



What is going on in the EUCAST Development laboratory (EDL)?

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EUCAST Breakpoint Tables v. 8.0

- News and changes
 - *P. aeruginosa*
 - Ceftolozane-tazobactam zone diameter breakpoints
 - *Aeromonas* spp.
 - Cefepime, ceftazidime, aztreonam, ciprofloxacin, levofloxacin and trimethoprim-sulfamethoxazole
 - Enterobacteriaceae (Enterobacterales) breakpoints validated for *Plesiomonas shigelloides*
 - Except for aminoglycosides
 - Macrolide breakpoints for *H. influenzae* removed
 - Fluoroquinolone breakpoints for *M. catarrhalis* revised
 - All dosages reviewed and several updated
 - A column for special situations (indications and/or organisms) added

EUCAST QC Tables v. 8.0

Table 1: Recommended QC strains for each organism or groups of organisms in the EUCAST Breakpoint Tables. The recommendations are based on using a strain of the same (or a similar) species as the organism to be tested (i.e. principal QC), but sometimes other QC strains have to be added to cover all agents.

Recommended strains for routine quality control

Recommendations for principal QC ¹		Recommendations for agents not covered by principal QC ¹	
Organism	QC strain	Agent	QC strain
Enterobacteriaceae (Enterobacterales ²)	<i>E. coli</i> ATCC 25922	Colistin (MIC)	Add <i>E. coli</i> NCTC 13846
<i>Pseudomonas</i> spp.	<i>P. aeruginosa</i> ATCC 27853	Piperacillin (zone diameter)	<i>E. coli</i> ATCC 25922
		Ticarcillin (zone diameter)	<i>E. coli</i> ATCC 25922
		Colistin (MIC)	Add <i>E. coli</i> NCTC 13846
<i>Stenotrophomonas maltophilia</i>	<i>E. coli</i> ATCC 25922		
<i>Acinetobacter</i> spp.	<i>P. aeruginosa</i> ATCC 27853	Trimethoprim-sulfamethoxazole (MIC and zone diameter)	<i>E. coli</i> ATCC 25922
		Colistin (MIC)	Add <i>E. coli</i> NCTC 13846
<i>Staphylococcus</i> spp.	<i>S. aureus</i> ATCC 29213	Roxithromycin (MIC)	<i>H. influenzae</i> ATCC 49766
<i>Enterococcus</i> spp.	<i>E. faecalis</i> ATCC 29212	Ampicillin-sulbactam (MIC)	See table 2
		Amoxicillin (MIC)	<i>E. coli</i> ATCC 25922
		Amoxicillin-clavulanic acid (MIC)	See table 2

Table 2: EUCAST recommended QC strains for control of β -lactam inhibitor combinations.

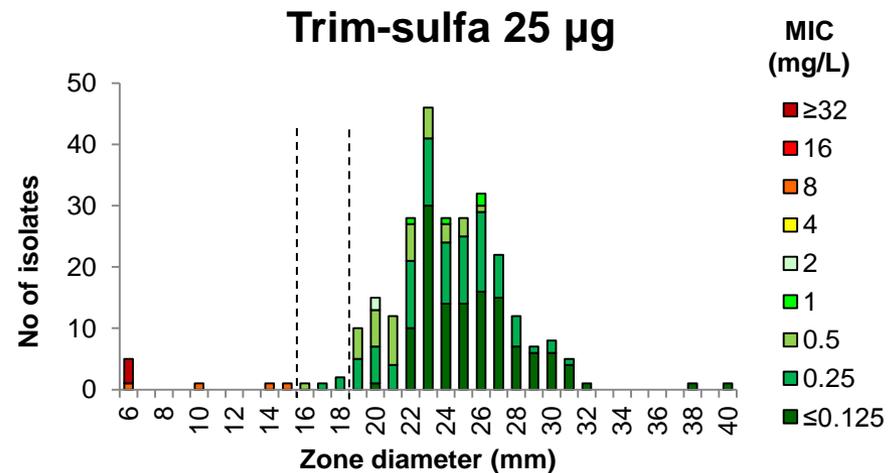
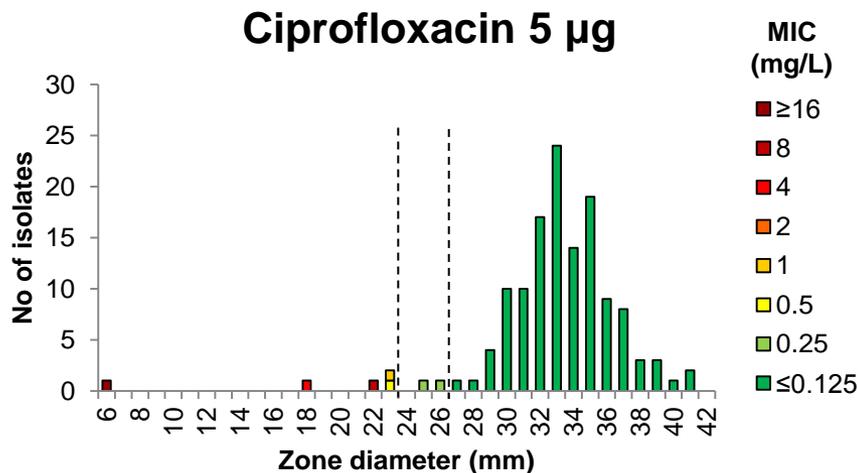
Organisms with recommendations added since 2012

