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

EUCAST
Frequently Asked Questions

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Objectives

- To summarise the EUCAST frequently asked questions section on the EUCAST website
- To discuss questions frequently asked by laboratories regarding EUCAST breakpoints and the EUCAST disk diffusion method
- To highlight significant changes in EUCAST breakpoints and the EUCAST disk diffusion method over the past year

EUCAST Frequently Asked Questions

- Several questions per day via telephone or e-mail
- Answers to difficult questions are often prepared with input from several EUCAST colleagues
- Each question is given a personal e-mail reply
- Common “Questions and Answers” are anonymised and added to the EUCAST website “FAQ”, which is updated regularly

Frequently Asked Questions (FAQ)

search term Search
Frequently Asked Questions (FAQ)

Organization
EUCAST News
Clinical breakpoints
Expert rules
Resistance mechanisms
MIC distributions ECOFFs
Zone distributions ECOFFs
AST of bacteria
AST of fungi
AST of veterinary pathogens
Frequently Asked Questions (FAQ)
Meetings
EUCAST Presentations
Documents
Translations
Information for industry
Links
Contacts

Website changes

Frequently Asked Questions (FAQ)

Updated March 23, 2015

Frequently Asked Questions - valid from 2015-03-23

The EUCAST secretariat receives many questions on subjects ranging from how we determine breakpoints, the MIC-distribution website, and the new disk diffusion methodology. We try to answer each question individually but also publish frequently asked questions and answers in a classical FAQ document. The file is updated at regular intervals.

Previous version of FAQ:

Frequently Asked Questions - valid 2014-02-26 - 2015-03-23

Frequently Asked Questions - valid 2013-04-24 - 2014-02-26

Sub-headings in the FAQ document

- EUCAST Disk Diffusion Test
  - Medium
  - Disks
  - Inoculum preparation
  - Reading zones of inhibition
  - General methodology
- Breakpoints –general
- Breakpoints –zone diameter
- Quality Control
- Other questions

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Questions? Please contact
erika.matuschek@escmid.org

EUCAST Frequently Asked Questions

Click on question to read answer

1. EUCAST disk diffusion test - Medium

1 Which manufacturer of Mueller-Hinton agar does EUCAST recommend?

▲ Back to top of page

EUCAST does not recommend a particular manufacturer of Mueller-Hinton agar. We have tested batches of Mueller-Hinton agar from four manufacturers (BBL, Oxoid, bioMérieux and Bio-Rad) repeatedly and have evaluated other media occasionally. We have also tested batches of pre-poured commercial MH-F (Mueller-Hinton Fastidious organisms; which is Mueller-Hinton agar with 5% horse blood and 20 mg/L β-NAD) from the manufacturers mentioned above. Irrespective of the manufacturer used, each user should ensure that batches of media meet the internal quality control ranges published by EUCAST. These ranges have been checked with media from several manufacturers. For users of pre-poured commercial plates, it should be noted that the plate manufacturer not necessarily is the same as the Mueller-Hinton powder manufacturer.

2 What is the difference between Mueller-Hinton agar and Mueller-Hinton II agar?

▲

The original specification of Mueller-Hinton agar did not define cation content, which is known to affect the activity of several agents, particularly aminoglycosides. Furthermore, the content of thymidine, which affects trimethoprim and trimethoprim-sulfamethoxazole activity, was undefined. Mueller-Hinton II agar is manufactured to contain a low concentration of thymidine and controlled concentrations of calcium and magnesium ions. Today, all Mueller-Hinton agars for susceptibility testing should be produced to meet the current CLSI performance standard (soon to be superseded by an ISO standard). Therefore, all Mueller-Hinton agars that yield inhibition zones within the acceptable ranges for EUCAST internal quality control strains can be used and EUCAST does not distinguish between MH and MH II.

3 Do we need to quality control each new batch of Mueller-Hinton agar?

▲

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EUCAST Frequently Asked Questions

DB

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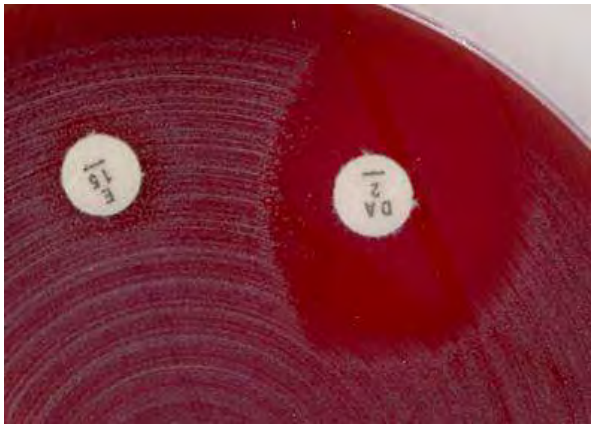
Frequently Asked Questions

How should we report inducible clindamycin resistance in staphylococci and streptococci?

DB

Inducible clindamycin resistance in staphylococci and streptococci

- Most resistance to macrolide, lincosamide and streptogramin type B (MLS_B) antimicrobial agents is mediated by the *erm* genes and is induced by erythromycin, clarithromycin and azithromycin, but not by clindamycin (dissociated resistance or MLS_B inducible resistance).



- Hence inducible strains are resistant to erythromycin but not to clindamycin in antimicrobial susceptibility tests.

Inducible clindamycin resistance in staphylococci and streptococci

- Inducible strains segregate clindamycin resistant mutants, which may be selected during treatment, possibly leading to treatment failure
- Should staphylococci and streptococci with inducible clindamycin resistance be reported resistant or susceptible?
- Guidance has been inconsistent leading to variation in reporting
  - S with note that should not be used for serious infections?
  - R with note that may be used for less serious infections?

Evidence for significance of inducible clindamycin resistance

S. aureus

- Mutant selected in vitro
- Mouse thigh model with high inoculum indicates bacteriostatic activity at 24h and some regrowth at 72h
- Several reports of clinical failures in serious infections

Streptococci

- Mutants selected in vitro
- Mouse thigh model indicating initial killing but then bacteriostatic and some regrowth after 72h.
- One report of 8 clinical failures

Current recommendations for reporting inducible clindamycin resistance

- EUCAST v 5.0 (2015)

  If MLSBi detected, then report as resistant. Consider adding this comment to the report:

  "Clindamycin may still be used for short-term therapy of less serious skin and soft tissue infections as constitutive resistance is unlikely to develop during such therapy".

- Recently agreed to add comment for streptococci

  "The clinical importance of inducible clindamycin resistance in combination treatment of severe *S. pyogenes* infections is not known".

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Frequently Asked Questions

How should we control tests on β-lactam-β-lactamase inhibitor combinations?

DB

Control of β-lactam-β-lactamase inhibitor combinations

- β-lactamase inhibitors, particularly clavulanic acid, are not stable
- Quality control of inhibitor content of combined disks and inhibitors in MIC tests on inhibitor combinations should include a control for activity of the inhibitor

……obvious but rarely done

Control of β-lactam-β-lactamase inhibitor combinations

- The inhibitor component should be controlled with *E. coli* ATCC 35218 (TEM-1 β-lactamase-producing strain)
  - Should be part of routine QC

- *E. coli* ATCC 25922 is used to control the active component

Control of β-lactam-β-lactamase inhibitor combinations

EUCAST QC table v 5.0

Escherichia coli ATCC 35218

(NCTC 11954, CIP 102181, DSM 5923, CCUG 30600, CECT 943)

TEM-1 β-lactamase-producing strain (non-ESBL) used to check the inhibitor component of penicillin inhibitpr combination disks.

