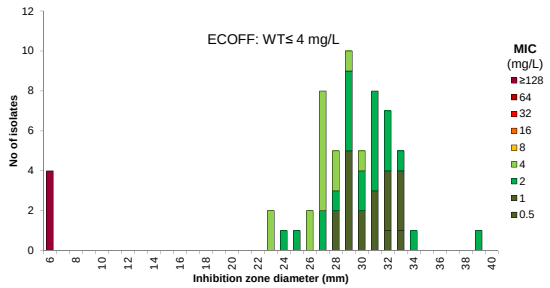
- Two collections of isolates with known MIC values (BMD)
  - NL collection (15 *C. jejuni* and 15 *C. coli*)
  - FI collection (17 *C. jejuni* and 12 *C. coli*, including ”difficult” isolates)
- Data submission via EUCAST website
  - QC data from 15 labs
  - Clinical data from 2 labs (…more data to come!)

Ciprofloxacin 5 µg vs. MIC, *Campylobacter jejuni* and *coli*
57 clinical isolates tested in duplicate



NL and FI isolates read in Växjö.
All isolates tested in duplicate on in-house MH-F plates from Oxoid and BBL MH.

Erythromycin 15 µg vs. MIC, *Campylobacter jejuni*
30 clinical isolates tested in duplicate



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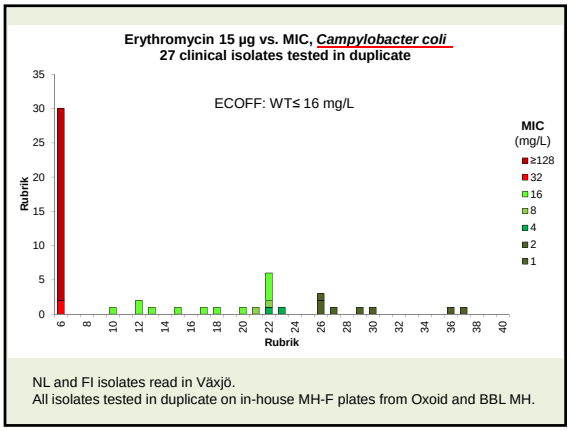

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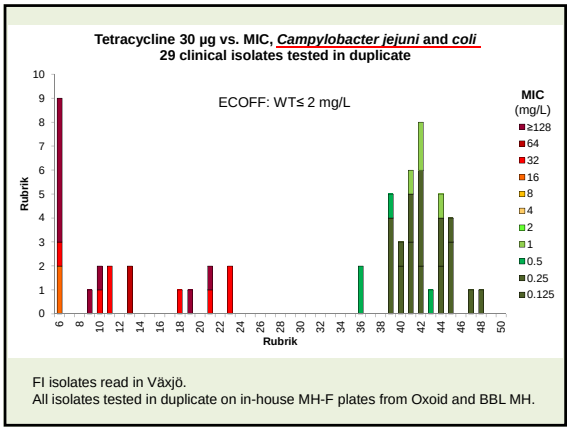


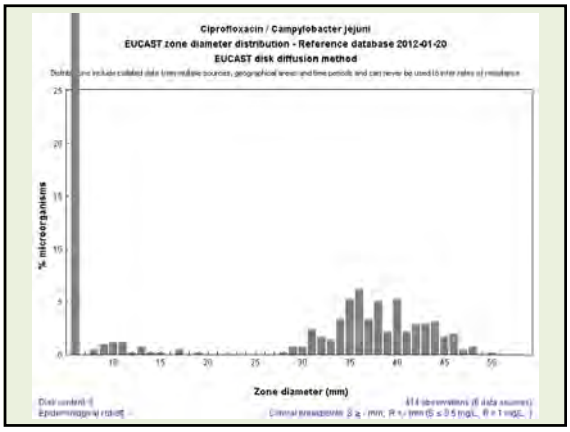
FI isolates read in Växjö.
All isolates tested in duplicate on in-house MH-F plates from Oxoid and BBL MH.

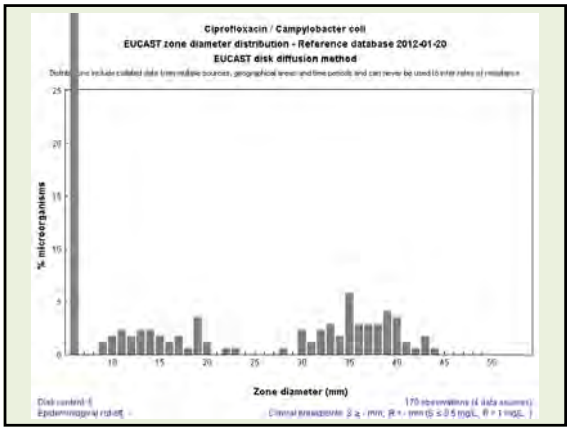
Zone distributions on EUCAST website

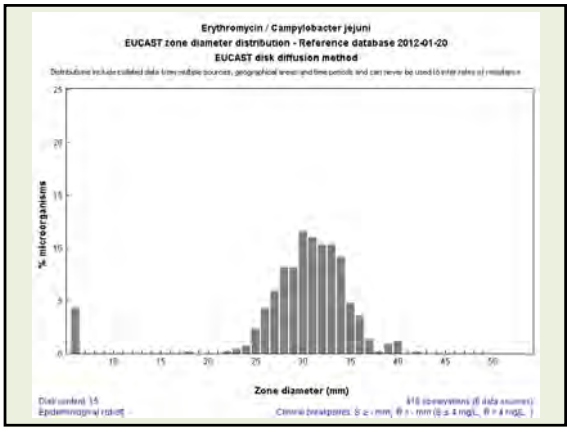
Data from several sources:

- NL and FI isolates tested at three sites (on different MH-F plates)
- Routine data from Växjö
- Data submission project (one lab)

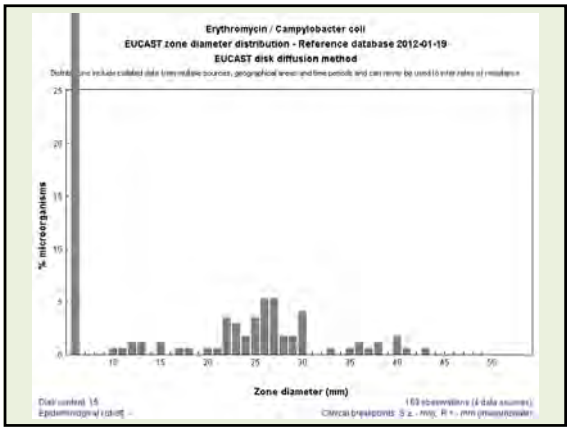
Kahlmeter – EUCAST and fastidious micro-organisms

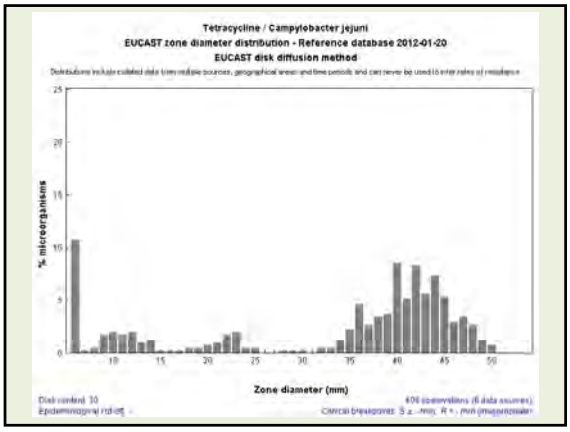


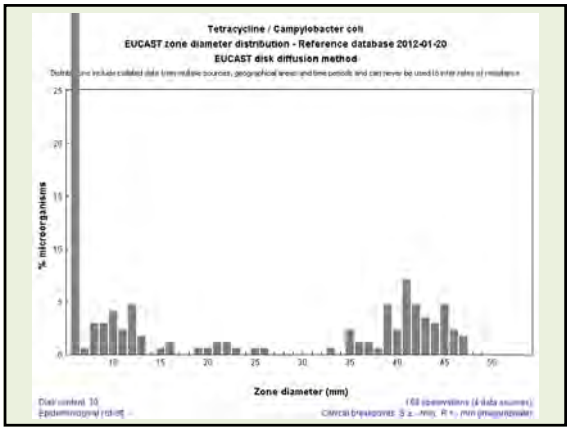




Kahlmeter – EUCAST and fastidious micro-organisms







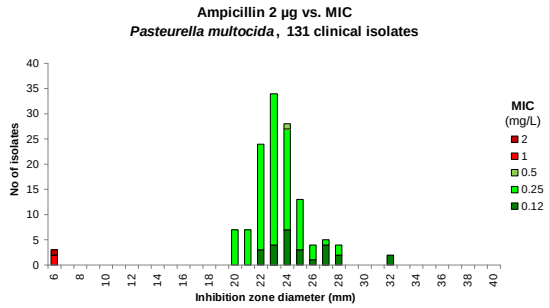
Pasteurella multocida

- In-house isolates and isolates from NL
  - 53 clinical isolates + resistant veterinary isolates
- SENTRY collection
  - 70 clinical isolates