Organism	Year
<i>Helicobacter pylori</i>	2012
<i>Listeria monocytogenes</i>	2012
<i>Pasteurella multocida</i>	2013
<i>Campylobacter jejuni</i> and <i>coli</i>	2013
<i>Corynebacterium</i> spp.	2014
<i>Aerococcus sanguincola</i> and <i>urinae</i>	2017
<i>Kingella kingae</i>	2017
<i>Aeromonas</i> spp.	2018

On going: *Nocardia* spp., *Bordetella pertussis* and disk diffusion for rapidly growing anaerobes

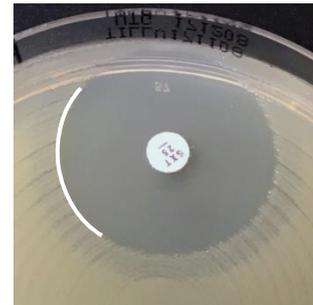
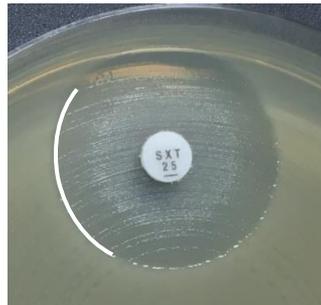
Aeromonas spp.

- MIC and zone diameter breakpoints available in EUCAST Breakpoint Tables v 8.0, 2018
 - Cefepime, ceftazidime, aztreonam, ciprofloxacin, levofloxacin and trimethoprim-sulfamethoxazole



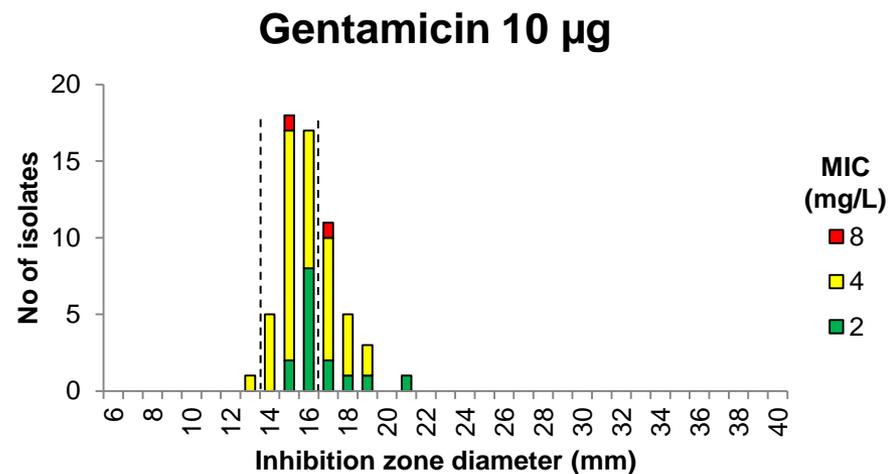
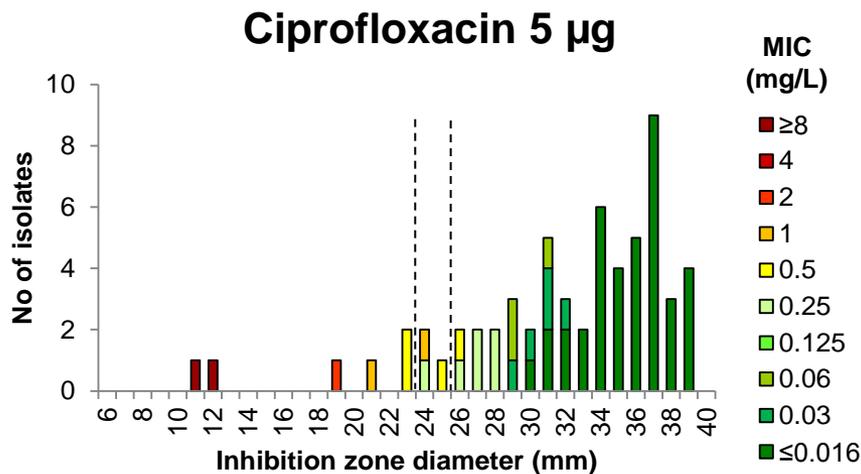
Aeromonas spp.

- EUCAST methodology for non-fastidious organisms
 - BMD with un-supplemented MH broth
 - Disk diffusion on un-supplemented MH agar
- Specific reading instructions for trimethoprim-sulfamethoxazole
 - Read the obvious zone edge and disregard haze or growth within the inhibition zone



Plesiomonas shigelloides

- Enterobacteriaceae (Enterobacterales) breakpoints validated
 - Except for aminoglycosides: “Breakpoints do not apply to *Plesiomonas shigelloides* since aminoglycosides have low intrinsic activity against this species”.