Disk diffusion methodology: Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target[1]	Range[2]		Target[1]	Range[2]
Amoxicillin-clavulanic acid[3]	***8-16***	***4-32***	20-10	20	17-22[4]
Ampicillin-sulbactam[5]	***32-64***	***16-128***	10-10	16	13-19[4]
Piperacillin-tazobactam[6]	1	0.5-2	30-6	***24***	***21-27***
Ticarcillin-clavulanic acid[3]	16	8-32	75-10	23	21-25

[3] The concentration of clavulanic acid is fixed at 2 mg/L.

[5] The concentration of sulbactam is fixed at 4 mg/L.

[6] The concentration of tazobactam is fixed at 4 mg/L.

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Frequently Asked Questions

We sometimes get susceptibility test results for *H. influenzae* that are susceptible for ampicillin but resistant to amoxicillin-clavulanic acid. How should we report these isolates?

EM

H. influenzae susceptible to ampicillin, resistant to amoxicillin-clavulanic acid

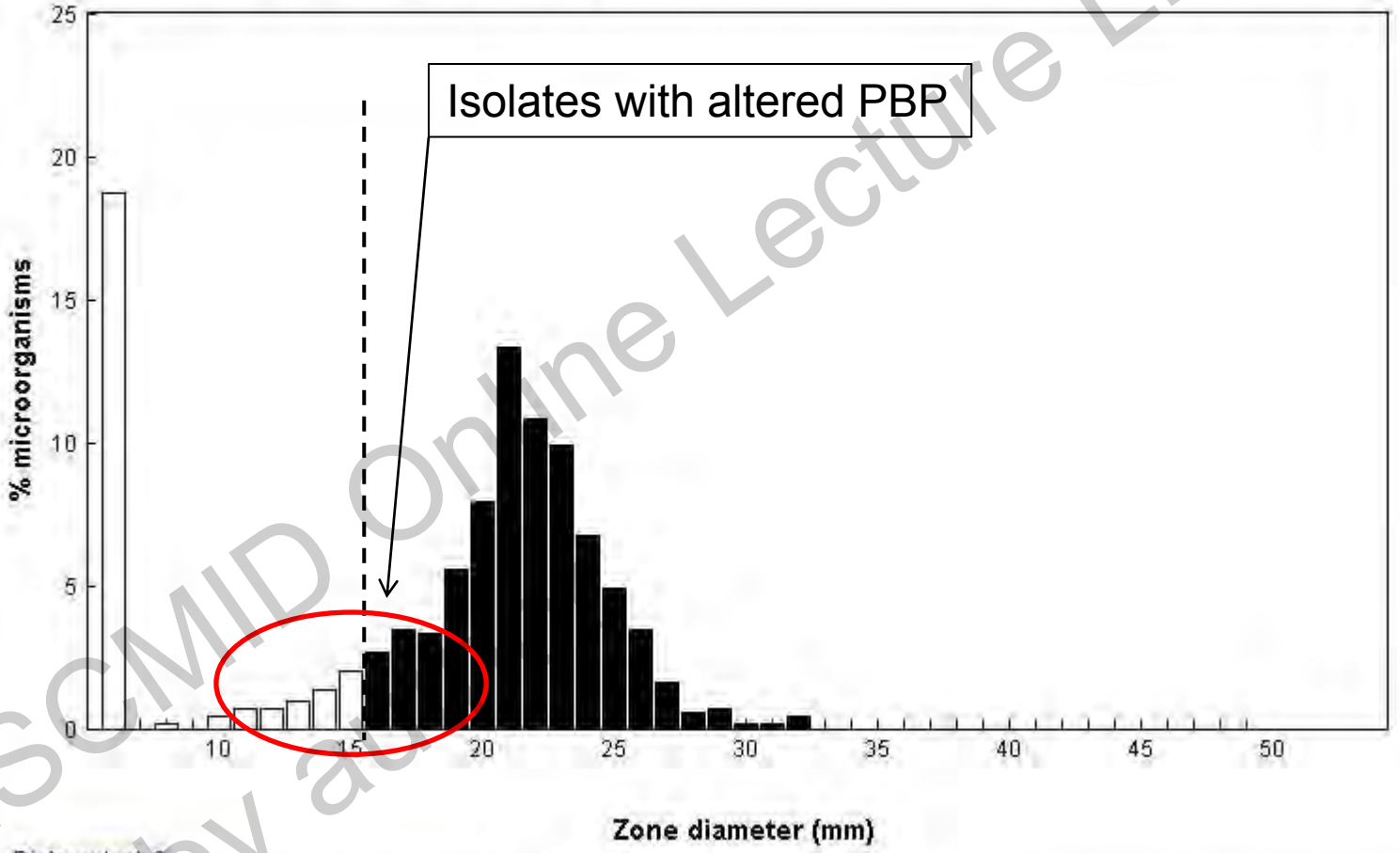
- Possible for β-lactamase negative isolates with borderline susceptibility due to altered PBPs
- Ampicillin-susceptible isolates should be reported susceptible to ampicillin, amoxicillin and amoxicillin-clavulanic acid
- Aminopenicillin susceptibility tests on *H. influenzae* with altered PBPs are difficult, and the zone diameter breakpoints will be reviewed further during 2015

Ampicillin / Haemophilus influenzae

International wild type zone diameter distribution - Reference database 2015-04-20