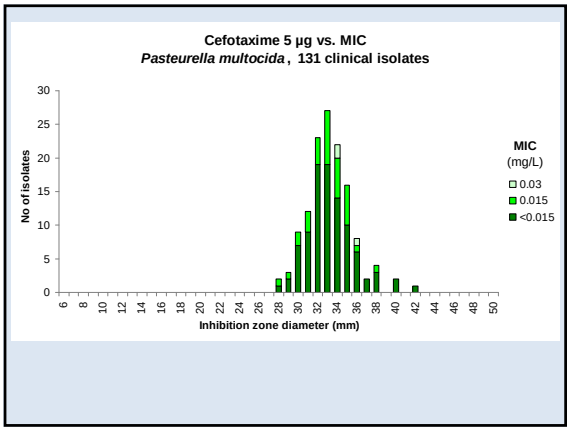
MICs and zone diameters by EUCAST
MH-F, ambient air, 35 C, 16 – 20 h

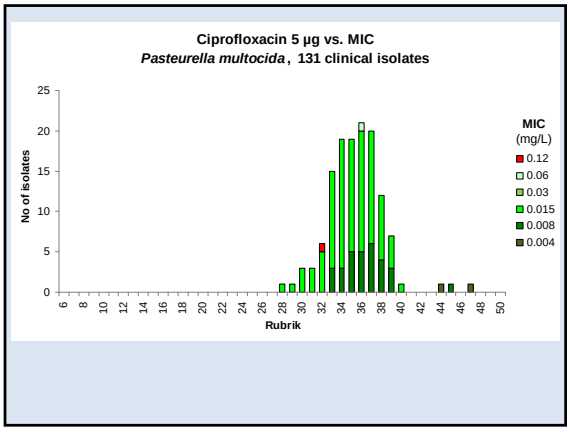
Pasteurella multocida

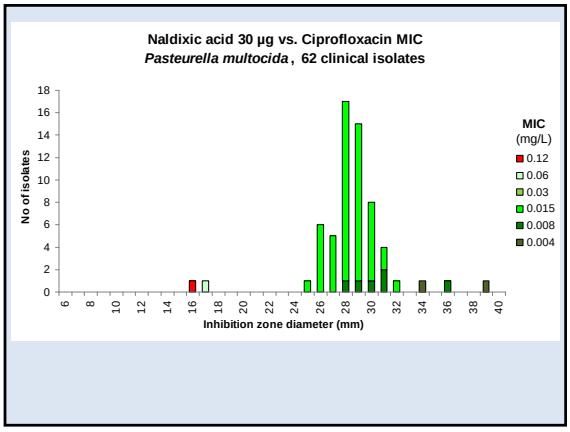
Antibiotics tested
- Benzylpenicillin
- Ampicillin
- Amoxicillin
- Amoxicillin-clavulanic acid
- Cefotaxime
- Ciprofloxacin
- Tetracycline
- Trimethoprim-sulfamethoxazole

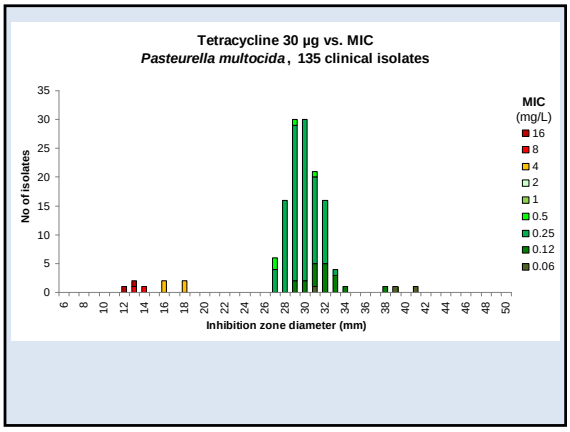


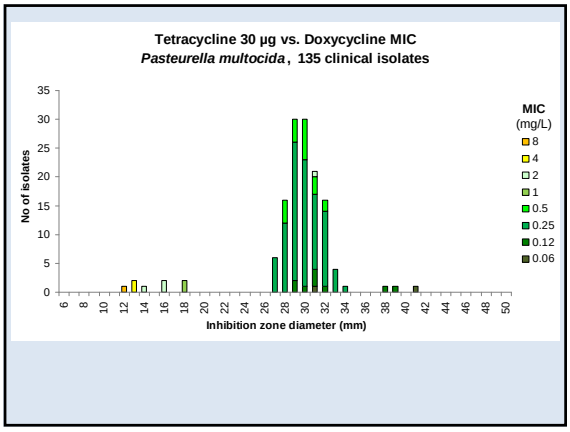
Kahlmeter – EUCAST and fastidious micro-organisms





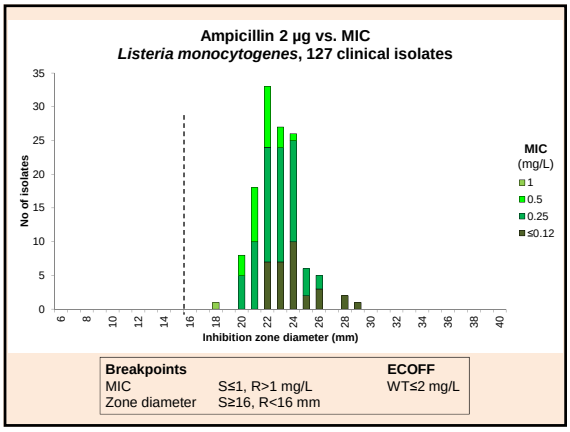


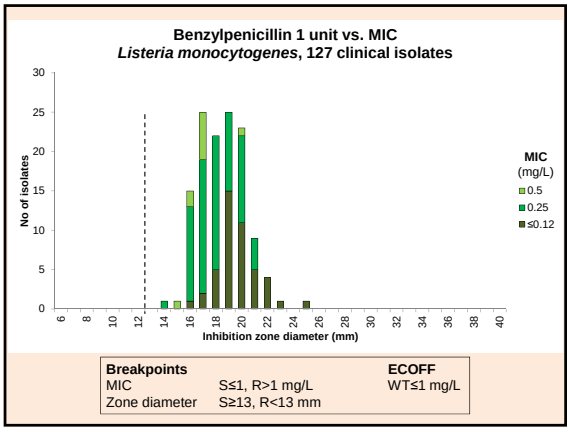


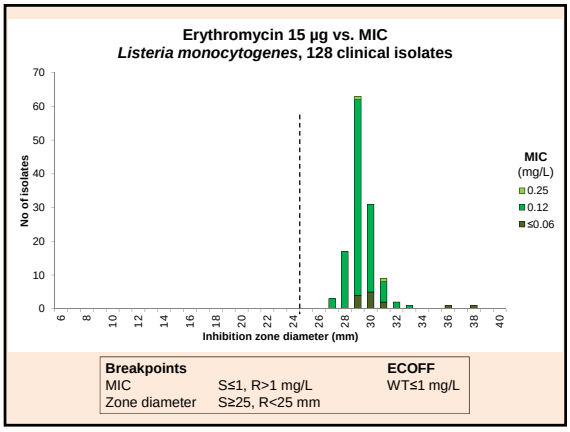


Listeria monocytogenes

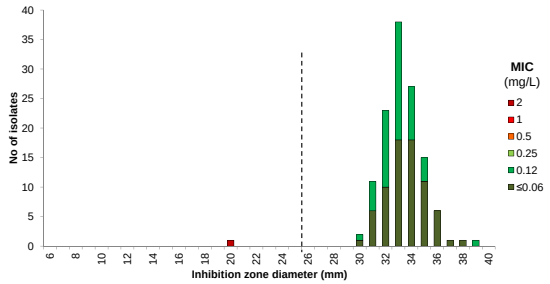
- 129 clinical isolates collected from 5 test sites:
  - Denmark
  - Israel
  - Norway
  - Sweden
  - United Kingdom
- Disk diffusion performed on all isolates at all sites
- MIC determination performed with broth microdilution and gradient tests on MH-F media at one site