Nocardia spp.

- EUCAST methodology under development.
- Preliminary results:
 - Good growth on MH-F agar
 - Zones can be read after 24-48 h
 - Poor growth in broth
 - Un-supplemented MH broth and MH-F broth
 - Suggestion to move forward with disk diffusion and agar dilution on MH-F agar

Bordetella pertussis

- Pertussis infections are usually treated with erythromycin
 - Erythromycin resistance has started to emerge
 - Other agents have in vitro activity
- Regan-Lowe charcoal agar or MHA + 5% blood recommended for Etest
- Pilot study performed at EDL
 - Growth on AST media is related to the growth phase of the bacteria
 - Non-sufficient growth on MH-F in many tests
 - Good growth on Regan-Lowe charcoal agar with cefalexin
 - Additional media for AST to be investigated
 - Erythromycin resistance can be detected by adding an erythromycin disk on the primary plate (an additional plate is needed due to large inhibition zones)

Can a substitute for fresh horse blood be used in MH-F agar plates?

- Difficulties to obtain horse blood of good quality in large parts of the world.
- Pilot study
 1. 70 clinical isolates (all species with EUCAST disk diffusion criteria on MH-F) were tested on standard MH-F and MH-F plates with the substitute (MH-FD).
 2. Parallel testing at clinical microbiology Växjö and Karlskrona on routine isolates.

MH-F with a substitute for fresh horse blood (MH-FD)

Table 1. Difference in zone diameters (mm) between standard MH-F and MH-FD

Antimicrobial agent	Disk content (µg)	>-2	-2	-1	0	1	2	>2
Benzylpenicillin	1 unit	0	2	5	25	12	7	0
Ampicillin	2	0	3	5	2	1	0	0
Oxacillin	1	0	0	1	3	1	0	0
Cefaclor	30	0	1	5	3	4	3	1
Cefotaxime	5	1	5	2	6	5	2	1
Meropenem	10	0	4	6	6	8	1	2
Ciprofloxacin	5	1	0	0	4	0	1	0
Nalidixic acid	30	0	1	1	8	5	2	0
Norfloxacin	10	0	2	1	5	1	1	1
Vancomycin	5	2	3	3	0	0	0	0
Erythromycin	15	0	2	5	13	9	3	2
Clindamycin	2	0	0	3	16	0	2	2
Tetracycline	30	19	7	16	16	1	0	0
Linezolid	10	2	2	1	1	1	1	0
Nitrofurantoin	100	0	0	2	2	0	2	0
Rifampicin	5	3	2	1	2	2	1	2
Trimethoprim-sulfamethoxazole	25	0	2	2	13	7	7	1
Total number (%)	-	28 (8)	36 (10)	59 (17)	125 (36)	57 (16)	33 (9)	12 (3)

69% of zones on MH-FD within ± 1 mm of zones on MH-F
 89% of zones on MH-FD within ± 2 mm of zones on MH-F

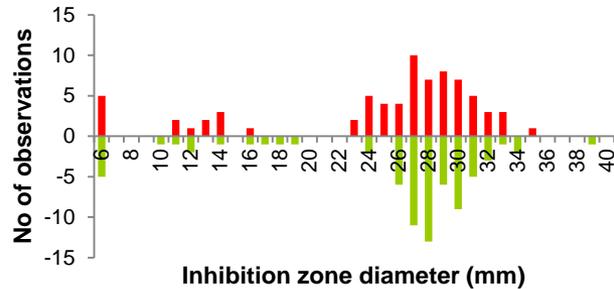
MH-F with a substitute for fresh horse blood (MH-FD)