EUCAST disk diffusion method

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

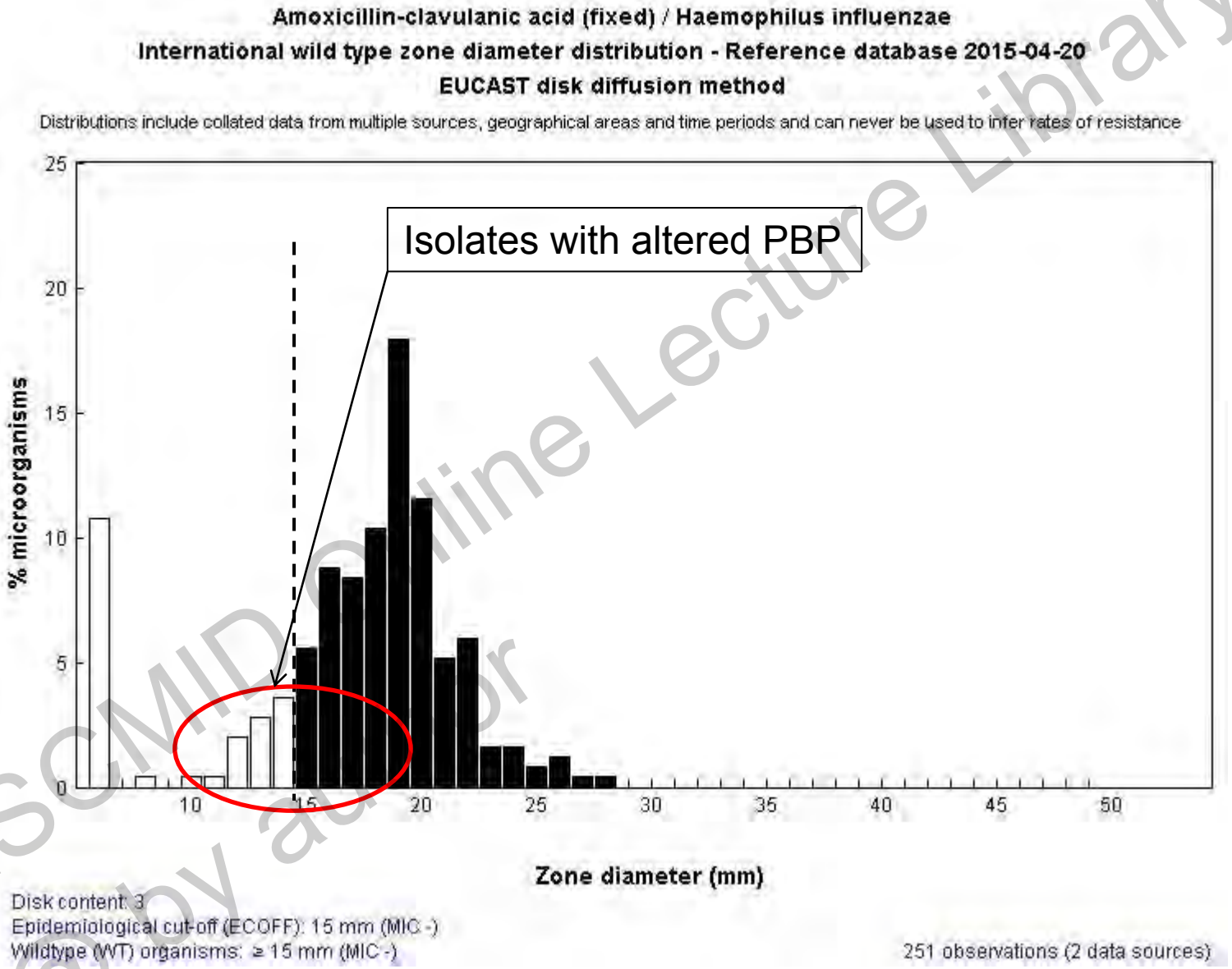


Disk content: 2

Epidemiological cut-off (ECOFF): 16 mm (MIC = 1 mg/L)

Wildtype (WT) organisms: ≥ 16 mm (MIC = 1 mg/L)

759 observations (3 data sources)



H. influenzae susceptible to ampicillin, resistant to amoxicillin-clavulanic acid

- Analyse QC data
  - Mean values from repeated measurements should optimally be on target ± 1 mm

- Don't test / interpret both ampicillin and amoxicillin-clavulanic acid
  - β-lactamase negative isolates: Report ampicillin
  - β-lactamase positive isolates: Report amoxicillin-clavulanic acid

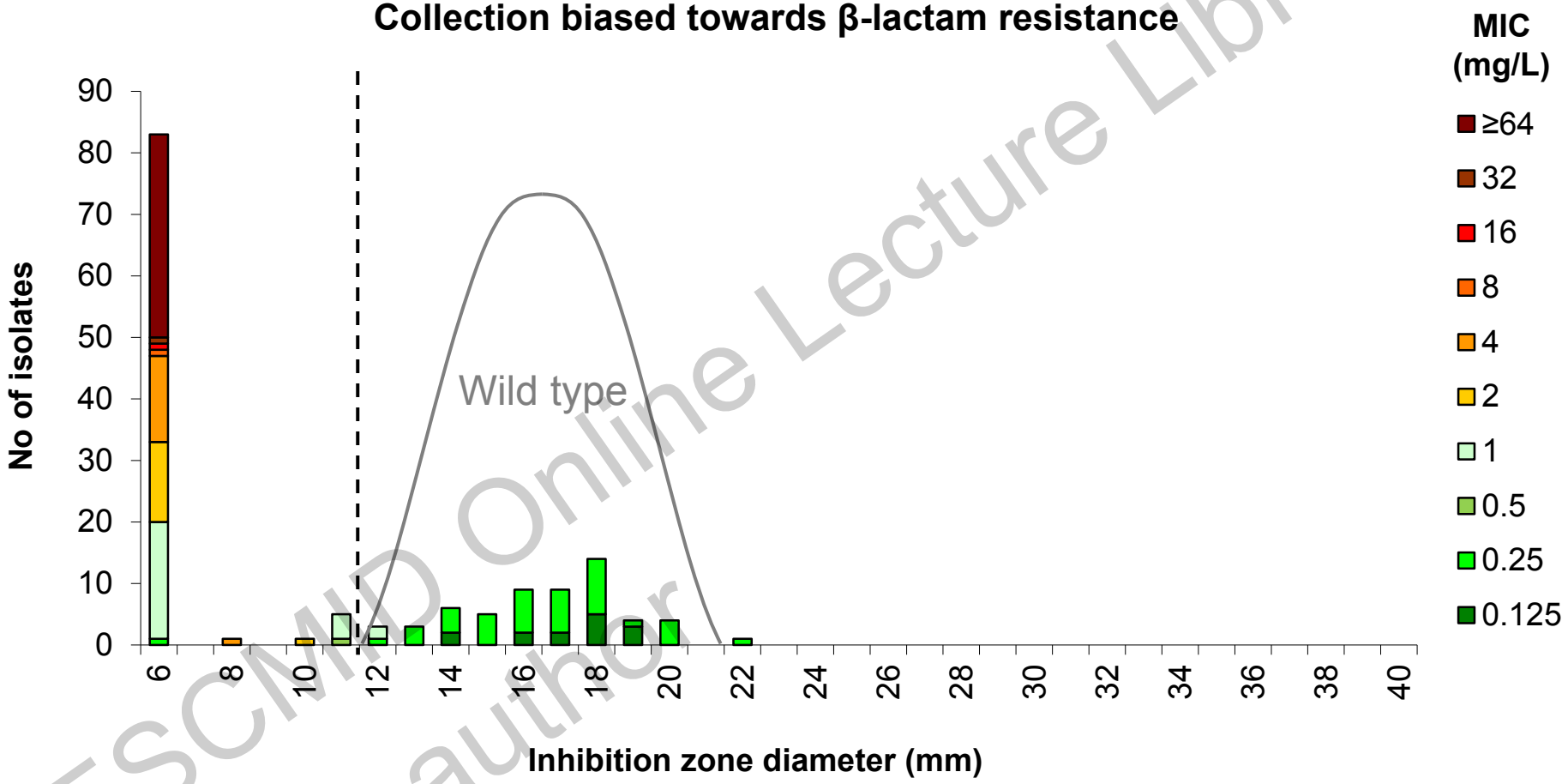
- Use the benzylpenicillin 1unit screening test

H. influenzae
screen for β-lactam resistance

Benzylpenicillin 1 unit	β-lactamase	Further testing and/or interpretation
≥ 12 mm	Do not test	Report S to all β-lactam agents with clinical breakpoints
< 12 mm	Negative	Test susceptibility to individual agents
	Positive	Ampicillin, amoxicillin and piperacillin: Report R
		Other agents: Test susceptibility