Ampicillin 2 µg vs. MIC
Listeria monocytogenes, 127 clinical isolates
No of isolates
Inhibition zone diameter (mm)
MIC (mg/L)
1
0.5
0.25
≤0.12
Breakpoints
MIC S≤1, R>1 mg/L
Zone diameter S≥16, R<16 mm
ECOFF
WT≤2 mg/L


Benzylpenicillin 1 unit vs. MIC
Listeria monocytogenes, 127 clinical isolates
No of isolates
Inhibition zone diameter (mm)
MIC (mg/L)
0.5
0.25
≤0.12
Breakpoints
MIC S≤1, R>1 mg/L
Zone diameter S≥13, R<13 mm
ECOFF
WT≤1 mg/L

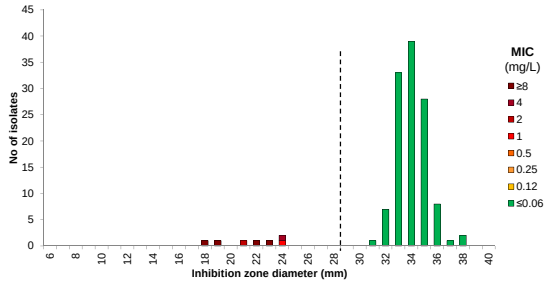

Erythromycin 15 µg vs. MIC
Listeria monocytogenes, 128 clinical isolates
No of isolates
Inhibition zone diameter (mm)
MIC (mg/L)
0.25
0.12
≤0.06
Breakpoints
MIC S≤1, R>1 mg/L
Zone diameter S≥25, R<25 mm
ECOFF
WT≤1 mg/L

Meropenem 10 µg vs. MIC
***Listeria monocytogenes*, 126 clinical isolates**



Breakpoints		ECOFF
MIC	S≤0.25, R>0.25 mg/L	WT≤0.25 mg/L
Zone diameter	S≥26, R<26 mm	

Trimethoprim-sulfamethoxazole 1.25-23.75 µg vs. MIC
***Listeria monocytogenes*, 126 clinical isolates**



Breakpoints	
MIC	S≤0.06, R>0.06 mg/L
Zone diameter	S≥29, R<29 mm

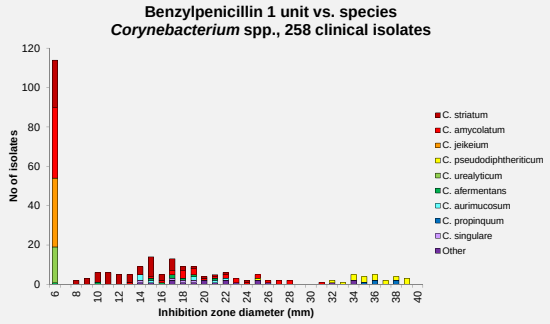
Corynebacterium spp.

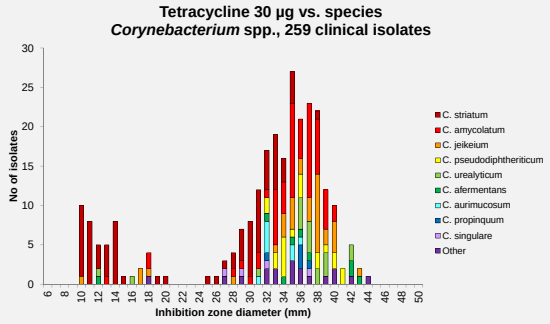
- A collection of 259 species-identified (Maldi-tof) *Corynebacterium* spp. from Spain, Sweden and USA (SENTRY)
- Disk diffusion and MIC (BMD with MH-F broth)
  - Isolates with non-sufficient growth after 16-20 h incubation was re-incubated and read after a total of 40-44 h incubation

Corynebacterium spp.

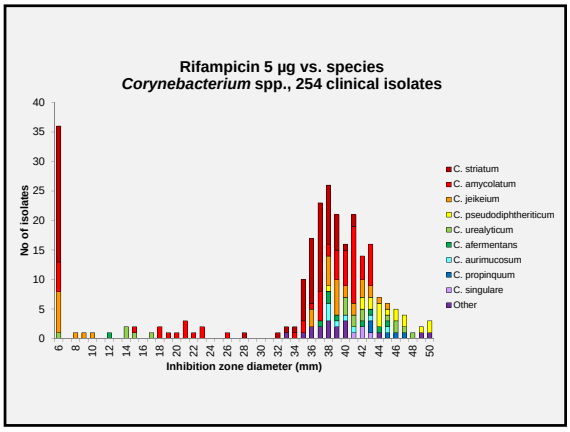
Antibiotics tested

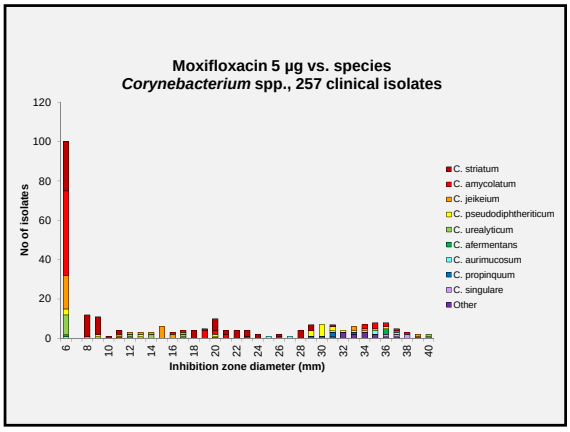
- Benzylpenicillin
- Ciprofloxacin
- Moxifloxacin
- Gentamicin
- Vancomycin
- Tetracycline
- Clindamycin
- Linezolid
- Rifampicin
- Trimethoprim-sulfamethoxazole

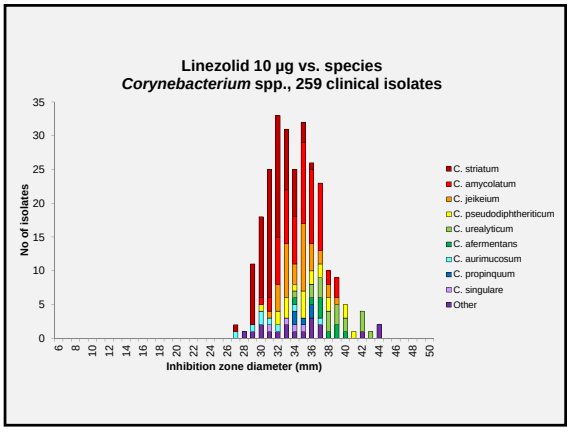




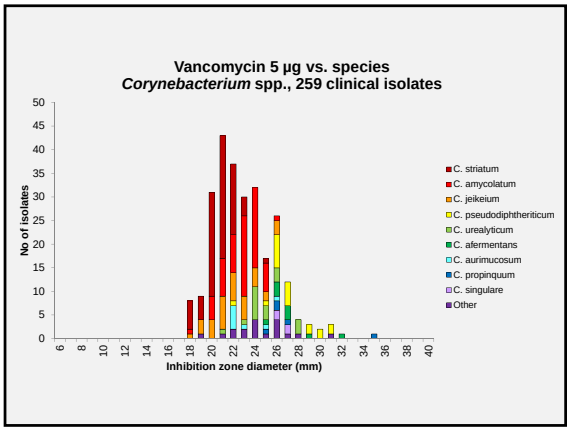
Kahlmeter – EUCAST and fastidious micro-organisms







Kahlmeter – EUCAST and fastidious micro-organisms



Fastidious organisms Summary

MH-F:

√ *Haemophilus influenzae* v 2.0 (Jan 2012)
√ *Moraxella catarrhalis* v 2.0 (Jan 2012)
√ Streptococci A, B, C, G v 2.0 (Jan 2012)
√ Viridans group streptococci v 2.0 (Jan 2012)
√ *Streptococcus pneumoniae* v 2.0 (Jan 2012)
√ *Listeria* v 2.0 (Jan 2012)

Campylobacter v 3.0 (Jan 2013)
Corynebacterium v 3.0 (Jan 2013)
Pasteurella v 3.0 (Jan 2013)

Other media:

Clostridium difficile v 3.0 (Jan 2013)
Neisseria gonorrhoeae (2013)