Green bars = standard MH-F

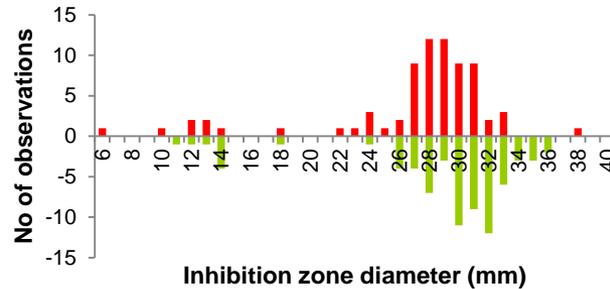
Red bars = MH-FD

S. pneumoniae, consecutive clinical isolates

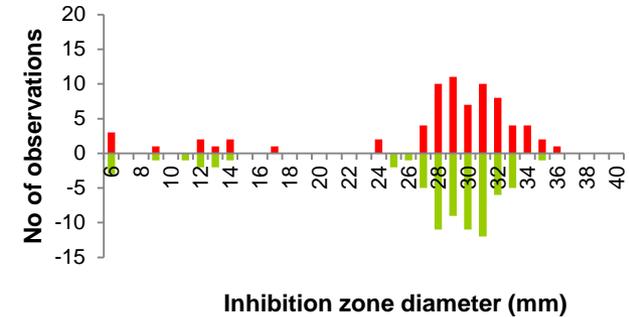
Oxacillin 1 μ g



Tetracycline 30 μ g

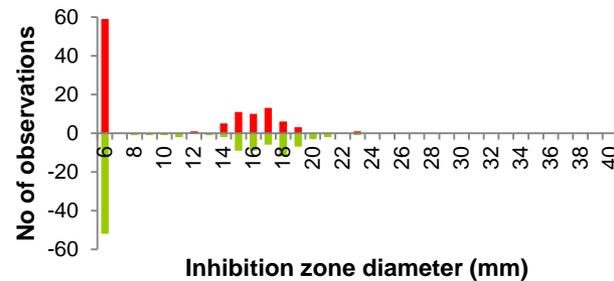


Erythromycin 15 μ g

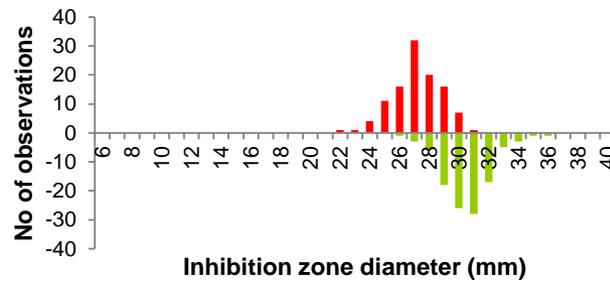


H. influenzae, consecutive clinical isolates

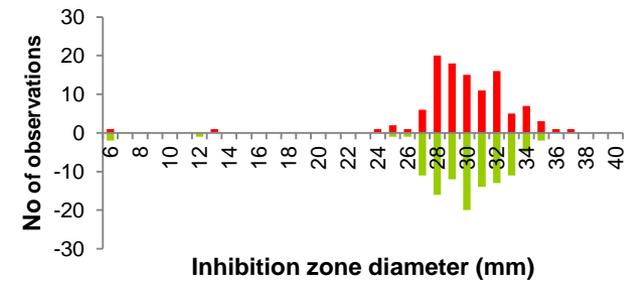
Benzylpenicillin 1 unit



Tetracycline 30 μ g



Nalidixic acid 30 μ g



Combination disks for confirmation of ESBL in Enterobacterales

- Current recommendation: (CLSI and EUCAST)
 - Cefotaxime 30 µg ± clavulanic acid 10 µg and
 - Ceftazidime 30 µg ± clavulanic acid 10 µg
- Can EUCAST disk potencies for cefotaxime and ceftazidime be used?
 - Cefotaxime 5 µg ± clavulanic acid 10 µg and
 - Ceftazidime 10 µg ± clavulanic acid 10 µg
- Disks from Mast and Liofilchem were evaluated on 50 Enterobacterales

Resistance mechanism	None	ESBL			AmpC		ESBL + AmpC
Resistance genes	-	CTX-M group 1	CTX-M group 9	CTX-M groups 1+9	CIT	DHA	CTX-M group 1 + CIT
<i>E. coli</i>	6	10	23	1	2	1	2
<i>K. pneumoniae</i>	0	5	0	0	0	0	0

Evaluation of antimicrobial disks

- 16 disks from 9 manufacturers

Bio-Rad	Abtek	HiMedia
Liofilchem	SirScan	Bioanalyse
BD	Oxoid	Mast

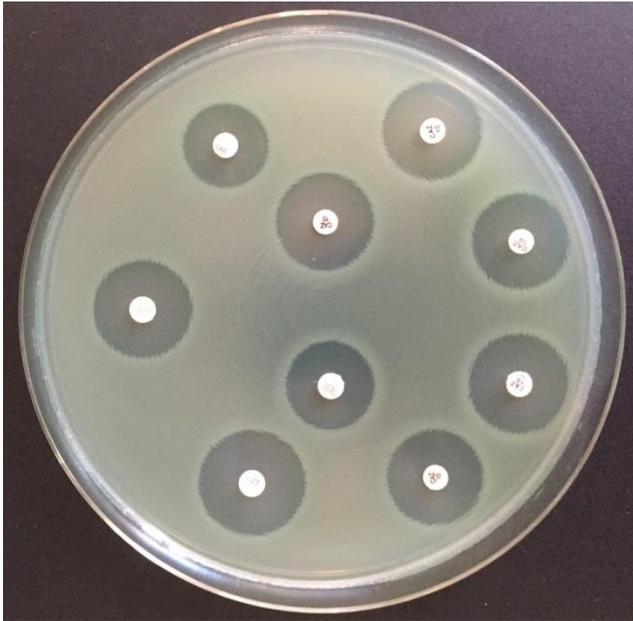
- Study performed in 2014 and repeated in 2017
- Each disk-strain combination was tested in triplicate using three separate inoculum suspensions.
- Mean values from triplicate tests of each disk were evaluated against targets and ranges in EUCAST QC Tables.