Benzylpenicillin 1 unit vs. Ampicillin MIC
H. influenzae, 148 clinical isolates

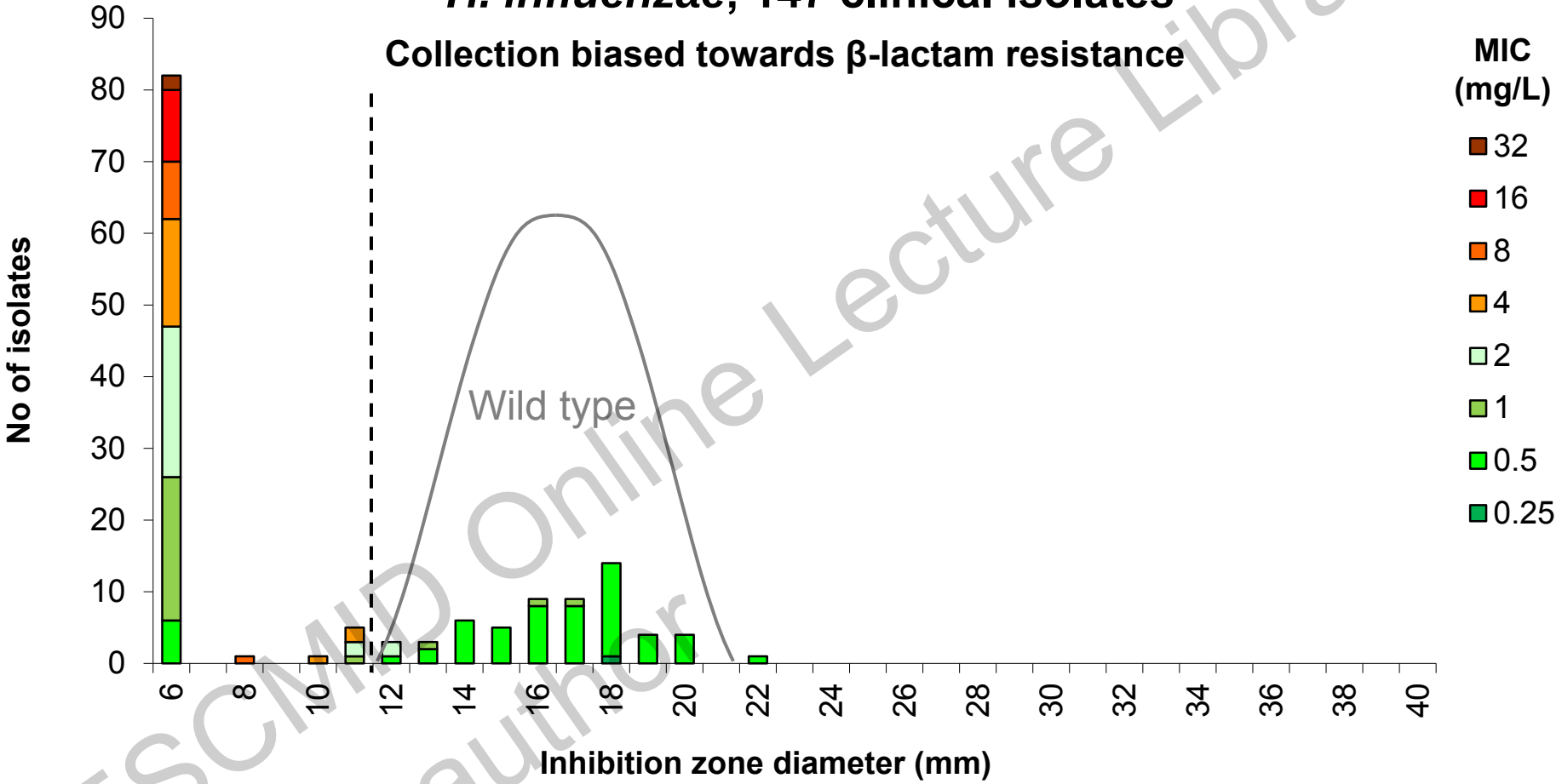
Collection biased towards β-lactam resistance



Breakpoints		**ECOFF**
Ampicillin MIC	S≤1, R>1 mg/L	1 mg/L
Benzylpenicillin zone diameter (screen)	S≥12 mm	

Benzypenicillin 1 unit vs. Amoxicillin-clavulanic acid MIC

H. influenzae, 147 clinical isolates



Breakpoints		**ECOFF**
Amoxicillin-clavulanic acid MIC	S≤2, R>2 mg/L	2 mg/L
Benzylpenicillin zone diameter (screen)	S≥12 mm	

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Frequently Asked Questions

Why has EUCAST changed the *H. influenzae* QC strain?

EM

New *H. influenzae* QC strain

- *H. influenzae* NCTC 8468
  - Growth characteristics different from most clinical isolates (MH-F plates and MH-F broth)
  - Difficult to access in some countries

- *H. influenzae* ATCC 49766
  - Growth characteristics similar to most clinical isolates (MH-F plates and MH-F broth)
  - Easier to access
  - Already widely used (in CLSI method)

EUCAST current recommendations

EUCAST QC Tables v 5.0, 2015

- *H. influenzae* ATCC 49766
  - MIC ranges for all agents
  - Zone diameter ranges for most agents

- *H. influenzae* NCTC 8468
  - No MIC ranges
  - Zone diameter ranges for all agents
  - Will be excluded from 2016

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Frequently Asked Questions

When will EUCAST publish a disk diffusion method for anaerobes?

DB

EUCAST method for anaerobes

- Should be possible for rapidly growing anaerobes with some agents

- Major issues with medium
  - Growth inadequate on MH-F
    - Addition of hemin and vitamin K did not improve growth
  - Brucella Blood Agar results variable depending on the source and supplements
  - Alternative media are being evaluated

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Frequently Asked Questions

When will EUCAST publish a disk diffusion method for *N. gonorrhoeae*?

DB

EUCAST disk diffusion for *N. gonorrhoeae?*

- Agar dilution by the CLSI method recognised by reference labs as the gold standard.
- The Etest is simpler than agar dilution and in general results are similar to agar dilution.
- Disk diffusion generally does not work well although CLSI, BSAC and CDS describe methods. Poor growth of some strains and too high disk contents may be problems.
- A study of disk diffusion methods has been proposed.
  - 100-200 isolates with different genotypes and phenotypes
  - A range of media will be examined as good growth is required
  - Growth inadequate on MH-F and a range of supplements is being investigated

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Frequently Asked Questions

There are no breakpoints for several microorganisms – does EUCAST have a plan for these?

EM

”Missing” EUCAST breakpoints

- Several organisms on the list
  - *Aerococcus* spp. — 2015
  - *Kingella kingae* — 2015
  - *Nocardia* spp. — 2016
  - *Streptomyces* spp. — 2016
  - *Aeromonas* spp. — 2016
  - *Vibrio* spp. — 2016
  - *Leuconostoc* spp. — 2016
  - *Lactobacillus* spp. — 2016
  - *Pediococcus* spp. — 2016