Saturday 15.30 – 16.30

P671 Adjusting EUCAST zone diameter breakpoints for *Haemophilus influenzae* on the Mueller-Hinton fastidious media. *J. Åhman*, E. Matuschek, P.R. Rhomberg, R.N. Jones, G. Kahlmeter*

P676 Antimicrobial susceptibility testing of *Listeria monocytogenes* with EUCAST breakpoints: a multi-laboratory study. *K. Bowker, J. Åhman, O. Natås, I. Nissan, P. Littauer, E. Matuschek**

P677 Susceptibility testing of ten antibiotics against *Corynebacterium* spp. Determined by broth microdilution, Etest and EUCAST disc diffusion methods. *C. Salas*, C. Karlsson, A. Akerlund, C. Rodriguez-Mirones, E. Matuschek, L. Martinez-Martinez, G. Kahlmeter*

P680 The *Brucella* blood agar for disc diffusion antimicrobial susceptibility testing – reproducibility results for *Clostridium difficile* ATCC 700057. *U.S. Justesen, L.T. Erikstrup*, T.K. Danielsen, E. Matuschek, G. Kahlmeter*

P682 EUCAST standardised disc diffusion methodology for *Campylobacter jejuni* and *C. coli.* *E. Matuschek*, K. Veldman, A. Hakanen, S. Bengtsson, M. Lehtopolku, D. Mevius, G. Kahlmeter*

Sunday 12.30 – 13.30

P1092 Antimicrobial susceptibility and species identification of *Corynebacterium* spp. strains collected in Europe and USA medical centres, 2006–2010. *H. Sader*, P. Rhomberg, R. Jones, J. Åhman, E. Matuschek, G. Kahlmeter*

Other EUCAST posters

P675 Contemporary doxycyline and tetracycline susceptibility testing using CLSI and EUCAST criteria for Gram-positive pathogens: results from SENTRY programme. *R. Jones*,M. Stilwell (North Liberty, US)*

P684 Comparison of Neo-sensitabs (ROSCO) tablets with paper discs (OXOID) for antimicrobial susceptibility testing of Gram-negative clinical isolates according to the EUCAST recommendations
H. Rodriguez-Villalobos, A. Boeras (Brussels, BE)*

P 686 New EUCAST breakpoint for detection of antimicrobial susceptibility in *Helicobacter pylori*
T. Alarcon, A. Somodevilla, M. (Madrid, ES)*

Acknowledgements

- Erika Matuschek, Jenny Åhman, Anna Petersson, Stina Bengtsson in Växjö, Sweden
- Derek Brown, scientific secretary of EUCAST
- Luis Martinez-Martinez (*Corynebacterium* spp.)
- JMI Laboratories – especially Ron Jones, Helio Sader, Paul Rhomberg (*H. influenzae*, *Corynebacterium* spp., *Pasteurella multocida*)

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

www.EUCAST.org

EXPERT RULES IN SUSCEPTIBILITY TESTING – RATIONALE, ADVANTAGE AND DISADVANTAGES

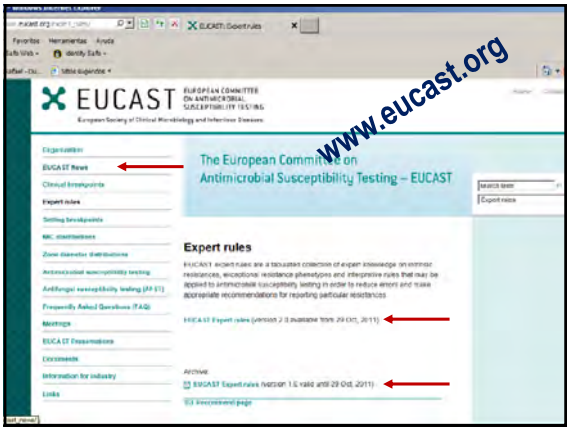
Dr. Rafael Cantón
Hospital Universitario Ramón y Cajal
SERVICIO DE MICROBIOLOGÍA Y PARASITOLOGÍA

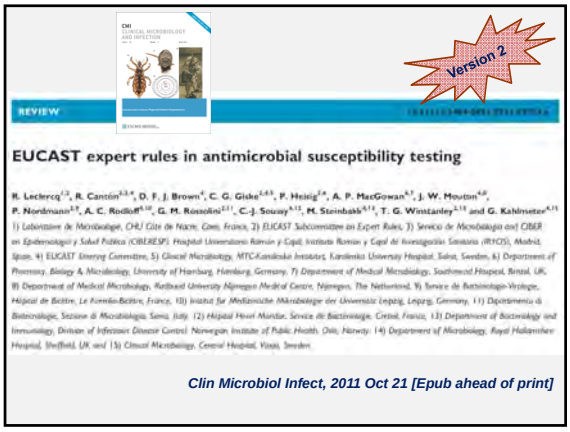
Departamento de Microbiología II
Universidad Complutense. Madrid

EUCAST expert rules

- **Expert rules** in **antimicrobial susceptibility testing (AST)**
  - describe actions to be taken on the basis of specific AST results
  - based on clinical breakpoints & resistance mechanism knowledge
  - assist clinical microbiologists in the interpretation of AST results
  - contribute to quality assurance by highlighting anomalous results
  - should be in agreement with clinical breakpoints

Winstanley T, Courvalin P. Clin Microbiol Rev 2011; 24: 515–56
Leclercq R et al. Clin Microbiol Infect 2011 Oct 21 [Epub ahead of print]
http://www.eucast.org





Clin Microbiol Infect, 2011 Oct 21 [Epub ahead of print]

EUCAST expert rules

- The **EUCAST expert rules** in antimicrobial susceptibility testing are divided into:

  - intrinsic resistances

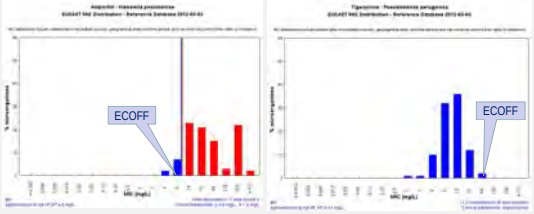
  - exceptional phenotypes

  - interpretive rules

Leclercq R et al. Clin Microbiol Infect 2011 Oct 21 [Epub ahead of print]
http://www.eucast.org

EUCAST expert rules *v2:* intrinsic resistance

- Characteristic of all or almost all isolates of the bacterial specie
- The antimicrobial activity of the drug is clinically insufficient or antimicrobial resistance is innate, rendering it clinically useless
- Antimicrobial susceptibility is unnecessary



www.eucast.org

EUCAST expert rules *v2:* intrinsic resistance

www.eucast.org

EUCAST expert rules *v2:* exceptional phenotypes

- Phenotypes of resistance of bacterial species to particular antimicrobial agents that have not yet reported or are very rare
- They may change over time and should be define locally

Rule no.	Organisms	Exceptional phenotypes
5.1	Any *Enterobacteriaceae* (except *Proteae*)	Resistant to meropenem and/or imipenem*
5.2	*Serratia marcescens* and *Proteae*	Susceptible to colistin
5.3	*Pseudomonas aeruginosa* and *Acinetobacter* spp.	Resistant to colistin
5.4	*Haemophilus influenzae*	Resistant to any third-generation cephalosporin, carbapenems, and fluoroquinolones
5.5	*Moraxella catarrhalis*	Resistant to ciprofloxacin and any third-generation cephalosporin
5.6	*Neisseria meningitidis*	Resistant to any third-generation cephalosporin and fluoroquinolones
5.7	*Neisseria gonorrhoeae*	Resistant to third-generation cephalosporin and spectinomycin

*Except in countries in which carbapenemase-producing *Enterobacteriaceae* are not rare.

EUCAST expert rules *v2:* interpretive rules

- Actions to be taken on the basis of specific AST results

Rule no.	Organisms	Agents tested	Agents affected	Rule	Exceptions, scientific basis and comments	Evidence grade	References
8.1	*Staphylococcus* spp.	Oxacillin, cefoxitin (disk diffusion) or detection of *mecA* gene or PBP2a	All β-lactams	IF resistant to isoxazolyl-penicillins (as determined with oxacillin, cefoxitin, or by detection of *mecA*-gene or of PBP2a) THEN report as resistant to all β-lactams except those specifically licensed to treat infections caused by methicillin-resistant staphylococci due to low affinity for PBP2a	Production of PBP2a (encoded by mecA) leads to cross resistance to β-lactams except ceftobiprole and ceftaroline.	A	Page et al. 2006 Chambers et al. 1990

IF ... THEN ...