Antimicrobial disks and QC strains

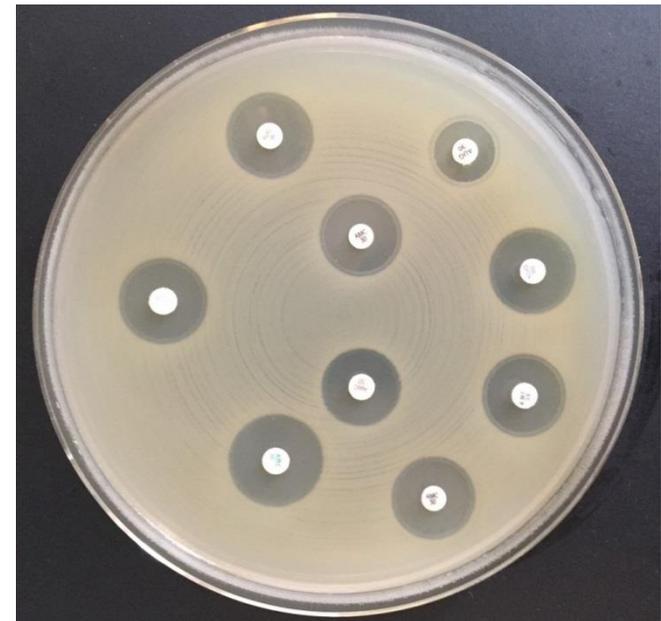
Antimicrobial agent	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>S. aureus</i> ATCC 29213	<i>E. faecalis</i> ATCC 29212	<i>S. pneumoniae</i> ATCC 49619	<i>H. influenzae</i> ATCC 49766	<i>E. coli</i> ATCC 35218	<i>K. pneumoniae</i> ATCC 700603
Bencylpenicillin 1 unit			•		•	•		
Amoxicillin-clavulanic acid 20-10 µg	•						•*	
Piperacillin-tazobactam 30-6 µg	•	•						•*
Oxacillin 1 µg			•*		•			
Mecillinam 10 µg	•							
Cefotaxime 5 µg	•				•	•		
Cefoxitin 30 µg			•					
Ceftazidime 10 µg	•	•						
Meropenem 10 µg	•	•			•			
Ciprofloxacin 5 µg	•		•	•				
Norfloxacin 10 µg	•		•	•				
Pefloxacin 5 µg	•							
Gentamicin 10 µg	•		•					
Tobramycin 10 µg	•		•					
Erythromycin 15 µg			•		•			
Tetracycline 30 µg			•		•	•		

* Only present in second study.

Examples



Ceftzidime 10 µg
P. aeruginosa ATCC 27853



Amoxicillin-clavulanic acid 20-10 µg
E. coli ATCC 25922

For each agent, one disk from each manufacturer was placed on the same agar plate to get identical testing conditions.

Results from first study

Year of evaluation	2014								
Manufacturer / Antimicrobial disk	Bio-Rad	Liofil-chem	BD	Abtek	Sir-Scan	Oxoid	Hi-Media	Bioanalyse	Mast
Benzylpenicillin 1 unit			L		H		NA	H	
Amoxicillin-clav. 20-10 µg	H			L			H		
Piperacillin-tazo. 30-6 µg				L	H		NA		
Oxacillin 1 µg		L	L		L		H	L	
Mecillinam 10 µg				L	H		H	H	
Cefotaxime 5 µg				NA			NA		
Cefoxitin 30 µg	H*	H		NA			L*		
Ceftazidime 10 µg				L			L		
Meropenem 10 µg	H	H*		L		H	H		
Ciprofloxacin 5 µg			L				H		L
Norfloxacin 10 µg				L	L		H*		
Pefloxacin 5 µg		L	L	NA	NA		H		
Gentamicin 10 µg			H		NA		H		
Tobramycin 10 µg	NA	H					H*		
Erythromycin 15 µg		L	L	L	L		H	L*	
Tetracycline 30 µg		L	L*	L	L*			L	L

Data from the first study reanalysed due to changes in QC criteria since 2014.

Mean value within ± 1 mm of the target value
Mean value >1 mm but within ± 2 mm of the target value
Mean value >2 mm from target value but still within the QC range
Mean value out of the QC range