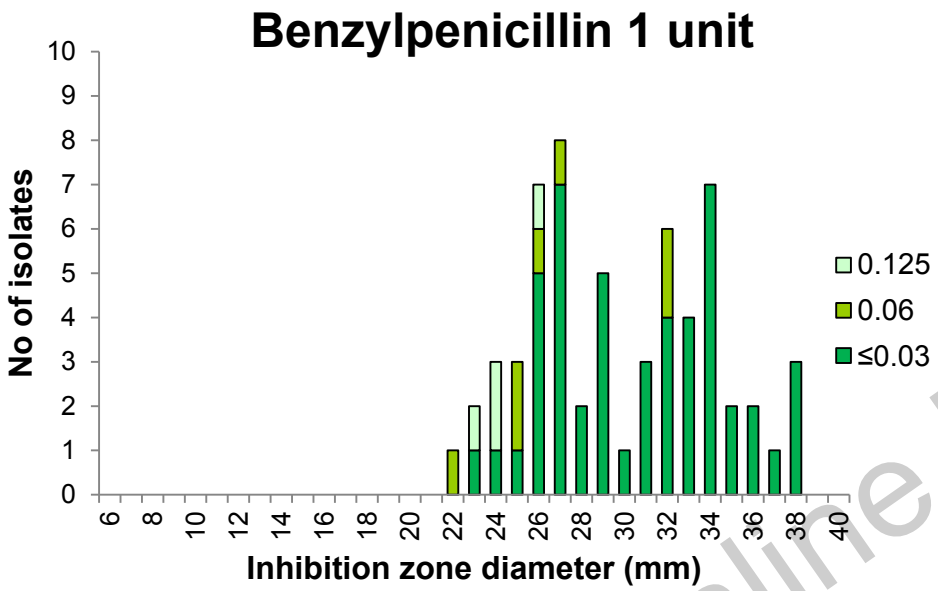
Aerococcus spp. and *Kingella kingae*

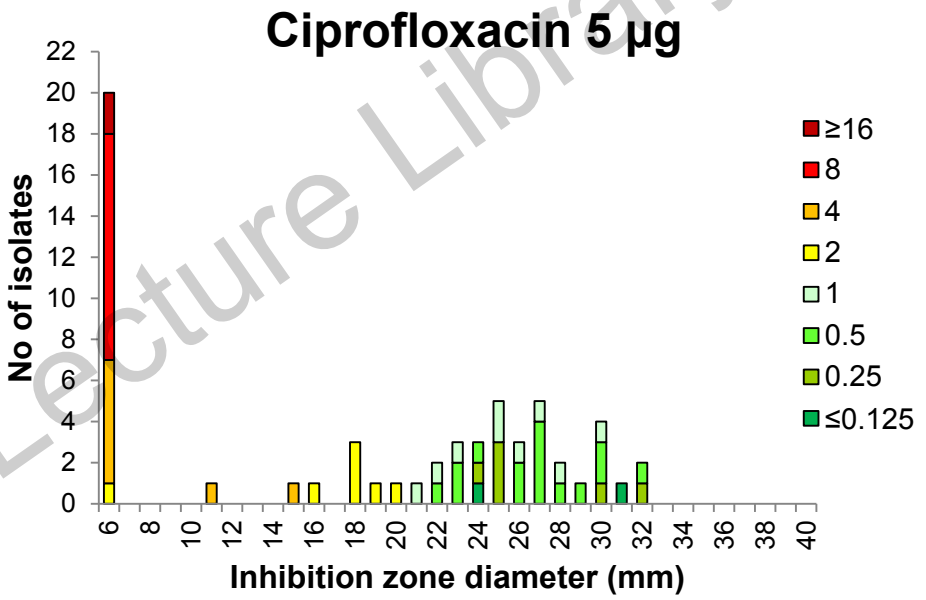
- Clinical breakpoints are under discussion in EUCAST
- Corresponding zone diameter breakpoints will be published
- Preliminary work suggests that AST of *A. urinae*, *A. sanguinicola* and *Kingella kingae* on EUCAST media will not be a problem

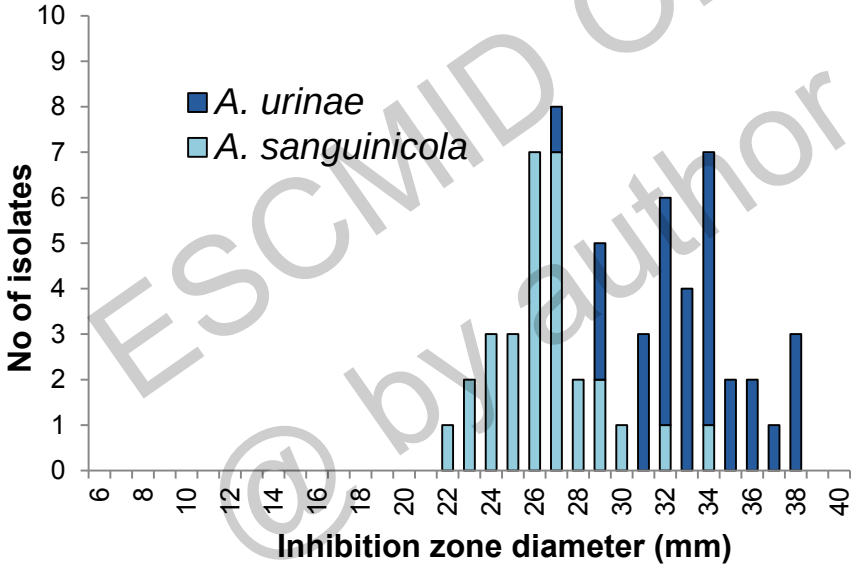
Aerococcus spp. and *Kingella kingae* methods

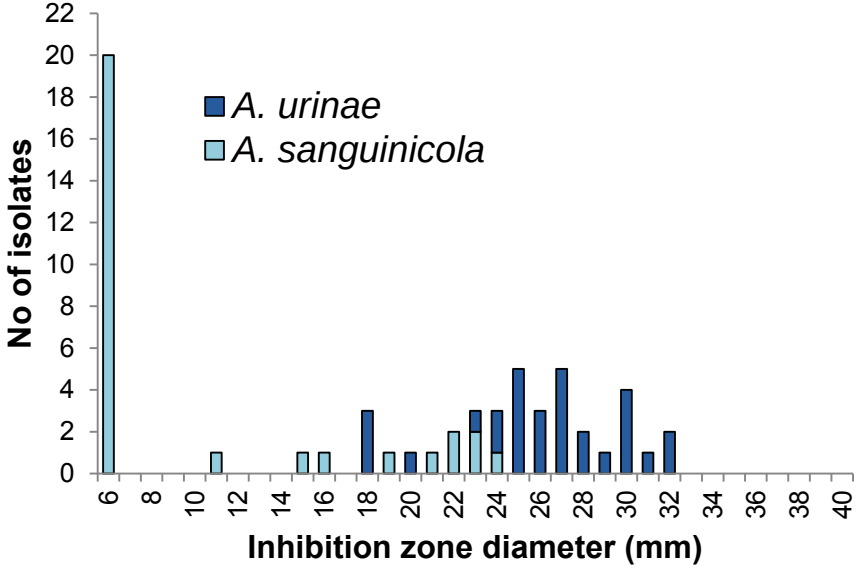
- EUCAST disk diffusion method for fastidious organisms
  - MH-F agar
  - McFarland 0.5
  - 5% CO_2
  - 16-20 h incubation
    - with possibility to prolong incubation to 40-44 h if growth is non-sufficient
- Calibration vs. broth microdilution
  - MH-F broth