EUCAST expert rules *v2:* interpretive rules

Evidences of expert rules

A. There **is good clinical evidence** that reporting the test results as susceptible leads to clinical failures

B. Evidence is weak and based only on a **few case reports** or on **experimental models**. It is presumed that reporting the test result as susceptible may lead to clinical failures

C. There is **no clinical evidence**, **but microbiological data** suggest that clinical use of the agent should be discouraged

Leclercq R et al. Clin Microbiol Infect 2011 Oct 21 [Epub ahead of print]
http://www.eucast.org

EUCAST expert rules *v2:* major modifications

β-lactam antibiotics

- **Deletion of ESBL expert rule from** *v1*

  - ESBL detection and clinical category modification in extended spectrum cephalosporins no longer exist (*report as found*)

The cephalosporin breakpoints for Enterobacteriaceae will detect all clinically important resistance mechanisms (including ESBL and plasmid mediated AmpC). Some strains that produce β-lactamases are S or I to 3rd or 4th gen. cephalosporins with these breakpoints and should be reported as found, i.e. the presence or absence of an ESBL does not in itself influence the categorization of susceptibility. In many areas, ESBL detection and characterization is recommended or mandatory for infection control purposes

EUCAST Clinical Breakpoint Table v. 2.0, valid from 2012-01-01

EUCAST expert rules *v2*

Q1

EUCAST expert rules *v2*

Q2

CLSI & EUCAST carbapenem clinical breakpoints

- *The current situation on Enterobacteriaceae …*

	FDA	CLSI (2011)		EUCAST (EMA) (2011)		
	S	S	R	S	R	ECOFF
Imipenem	≤4	≤1 (4)*	≥4 (16)	≤2	>8	≤0.5; ≤1**
Meropenem	≤4	≤1 (4)	≥4 (16)	≤2	>8	≤0.125
Ertapenem	≤2	≤0.25 (2)	≥1 (8)	≤0.5	>1	≤0.06
Doripenem	≤0.5	≤1 (ND)	≥4 (ND)	≤1	>4	≤0.12

*2009; ***E. coli* y *K. pneumoniae;* ND: not defined

EUCAST breakpoint are higher than those of CLSI!

CLSI & EUCAST carbapenem clinical breakpoints

CLSI

- New breakpoints published in June 2010 and January 2011*
  - to capture carbapenemase (mainly KPCs) producers
  - Rationale:
    - Pk/Pd tools avoiding PK subject variability (*inflated variance*)
- Modified Hodge test no longer necessary unless for infection control and epidemiological purposes

*Documents M100-S20-U; M100-S21

EUCAST

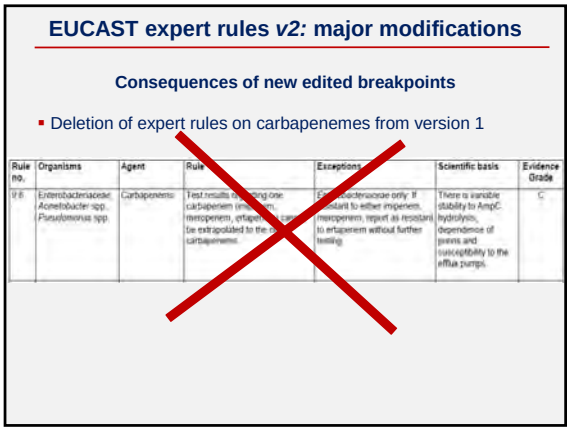
- Breakpoints published in 2006 and with doripenem in 2008*
  - define as "clinical breakpoints" not to detect carbapenemases
  - Rationale:
    - MIC distribution of wild-type isolates, MBL-KPC producers
    - Pk/Pd data
    - Review of clinical data
- Carbapenemase detection no longer necessary for clinical categorization unless for infection control purposes

*Version 1.3, January 2011

EUCAST expert rules *v2:* major modifications

Consequences of new edited breakpoints

- Deletion of expert rules on carbapenemes from version 1



Rule no.	Organisms	Agent	Rule	Exceptions	Scientific basis	Evidence Grade
9.6	Enterobacteriaceae, *Acinetobacter* spp., *Pseudomonas* spp.	Carbapenems	Test results for only one carbapenem (imipenem, meropenem, ertapenem) can be extrapolated to the other carbapenems.	Enterobacteriaceae only. If resistant to either imipenem, meropenem, report as resistant to ertapenem without further testing	There is variable stability to AmpC hydrolysis, dependence of porins and susceptibility to the efflux pumps.	C

EUCAST expert rules *v2:* major modifications

Consequences of new edited breakpoints

- Deletion of expert rules from version 1 on carbapenemes

Rule no.	Organisms	Agent	Rule	Scientific basis	Evidence Grade
9.7	Enterobacteriaceae, *Acinetobacter* spp., *Pseudomonas* spp.	Carbapenems	If production of metallo-β-lactamase is confirmed, report the susceptible results as intermediate and the intermediate results as resistant for any β-lactam except aztreonam which should be reported as found.	Metallo-β-lactamases can hydrolyse all β-lactams except monobactams.	B
9.8	Enterobacteriaceae	Carbapenems, oxyimino cephalosporins, aztreonam	If reduced susceptibility to carbapenems AND oxyimino cephalosporins AND aztreonam, resistance may reflect either KPC, VIM, GES β-lactamases or combinations of AmpC or ESBL plus impermeability. In either case ertapenem tends to be the most affected carbapenem. Synergy between carbapenems and clavulanate may arise with either KPC enzymes or with combinations of ESBL and impermeability.	KPC carbapenemase or combinations of ESBL or AmpC and impermeability.	C

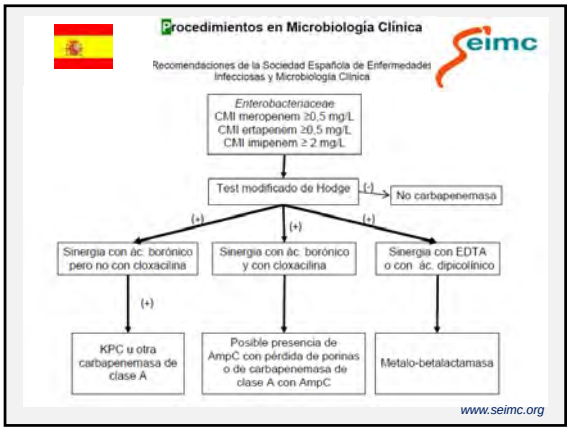
EUCAST Version 2.0, January 2012

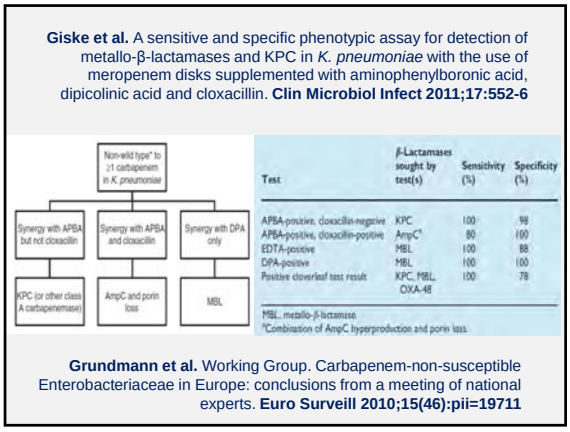
Breakpoint table for interpretation or MICs and zone diameters

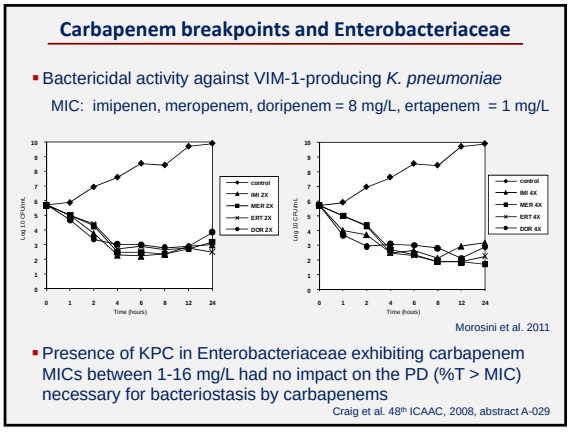
Carbapenems	MIC breakpoint (mg/L)	
	S ≤	R >
Doripenem	1	4
Ertapenem	0.5	1
Imipenem	2	8
Meropenem	2	8