NA = Not Available

H = High, mean value > 1 mm above target

L = Low, mean value > 1 mm below target

* One or more readings out of QC range

Results from both studies

Year of evaluation	2014									2017								
Manufacturer / Antimicrobial disk	Bio-Rad	Liofil-chem	BD	Abtek	Sir-Scan	Oxoid	Hi-Media	Bioanalyse	Mast	Bio-Rad	Liofil-chem	BD	Abtek	Sir-Scan	Oxoid	Hi-Media	Bioanalyse	Mast
Benzylpenicillin 1 unit			L		H		NA	H				L		NA				
Amoxicillin-clav. 20-10 µg	H			L			H			H	L		L			L		
Piperacillin-tazo. 30-6 µg				L	H		NA						L			L	H	H
Oxacillin 1 µg		L	L		L		H	L		H						H		
Mecillinam 10 µg				L	H		H	H										
Cefotaxime 5 µg				NA			NA											
Cefoxitin 30 µg	H*	H		NA			L*					L				L	H	
Ceftazidime 10 µg				L			L						L			L*		
Meropenem 10 µg	H	H*		L		H	H									L*		
Ciprofloxacin 5 µg			L				H		L									
Norfloxacin 10 µg				L	L		H*											
Pefloxacin 5 µg		L	L	NA	NA		H				L					H		
Gentamicin 10 µg			H		NA		H									H		H
Tobramycin 10 µg	NA	H					H*									H		
Erythromycin 15 µg		L	L	L	L		H	L*										
Tetracycline 30 µg		L	L*	L	L*			L	L							L		

Data from the first study reanalysed due to changes in QC criteria since 2014.

Mean value within ± 1 mm of the target value
Mean value >1 mm but within ± 2 mm of the target value
Mean value >2 mm from target value but still within the QC range
Mean value out of the QC range

NA = Not Available

H = High, mean value > 1 mm above target

L = Low, mean value > 1 mm below target

* One or more readings out of QC range

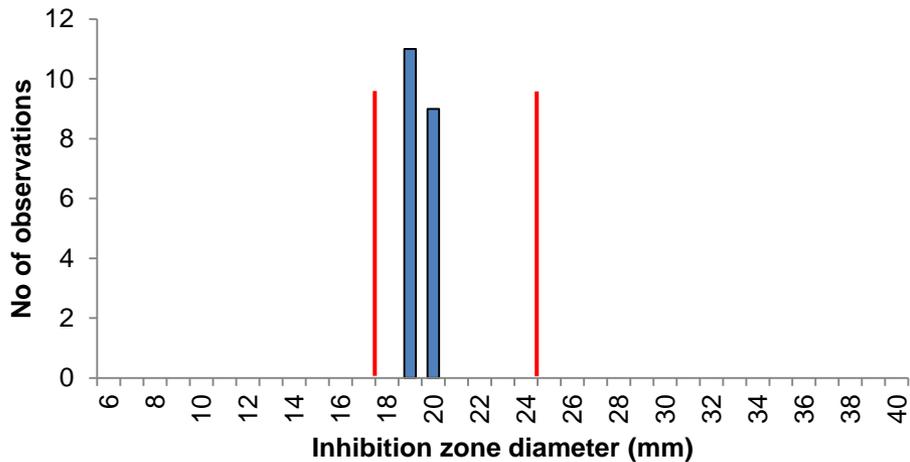
Disk-to-disk variation

- Variation in potency between disks within a vial
- Tobramycin, amoxicillin-clavulanic acid and piperacillin-tazobactam.
- 20 consecutive disks from one vial were tested simultaneously using the same inoculum suspension.
- *E. coli* ATCC 25922 plus β -lactamase producing QC strains to control the clavulanic acid (*E. coli* ATCC 35218) and tazobactam (*K. pneumoniae* ATCC 700603).