A. urinae and *A. sanguinicola*

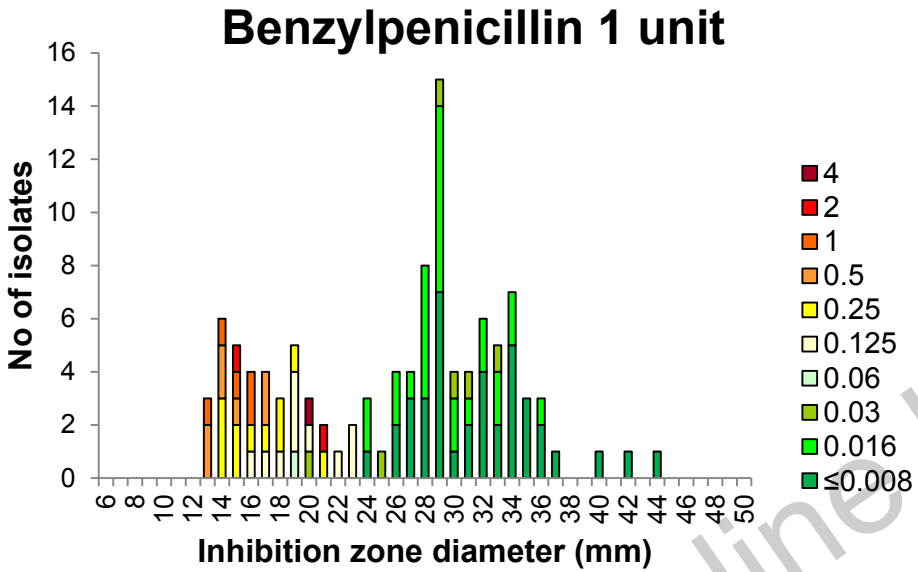


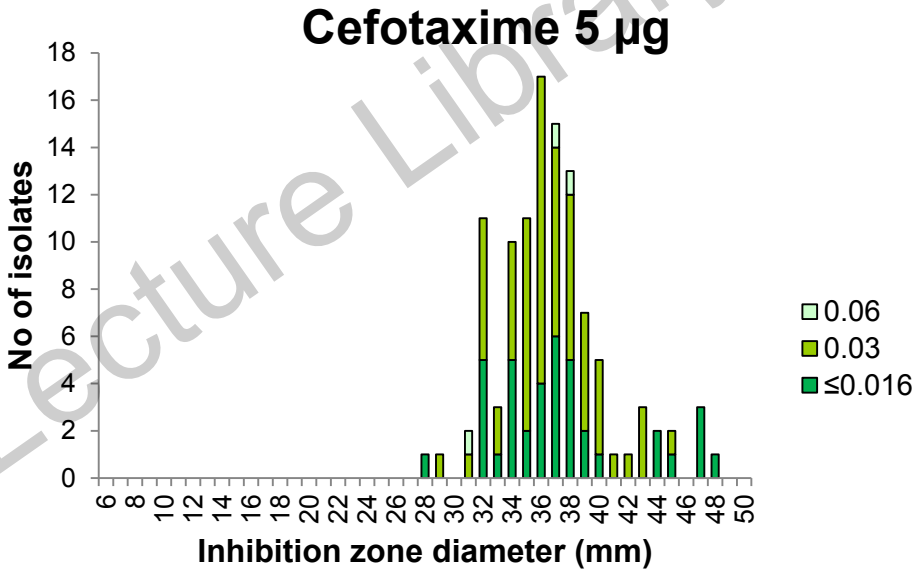


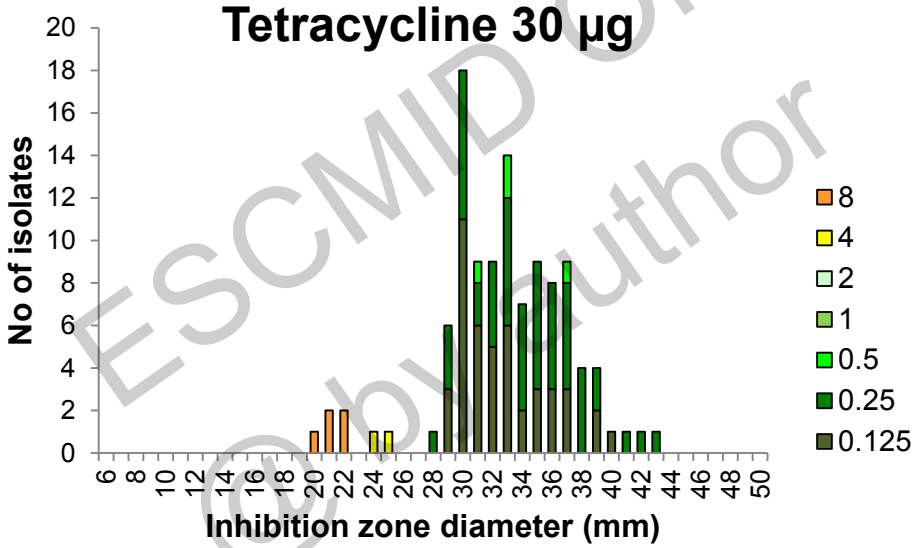


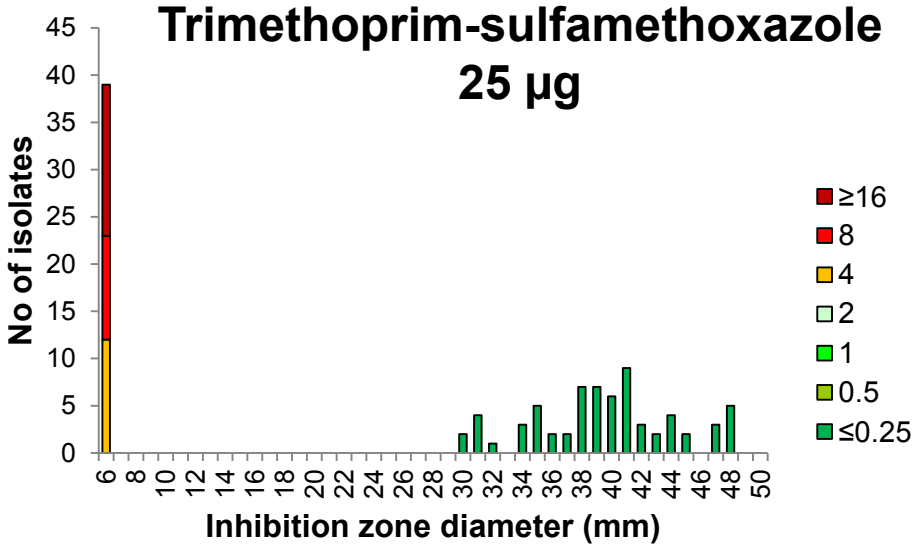


Kingella kingae









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Frequently Asked Questions

Can you clarify the expert rules on aminoglycosides and *Serratia marcescens*?

DB

Expert rules on aminoglycosides and *Serratia marcescens*

- Rule 1.14 Intrinsic resistance table

Rule no.	Organisms											Colistin	Nitrofurantoin
1.1	Citro												
1.2	Citro												
1.3	Ente												
1.4	Ente												
1.5	Esc												
1.6	Hafn												
1.7	Kleb												
1.8	Mor											R	R
1.9	Prot											R	R
1.10	Proteus vulgaris	R				R		R	R		R	R	R
1.11	Proteus penneri	R				R		R	R		R	R	R
1.12	Providencia rettgeri	R	R			R					R	R	R
1.13	Providencia stuartii	R	R			R				Note[2]	R	R	R
1.14	Serratia marcescens	R	R			R		R	R	Note[3]		R	R
1.15	Yersinia enterocolitica	R	R	R		R	R	R					
1.16	Yersinia pseudotuberculosis											R	

Note 3: All *Serratia marcescens* produce a chromosomal AAC(6')-Ic enzyme that affects the activity of all aminoglycosides except streptomycin and gentamicin.

Expert rules on aminoglycosides and *Serratia marcescens*

- Variation in amount of enzyme and activity against particular aminoglycosides so phenotypic susceptibility may vary from one isolate to another
- No evidence that clinically significant when MIC is in the susceptible category
- Rule 1.14 **does not** recommend reporting all isolates as resistant to all aminoglycosides except streptomycin and gentamicin

Expert rules on aminoglycosides and *Serratia marcescens*

- UKNEQAS results for *Serratia marcescens* 2433
  - Gentamicin MIC 1 mg/L (Susceptible)
  - Tobramycin MIC 1-2 mg/L (Susceptible)
  - Amikacin MIC 2-8 mg/L (Susceptible)

Participants	N	Percent reporting		
		S	I	R
All	509	85.5	0.8	13.7
CLSI	86	97.6	1.2	1.2
EUCAST automated	240	77.1	0.4	22.5
EUCAST non-automated	183	90.7	1.1	8.2

Expert rules on aminoglycosides and Enterobacteriaceae

- Rule 12.7

Rule no.	Organisms	Agent tested	Agents affected	Rule	Exceptions, scientific basis and comments	Evidence grade
12.7	All Enterobacteriaceae Pseudomonas aeruginosa,	Tobramycin, gentamicin, amikacin	Amikacin	IF intermediate or resistant to tobramycin and susceptible to gentamicin and amikacin, THEN report amikacin as intermediate for	Production of acquired AAC(6')I may not confer phenotypic resistance despite modification of amikacin.	C

IF intermediate or resistant to tobramycin and susceptible to gentamicin and amikacin, THEN report amikacin as intermediate for Enterobacteriaceae or resistant for *Pseudomonas* spp. and *Acinetobacter* spp.

Production of acquired AAC(6')I may not confer phenotypic resistance despite modification of amikacin.