Low-level resistance is common in *Morganella spp., Proteus spp. and Providencia spp.*

The carbapenem breakpoints for Enterobacteriaceae will detect all clinically important resistance mechanisms (including the majority of carbapenemases). Some isolates that produce carbapenemase are categorised as S with these breakpoints and **should be reported as tested,** i.e. the presence or absence of a carbapenemase does not in itself influence the categorisation of susceptibility. **In many areas, carbapenemase detection and characterisation is recommended or mandatory for infection control purposes.**


Procedimientos en Microbiología Clínica
Recomendaciones de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica
seimc
Enterobacteriaceae
CMI meropenem ≥0,5 mg/L
CMI ertapenem ≥0,5 mg/L
CMI imipenem ≥ 2 mg/L
Test modificado de Hodge
(-)
No carbapenemasa
(+)
(+)
(+)
Sinergia con ác. borónico pero no con cloxacilina
Sinergia con ác. borónico y con cloxacilina
Sinergia con EDTA o con ác. dipicolínico
(+)
KPC u otra carbapenemasa de clase A
Posible presencia de AmpC con pérdida de porinas o de carbapenemasa de clase A con AmpC
Metalo-betalactamasa
www.seimc.org

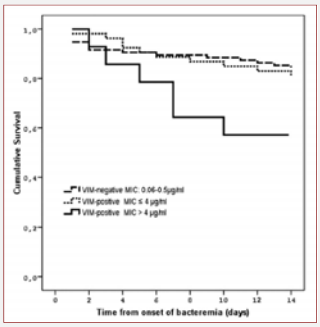

Giske et al. A sensitive and specific phenotypic assay for detection of metallo-β-lactamases and KPC in *K. pneumoniae* with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. **Clin Microbiol Infect 2011;17:552-6**
Non-wild type* to ≥1 carbapenem in K pneumoniae
Synergy with APBA but not cloxacillin
Synergy with APBA and cloxacillin
Synergy with DPA only
KPC (or other class A carbapenemase)
AmpC and porin loss
MBL
Test
β-Lactamases sought by test(s)
Sensitivity (%)
Specificity (%)
APBA-positive, cloxacillin-negative KPC 100 98
APBA-positive, cloxacillin-positive AmpC* 80 100
EDTA-positive MBL 100 88
DPA-positive MBL 100 100
Positive cloverleaf test result KPC, MBL, OXA-48 100 78
MBL, metallo-β-lactamase
*Combination of AmpC hyperproduction and porin loss
Grundmann et al. Working Group. Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. **Euro Surveill 2010;15(46):pii=19711**


Carbapenem breakpoints and Enterobacteriaceae
▪ Bactericidal activity against VIM-1-producing *K. pneumoniae*
MIC: imipenen, meropenem, doripenem = 8 mg/L, ertapenem = 1 mg/L
control
IMI 2X
MER 2X
ERT 2X
DOR 2X
control
IMI 4X
MER 4X
ERT 4X
DOR 4X
Morosini et al. 2011
▪ Presence of KPC in Enterobacteriaceae exhibiting carbapenem MICs between 1-16 mg/L had no impact on the PD (%T > MIC) necessary for bacteriostasis by carbapenems
Craig et al. 48th ICAAC, 2008, abstract A-029

Imipenem / meropenem and metalo-β-lactamase (VIM)

- Survival probability of patients with VIM-producing *K. pneumoniae* blood stream infections according with susceptibility to carbapenems

Patients infected with a VIM-(+) organism for which the MICs of both imipenem and meropenem were >4 mg/L were more likely to die than those infected with a VIM-(+) carbapenem-susceptible of VIM-(-) organisms (P 0.044)

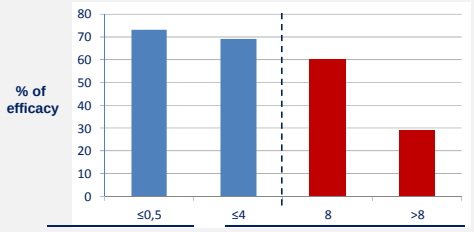


Not all patients were treated with carbapenems

Daikos et al. Antimicrob Agents Chemother 2009; 53: 1868-73

Carbapenemase isolates and carbapenem treatment

Clinical outcomes of carbapenem monotherapy treatment



22 patients with non-carbapenemase-producing *K. pneumoniae* isolates

44 patients with VIM, NDM or KPC producing *K. pneumoniae* isolates

Daikos et al.Clin Microbiol Infect 2011; 17: 1135-41

EUCAST expert rules *v2:* major modifications

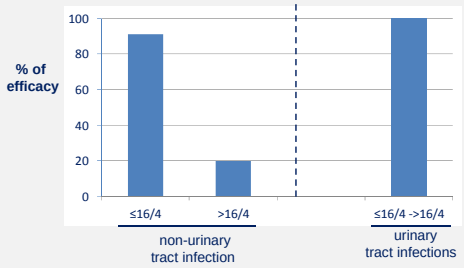
β-lactam-β-lactamase inhibitor combinations and Enterobacteriaceae (expert rule 9.1)

Agents tested	Agents affected	Rule	Exceptions, scientific basis and comments
Cefotaxime, ceftriaxone, ceftazidime, cefepime, amoxicillin-clavulanate, ampicillin- sulbactam, piperacillin-tazobactam	Amoxicillin-clavulanate, ampicillin-sulbactam piperacillin-tazobactam	**IF** I or R to any 3rd gen. (cefotaxime, ceftriaxone, ceftazidime) or 4th gen. (cefepime) oxyimino-cephalosporin, **AND susceptible to amoxicillin-clavulanate, ampicillin-sulbactam or piperacillin-tazobactam THEN report as tested and enclose a warning on uncertain therapeutic outcome for infections other than urinary tract infections.**	ESBL producers are often categorized as S to combinations of a penicillin plus a β-lactamase inhibitor. With the exception of urinary tract infections and blood stream infections secondary to this origin, the use of these combinations in infections caused by ESBL producers remains controversial, and should be approached with caution. No evidence with ticarcillin-clavulanate has been published

Grade B

β-lactam - β-lactamase inhibitor combinations

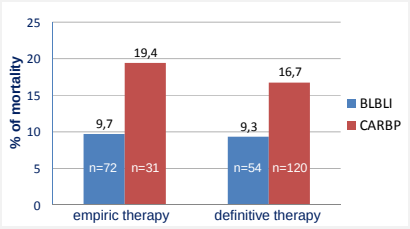
Clinical outcomes of **piperacillin-tazobactam** (alone or in combination for at least 72 h) in infection caused by ESBL-Enterobacteriaceae



Gavin et al.Antimicrob Agents Chemother 2012; 50: 2244-7

β-lactam - β-lactamase inhibitor combinations (BLBLI)

Mortality rates at day 30 in ESBL-*Escherichia coli* bacteriemic patients treated with BLBLI or carbapenems



Rodriguez-Baño et al.Clin Infect Dis 2012; 54: 167-174

β-lactam - β-lactamase inhibitor combinations (BLBLI)

Mortality rates at day 30 in ESBL-*Escherichia coli* bacteriemic patients who received empirical terapy with an active BLBLI

	Minimum Inhibitory Concentration, mg/L				
Antimicrobial	≤1	2	4	8	16
Piperacillin-tazobactam	0/10	0/8	1/4	2/6	1/7
Amoxicillin-clavulanate	…	…	1/12	2/25	…

[a] Data are expressed as No. of patients who died/No. of patients treated.

PTZ and AMC are suitable alternatives to carbapenems in ESBL-*E. coli* bacteriemic patients if active *in vitro* and would be particularly useful as definitive therapy

Rodriguez-Baño et al.Clin Infect Dis 2012; 54: 167-174

EUCAST expert rules *v2:* major modifications

β-lactams and *Haemophilus influenzae*

Rule 10.1 *v2* (evidence grade A)

Agents tested	Agents affected	Rule	Exceptions, scientific basis and comments
Ampicillin or amoxicillin (and β-lactamase detection)	Ampicillin, amoxicillin and piperacillin	IF β-lactamase positive THEN report as R to ampicillin, amoxicillin and piperacillin	Ampicillin is the class representative for amoxicillin Resistance to ampicillin by production of β-lactamase may be misidentified by the disk diffusion technique. Production of β-lactamase should be examined with a chromogenic test.