Amoxicillin-clavulanic acid 20-10 µg

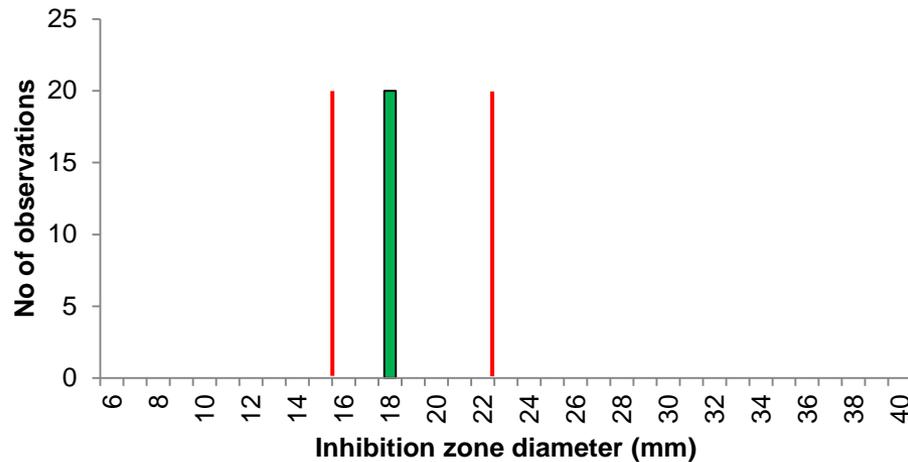
Control of active component

E. coli ATCC 25922, BD disks

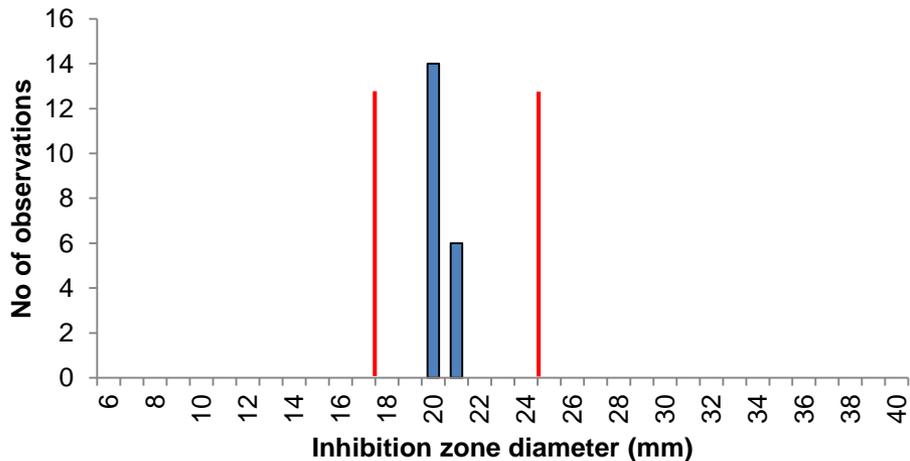


Control of inhibitor

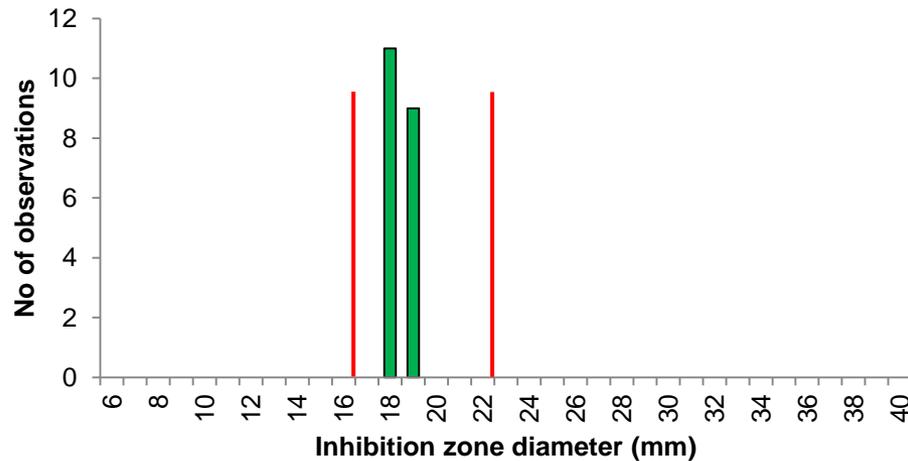
E. coli ATCC 35218, BD disks



E. coli ATCC 25922, Oxoid disks



E. coli ATCC 35218, Oxoid disks

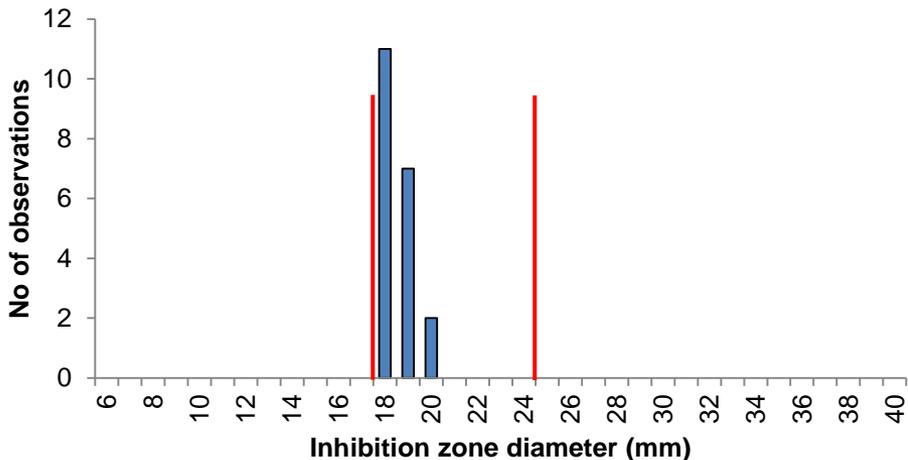


Amoxicillin-clavulanic acid 20-10 µg

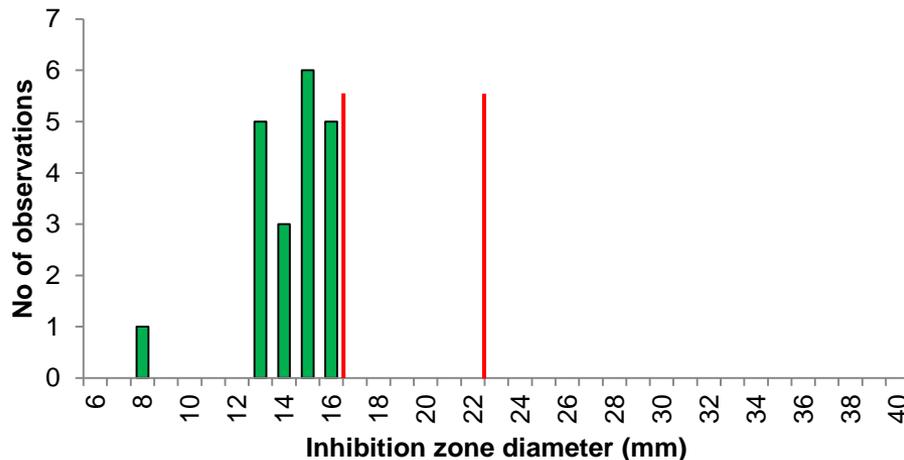
Control of active component

Control of inhibitor

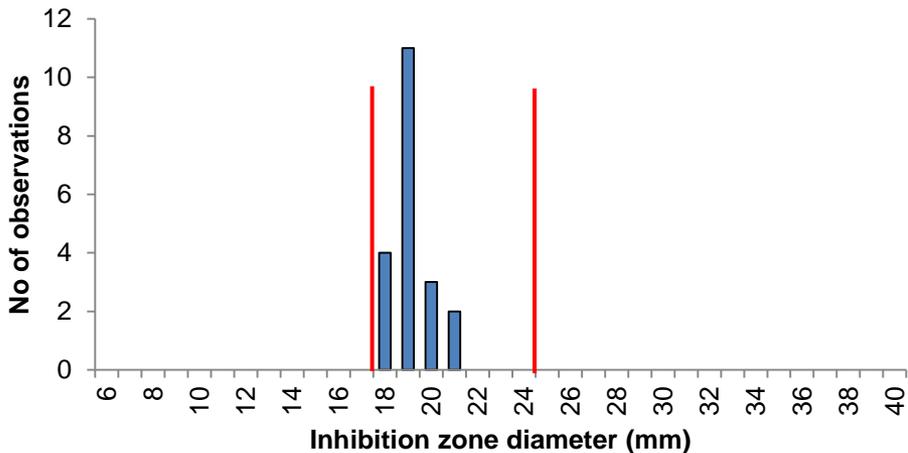
E. coli ATCC 25922, Abtek disks



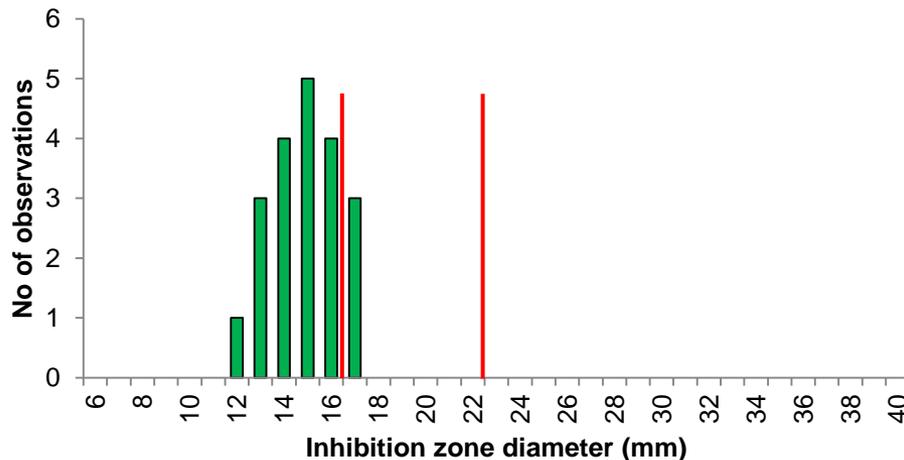
E. coli ATCC 35218, Abtek disks



E. coli ATCC 25922, Bioanalyse disks



E. coli ATCC 35218, Bioanalyse disks



Disk quality summary

- The quality of disks vary between manufacturers and sometimes even within a vial of disks.
- Disks should be produced to comply with EUCAST QC targets.
- Perform internal quality control
 - Daily or at least 4 times a week
 - Add β -lactamase producing strains to test the inhibitor component of combinations disks
- Report problems to the manufacturer, and when unresolved also to the EDL.

Evaluation of Mueller Hinton agar

Manufacturer	Product
Acumedia	MH agar
Alpha Biosciences	MH agar
Becton Dickinson	Difco MH agar
Becton Dickinson	BBL MH agar II
Biolab	MH II agar
Biolife	MH agar II
Biomerieux	MHE
BioRad	MH agar
E&O laboratories	MH agar
Hardy Diagnostics	MH agar CRITERION
HiMedia	MH agar No.2
HiMedia	MH agar
LabM	MH agar
Mast Diagnostic	MH agar
Mast Diagnostic	MH II agar (catjon adjusted)
Merck	MH agar
Merck	MH agar acc. To CLSI
Oxoid	Oxoid MH agar
Remel	Remel MH agar
Sifin	MH agar
Sigma	MH agar
Sigma	MH agar 2 (catjon adjusted)

- 23 agars from 17 manufacturers
- Testing with disk-QC strain combinations that can disclose agar differences.
- Disks from one manufacturer with high quality and previous stable results.

Questions and/or problem?

Contact us!

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