Expert rules on aminoglycosides and *Serratia marcescens*

- Same enzyme as rule 1.14 but **does** recommend reporting all isolates appearing amikacin susceptible as intermediate to amikacin when intermediate or resistant tobramycin and susceptible to gentamicin
- Same caveats apply….
  - Variation in amount of enzyme and activity against particular aminoglycosides so phenotypic susceptibility may vary from one isolate to another
  - No evidence that clinically significant when MIC is in the susceptible category
- Rules 1.14 and 12.7 are under review

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Frequently Asked Questions

Reading zone edges is difficult for some tests. How can we ensure that the are read correctly and consistently?

EM

How should zone edges be read in disk diffusion tests?

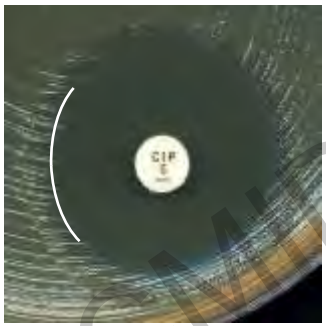
- MH plates
  Read zones from the back of the plate against a dark background and illuminated with reflected light.

- MH-F plates
  Read zones from the front of the plate with the lid removed and illuminated with reflected light.

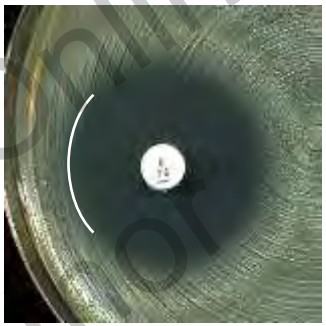
How should zone edges be read in disk diffusion tests?

Read zone edges at the point where no obvious growth is detected by the unaided eye with the plate held about 30 cm from the eye.

Examples:



E. coli
Ciprofloxacin



S. aureus
Erythromycin

CoNS
Trimethoprim

S. pneumoniae
Rifampicin

Reading guide available at www.eucast.org

How should zone edges be read in disk diffusion tests?

Important exceptions:

- *S. maltophilia* and trimethoprim-sulfamethoxazole
  - Ignore growth within the zone and read an inhibition zone if any zone edge can be seen

- Enterococci and vancomycin
  - Fuzzy zone edges indicate resistance

- *S. aureus* and benzylpenicillin
  - Sharp zone edges indicate resistance

Pictures in Reading Guide and Breakpoint Table

Harmonisation of reading of zones

- Let all laboratory staff read zones from the same plate

- Choose two strains with four disks respectively for each occasion
  - Repeat reading for the same organism twice
  - Compare results and discuss with all staff
  - Repeat the exercise with the same organisms until everyone gets the same results (mean ± 1 mm)

Reading exercise in Växjö

Staphylococcus aureus, clinical isolate
1st reading occasion

Cefoxitin	Erythromycin	Clindamycin	Fusidic acid	Norfloxacin	Rifampicin
26	27	26	29	20	30
26	28	28	29	22	31
26	29	28	29	25	31
29	28	28	30	23	31
26	27	27	28	19	30
26	28	29	30	23	32
26	29	29	30	21	31
26	27	27	29	23	29
25	27	26	29	20	32
25	25	26	28	25	32
28	27	26	27	22	30
Mean	**26**	**27**	**27**	**29**	**22**	**31**
SD	*1.2*	*1.1*	*1.2*	*0.9*	*2.0*	*1.0*

> 1 mm above mean
> 1 mm below mean

Reading exercise in Växjö

Staphylococcus aureus, clinical isolate
After analysis and discussion

Cefoxitin	Erythromycin	Clindamycin	Fusidic acid	Norfloxacin	Rifampicin
27	24	25	26	18	28
27	25	26	28	18	29
27	25	26	27	17	27
27	25	26	27	16	27
27	25	26	27	19	28
28	24	24	26	17	25
27	26	27	29	19	29
27	24	26	28	18	28
27	25	23	26	18	27
28	25	26	27	18	30
27	24	24	25	17	27
Mean	**27**	**25**	**25**	**27**	**18**	**28**
SD	*0.4*	*0.6*	*1.2*	*1.1*	*0.9*	*1.3*

> 1 mm above mean

> 1 mm below mean

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Frequently Asked Questions

Why are there no breakpoints for topical use of agents?

DB

Issues with breakpoints for topical agents

- What concentrations are relevant?
- For most agents there are no sound pharmacological data. Frequently assumed that topical concentrations are high at the infection site, but:
  - not known what the concentrations are
  - not known how long concentrations are maintained
  - Not known what variation there is in practice
- Multiple formulations, mixtures, administration schedules
- For most agents there are no data relating treatment to outcome other than anecdotal comment.

EUCAST breakpoints for topical agents Approach 1

Use systemic breakpoints when agent also available for systemic use. Use epidemiological cut-off values (ECOFFs) when systemic breakpoints are not available.

Problems:

- Systemic breakpoints often inconsistent in relation to ECOFFs, some being several dilutions above or below ECOFF.
- No clinical breakpoints for some agent-organism combinations where the agents are used both systemically and topically but are not recommended for systemic use against particular organisms which would be of relevance for topical use (e.g. ofloxacin with *P. aeruginosa*; ciprofloxacin, ofloxacin and fusidic acid with β-haemolytic streptococci).

EUCAST breakpoints for topical agents Approach 2

Use only epidemiological cut-off values (ECOFFs).

Would categorise isolates as wild type or non-wild type and would demonstrate the presence of phenotypically detectable resistance mechanisms, which may result in a higher probability of clinical failure.

Problems:

- Acceptable distributions are not available to establish ECOFFs for all topical agents
- If tissue is involved use of systemic treatment and systemic breakpoints should be considered
- For agents also used systemically it might be confusing to report ECOFF-based “topical” breakpoints which were only slightly different from systemic breakpoints.

EUCAST guidance note on breakpoints for topical agents

Organisms	ECOFF (mg/L) Systemic clinical breakpoint (S≤/R>, mg/L)	Gentamicin[1]	Ciprofloxacin[1]	Levofloxacin[1]	Ofloxacin[1]	Chloramphenicol[1]	Colistin[1] (for Polymyxin B)	Fusidic acid[1]	Neomycin (framycetin)	Bacitracin	Mupirocin	Retapamulin
Enterobacteriaceae	ECOFF	2	0.12	0.25	0.5	16	2	-	8	-	-	-
	Systemic clinical breakpoint	2/4	0.5/1	1/2	0.5/1	8/8	2/2	-	-	-	-	-
Acinetobacter spp.	ECOFF	4	1	0.5	1	-	2	-	ND	-	-	-
	Systemic clinical breakpoint	4/4	1/1	1/2	-	-	2/2	-	-	-	-	-
P. aeruginosa	ECOFF	8	0.5	2	2	-	4	-	ND	-	-	-
	Systemic clinical breakpoint	4/4	0.5/1	1/2	-	-	4/4	-	-	-	-	-
S. aureus	ECOFF	2	1	1	1	16	-	0.5	1	ND	1/1[2]	0.5
	Systemic clinical breakpoint	1/1	1/1	1/2	1/1	8/8	-	1/1	-	-	-	-
S. pneumoniae	ECOFF	-	2	2	4	8	-	32	ND	ND	-	-
	Systemic clinical breakpoint	-	0.12/2	2/2	0.12/4	8/8	-	-	-	-	-	-
β-haemolytic streptococci	ECOFF	-	2	2	4	8	-	32	ND	ND	0.5	0.12
	Systemic clinical breakpoint	-	-	1/2	-	8/8	-	IE	-	-	-	-
H. influenzae	ECOFF	4	0.06	0.06	0.12	1	-	ND	ND	-	-	-
	Systemic clinical breakpoint	IE	0.5/0.5	1/1	0.5/0.5	2/2	-	-	-	-	-	-
Moraxella spp.	ECOFF	0.25	0.12	0.12	0.25	2	-	ND	ND	-	-	-
	Systemic clinical breakpoint	IE	0.5/0.5	1/1	0.5/0.5	2/2	-	-	-	-	-	-

- = inappropriate combination; IE = insufficient evidence to set a clinical breakpoint; ND = No ECOFF defined on EUCAST MIC distribution website

[1]Agents also available for systemic use

[2]Breakpoints for nasal decontamination S≤1, R>256 mg/l.