EUCAST expert rules *v2:* major modifications

β-lactams and *Haemophilus influenzae*

Rule 10.2 *v2* (evidence grade C)

Agents tested	Agents affected	Rule	Exceptions, scientific basis and comments
Ampicillin or amoxicillin (and β-lactamase detection)	Ampicillin, amoxicillin, amoxicillin-c lavulanate, ampicillin-sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin and piperacillin-tazobactam.	IF BLNAR THEN report as R to ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, cefaclor, cefuroxime and cefuroxime axetil.	BLNAR isolates have reduced affinity of PBPs for β-lactams. Although piperacillin and piperacillin-tazobactam appear less affected by the PBP-mediated resistance mechanisms evidence regarding clinical efficacy is lacking.
BLNAR: β-lactamase negative but ampicillin resistant			

EUCAST expert rules *v2:* major modifications

β-lactams and *Haemophilus influenzae*

Rule 10.3 *v2* (evidence grade C)

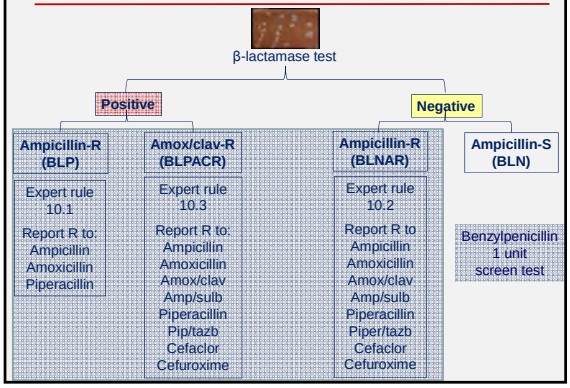
Agents tested	Agents affected	Rule	Exceptions, scientific basis and comments
Amoxicillin-clavulanate (and β-lactamase detection)	Ampicillin- sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin and piperacillin-tazobactam.	IF BLPACR THEN report as R to ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefaclor, piperacillin, piperacillin-tazobactam, cefuroxime and cefuroxime axetil.	BLPACR isolates produce β-lactamase and have reduced affinity of PBPs for β-lactams. Although piperacillin and piperacillin-tazobactam appear less affected by the PBP-mediated resistance mechanisms evidence regarding clinical efficacy is lacking.
BLPACR: β-lactamase positive and amoxicillin-clavulanate resistant			

EUCAST expert rules *v2:* major modifications

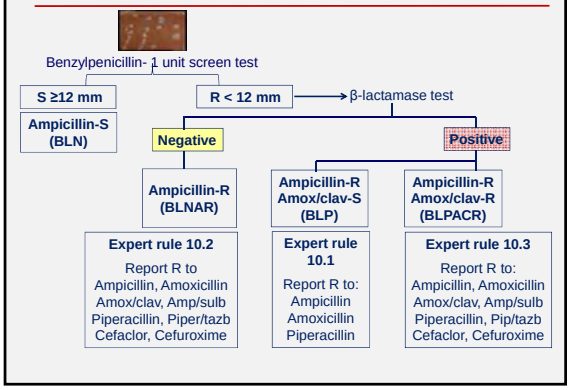
β-lactams and *Haemophilus influenzae:* practical issues

- Test for β-lactamase and report positive isolates R to penicillins without β-lactamase inhibitors
- Use ampicillin and amoxicillin breakpoints only to β-lactamase negative isolates
- Isolates may be R to penicillins, aminopenicillins and/or cephalosporins due to changes in PBPs (BLNAR)
- A few β-lactamase positive isolates may have also PBP changes (BLPACR)
- Isolates S to ampicillin and amoxicillin are also S to amoxicillin -clavulanate, piperacillin and piperacillin-tazobactam
- Isolates S to amoxicillin-clavulanate are also S to piperacillin tazobactam

EUCAST expert rules *v2:* major modifications



EUCAST expert rules *v2:* major modifications



EUCAST expert rules *v2*

Q3

EUCAST Expert Rules with Non-β-Lactams

Christian G. Giske, MD/PhD
Consultant physician / Associate professor
Karolinska University Hospital
and EUCAST Steering Committee
ECCMID 30 April 2012

Key topics

- Focus on interpretive rules for non-β-lactams (not exceptional and intrinsic resistance)
- Topics
  - →Fluoroquinolone resistance in Enterobacteriaceae
  - →Fluoroquinolone resistance in *H. influenzae*
  - →Fluoroquinolone resistance in *S. pneumoniae* and *S. aureus*
  - →MLS-resistance
- What is the future of expert rules?

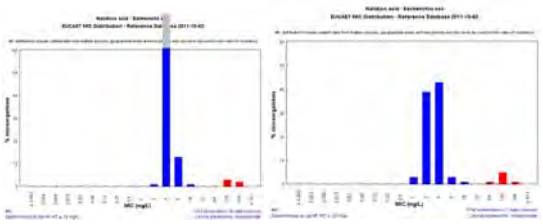
Fluoroquinolones and Enterobacteriaceae

The EUCAST expert rule

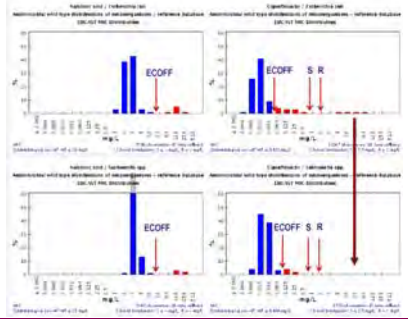
Class and drug	Rule	Rationale	Evidence grading
13:5 Enterobacteri-aceae and CIP	If resistant to CIP THEN report as resistant to all quinolones	Two target mutations (AAC(6')-Ib-cr may affect CIP but not LEV)	B
13:6 Salmonella AND CIP	If CIP MIC >0.06 mg/L THEN report as resistant to all quinolones	Evidence for clinical failure	A (S. Typhi) B non-Typhi

Q1

Nalidixic acid in Gram-negative bacteria



Epidemiological cut-off values

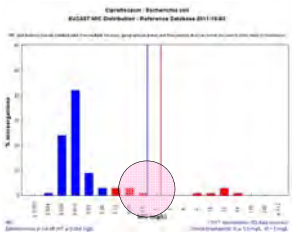


Nalidixic acid and chromosomal resistance

Mutations in			MIC (mg/L)		
gyrA	*parC*	Efflux	NAL	LEV	CIP
-	-	-	2	0.06	0.01
+	-	-	**32-256**	0.5	0.5
-	+	-	**64**	0.06	0.01
++	-	-	**>256**	**2**	**1**
+	-	+	**32-256**	**4**	**2**
++	+	-	**>256**	**32**	**64**

Nalidixic acid and ciprofloxacin vs plasmid-mediated quinolone resistance

Resistance mechanism	MIC (mg/L)	
	NAL	CIP
WT	2-16	0.004-0.064
QnrA	8-32	0.12-2
QnrB	16	0.25-1
QnrS	8-32	0.12-0.5
QnrD	4-8	0.12-0.25
QnrC	16	0.25
AAC(6')1b-CR	8-16	0.12-0.5
Qep	1-2	0.25
OfxcAB	64	0.12



Can nalidixic acid also fail in case of chromosomal mutations?

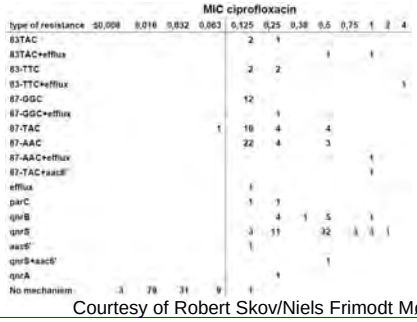
Table 2. Characteristics of the 11 *Salmonella enterica* serovar Typhi isolates belonging to subpopulation B, France, 2007–2009