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Summary of significant changes in EUCAST breakpoints and disk diffusion methodology 2014-2015

EM

New and/or revised breakpoints

EUCAST Breakpoint Table v 5.0, 2015

- Ceftobiprole MIC breakpoints
- Telavancin MIC breakpoints
  - Adjusted for tests with polysorbate-80
- Delamanid and bedaquiline (*M. tuberculosis*)
- Dalbavancin MIC breakpoints
- Oritavancin MIC breakpoints
- Tedizolid MIC breakpoints

Addendum April 2015

New recommendations

- Information on testing conditions added for telavancin, tigecycline, daptomycin and fosfomycin

- Reporting of inducible clindamycin resistance in streptococci

On-going work

- Method and breakpoints for *Aerococcus* spp. and *Kingella kingae*
- Zone diameter breakpoints for fosfomycin
- Breakpoints for temocillin and nitroxoline
- Review of breakpoints for carbapenems and fluoroquinolones
- Breakpoints for new agents
- Breakpoints for early reading (6-8 h) of disk diffusion tests

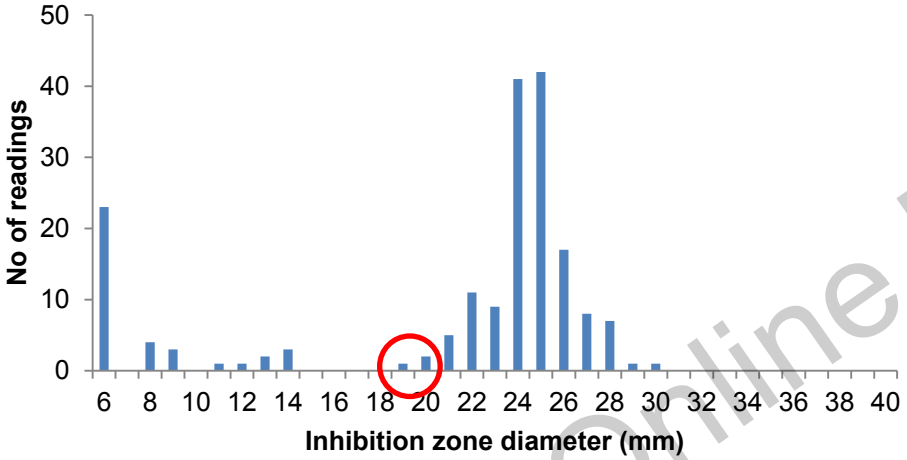
Early reading of disk diffusion test results

- Any method deviating from the standardised method must be calibrated vs the standardised method
- Be aware of that some resistance mechanisms might not be expressed during a shorter incubation time
- EUCAST is planning to publish zone diameter breakpoints for 6-8 h reading of disk diffusion tests for important organism-agent combinations at the end of 2015

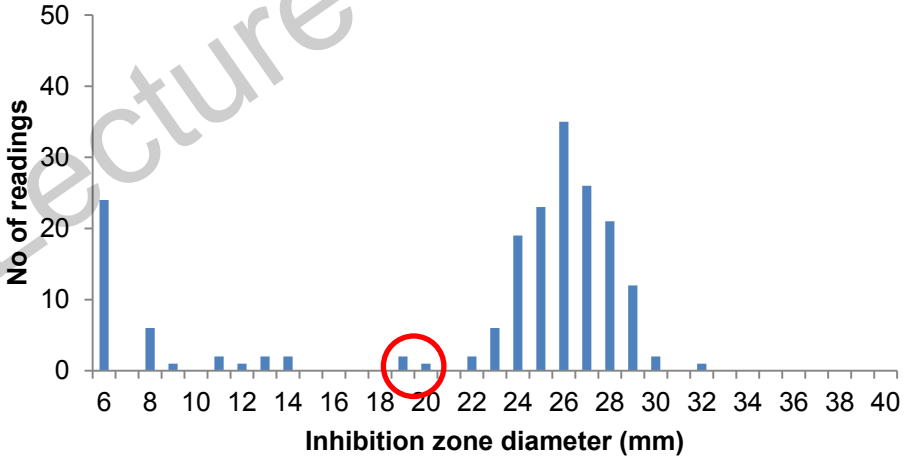
Early reading of disk diffusion tests

Examples with *E. coli* and cefotaxime 5 µg

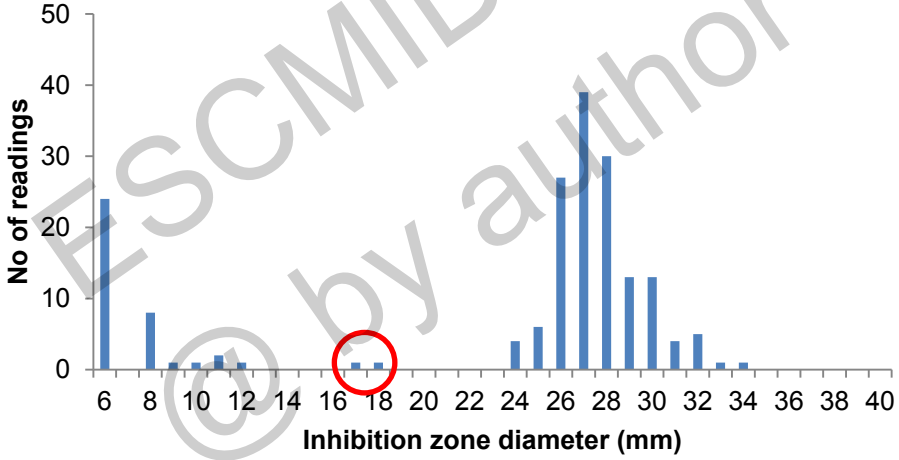
6 h incubation



8 h incubation



16-20 h incubation



- Poorer separation between wild type and non-wild type with short incubation time

- Additional isolates with known resistance mechanisms will be tested during 2015

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Frequently Asked Questions

Why do I get inhibition zones out of range for QC strains?

EM

Trouble shooting check list

- Inoculum
  - Too light, too heavy or uneven?
- Incubation
  - Always 16-20 h and 35°C (unless otherwise stated, e.g. Campylobacter)
- Reading
  - Sharp/fuzzy zone egdes,
  - Growth or haemolysis?
- Disks
- Agar depth
  - 4.0 mm ± 0.5 mm (occasional deviations)
- QC strains

Potential sources of error (1)

Medium	Storage of plates
	Not prepared to instructions
	Batch to batch variation or change of supplier of agar
	Supplements (batch to batch variations, incorrect amount or expired)
	pH
	Agar depth/Agar volume
	Expiry date
Test conditions	“15-15-15”-rule not adhered to (suspension used within 15 min, disks applied within15 min, incubation within 15 min)
	Incubation (temperature, atmosphere and time)
	Incorrect inoculation (too light, too heavy or uneven)
	Reading conditions
	Reading zone edges

Potential sources of error (2)

Disks	Incorrect disk (wrong agent or wrong disk content)
	Disk potency (incorrect storage, labile agent, expiry date)
	Disks not at room temperature when containers opened
	Too many disks on plate (interference between agents)
Control organisms	Incorrect QC strain
	Mutation
	Contamination
	Age of culture

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