Isolate	Year	Geographic origin	Antimicrobial drug resistance type	Disk diffusion, mm		MIC, µg/mL		gyrB	Haplotype	PFGE
				Nal	Cip	Nal	Cip			
97-5123	1997	Unknown	Cip[R]	18 [S]	28 [S]	8 [S/S]	0.125 [S/S]	Tyr464	Non-H58	X8
02-2759	2002	India	Cip[R]	18 [S]	26 [S]	4 [S/S]	0.125 [S/S]	Phe464	H58	X2
05-1578	2005	India	Pans susceptible	19 [I]	28 [S]	8 [S/S]	0.047 [S/S]	Asp466	Non-H58	X6
05-2556	2005	India	Cip[R]	17 [R]	31 [S]	16 [S/I]	0.19 [S/S]	Phe464	Non-H58	X7
05-9141	2005	India	Cip[R]	17 [R]	28 [S]	12 [S/I]	0.125 [S/S]	Tyr464	Non-H58	X3
06-426	2006	India	Cip[R]	20 [S]	25 [S]	8 [S/S]	0.125 [S/S]	Tyr464	Non-H58	X3
07-6086	2007	Tunisia	Pan susceptible	16 [I]	31 [S]	16 [S/I]	0.047 [S/S]	WT	ND	ND
08-7675†	2008	India	ASCSuTmpSXTCip[R]	18 [R]	28 [S]	8 [S/S]	0.125 [S/S]	Phe464	H58	X1
09-1986†	2008	India	ASCSuTmpSXTCip[R]	18 [R]	27 [S]	8 [S/S]	0.125 [S/S]	Phe464	ND	X1
09-0350	2009	Unknown	Cip[R]	18 [R]	27 [S]	8 [S/S]	0.125 [S/S]	Phe464	Non-H58	X5
09-2317	2009	French Guyana	Pansusceptible	19 [I]	32 [S]	8 [S/S]	0.032 [S/S]	Glu468	Non-H58	X4

*PFGE, pulsed-field gel electrophoresis; Nal, nalidixic acid; Cip, ciprofloxacin; ND, not determined; WT, wild type; A, ampicillin; S, streptomycin; C, chloramphenicol; Su, sulfamethoxazole; Tmp, trimethoprim; SXT, cotrimoxazole; Cip[R], decreased susceptibility to ciprofloxacin. Disk diffusion test was performed and interpreted ([S], susceptible; [I], intermediate) following recommendations of antibiogram committee of the French Society for Microbiology (CA-SFM); MICs were determined by Etest strips and categorization was made according to CA-SFM and Clinical and Laboratory Standards Institute.
†Previously described same patient (12).

Accaou-Demartin M. EID 2011

FQ-R Salmonellae vs MIC ciprofloxacin



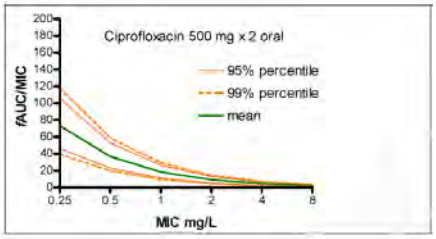
Courtesy of Robert Skov/Niels Frimodt Møller

Treatment failure in Salmonella related to CIP MIC

Parameter	Susceptibility to CIP		Significance
	CIP<0.12	CIP 0.12-1	
Antimicrobial-related fever clearance (h)	72	92	P=0.01
Ciprofloxacin-related fever clearance	64	90	p=0.153
Treatment failure	2/46	4/24	RR 2.5, 95% CI 1.2-5.1

Crump JA et al. AAC 2008; 52: 1278
Still no similar published data in *E. coli*

PK/PD in support of higher breakpoint than CIP 0.06 mg/L in non-Salmonellae (?)



www.eucast.org (rationale document ciprofloxacin)

Decision of EUCAST: ECOFF for *Salmonella* spp, PK/PD for other Enterobacteriaceae

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
Ciprofloxacin[1]	0.5	1	5	22	19	1 *Salmonella* spp. - there is clinical evidence for ciprofloxacin to indicate a poor response in systemic infections caused by *Salmonella spp.* with low-level fluoroquinolone resistance (MIC>0.064 mg/L). The available data relate mainly to *S. typhi* but there are also case reports of poor response with other *Salmonella* species.
Levofloxacin	1	2	5	22	19	
Moxifloxacin	0.5	1	5	20	17	
Nalidixic acid (screen)	NA	NA		NA	NA	

Fluoroquinolones and *H. influenzae*

H. influenzae and nalidixic acid

Class and drug	Rule	Rationale	Evidence grading
13:7 *H. influenzae* and NAL	If resistant to NAL THEN report determine MIC for the quinolone intended for clinical use	Target mutations can reliably be detected with NAL	C

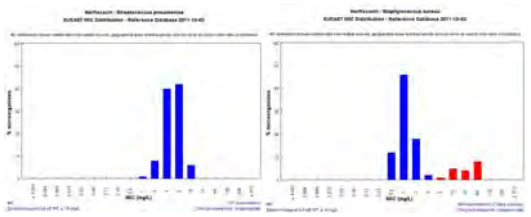
Fluoroquinolones and Gram-positives

Actually not expert rules….

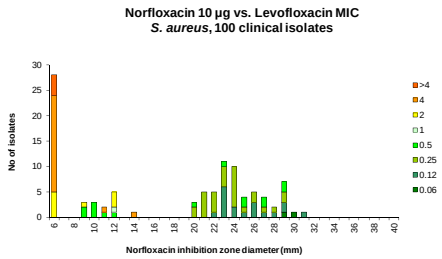
The norfloxacin disk diffusion test can be used to screen for fluoroquinolone resistance. Isolates categorised as susceptible can be reported susceptible to ciprofloxacin, levofloxacin, moxifloxacin and ofloxacin. Isolates categorised as resistant should be tested for susceptibility to individual agents.	**1/A.** The norfloxacin disk diffusion test can be used to screen for fluoroquinolone resistance. Isolates categorised as susceptible can be reported susceptible to levofloxacin and moxifloxacin and intermediate to ciprofloxacin and ofloxacin. Isolates categorised as resistant should be tested for susceptibility to individual agents.
Staphylococci	Pneumococci

Q2

Norfloxacin – a marker of low-grade FQ-resistance in Gram-positive bacteria

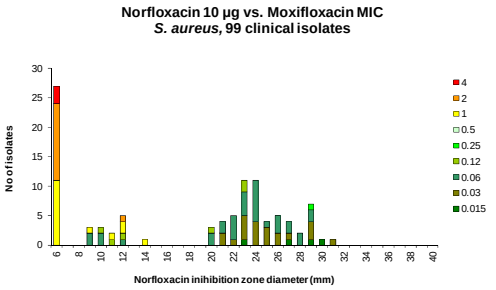


Norfloxacin disk screening



Courtesy of EUCAST, JMI Laboratories (US) and Statens Serum Institut (DK).

Norfloxacin disk screening



Courtesy of EUCAST, JMI Laboratories (US) and Statens Serum Institut (DK).

Norfloxacin and *S. pneumoniae*

Strains	Genotype	No. of strains	Mean zone diam[a] (mm) ± SD			
			NOR[b]	CIP	LVX	MXF
WT strains	Wild type	1,151	15 ± 3	24 ± 2	24 ± 2	32 ± 3
LLR mutants	*parC*/*parE*	46	**6 ± 0**	17 ± 3	20 ± 2	29 ± 3
	parC + efflux	3	**6 ± 0**	**8 ± 3**	17 ± 0	30 ± 1
	Efflux	14	**6 ± 0**	18 ± 2	22 ± 1	29 ± 3
	gyrA	16	15 ± 3	**21 ± 3**	21 ± 2	25 ± 3
HLR mutants	*parC* + *gyrA*	57	6 ± 0	6 ± 1	**7 ± 2**	17 ± 3
	parE + *gyrA*	7	6 ± 0	11 ± 4	**8 ± 2**	20 ± 2

Varon E et al. AAC 2006 Feb;50(2):572-9

The EUCAST expert rules (13.1-13.4)

- Apply for staphylococci and pneumococci
- If resistant to CIP or OFL: report warning for MOX and LEV (first step mutation)
- If resistant to MOX or LEV: report as resistant to all FQ

MLS-resistance in staphylococci

Q3

Rule 11.2

- IF resistant to erythromycin but susceptible to clindamycin, THEN test for inducible MLS_B resistance. IF negative, THEN report as susceptible to clindamycin. IF positive, THEN report as resistant to clindamycin or report as susceptible with a warning that clinical failure during treatment with clindamycin may occur
- Mutants with constitutive expression of Erm ribosomal methylases may selected during therapy (inducible resistance can be detected with D-test)
- Most data including recent animal studies (LaPlante KL et al. AAC 2008) indicate that clindamycin should be avoided in serious infections (class B evidence)

What is the future of the expert rules?

- Subcommittee has been closed following completion of task
- Intrinsic resistance: probably no major changes over time
- Exceptional resistance: probably needs revision
- Interpretive rules: probably also needed in the future although some class C rules might be removed in a new version
- Although breakpoints are mainly doing the job, interpretive support will most likely be needed