



What's new in susceptibility testing of anaerobes?

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Background

- EUCAST MIC breakpoints
 - Gram-negative anaerobes
 - Gram-positive anaerobes
- No disk diffusion method for anaerobic bacteria.
- MIC breakpoints for anaerobes were reviewed by EUCAST
→ species-specific breakpoints!
- In 2022, EUCAST published a disk diffusion method for selected anaerobes with species-specific MIC and zone diameter breakpoints.

Disk diffusion methodology

Medium	Fastidious Anaerobe Agar with 5% mechanically defibrinated horse blood (FAA-HB)
Inoculum	McF 1.0
Incubation	Anaerobic environment, 35-37°C for 16-20 h
Species	<i>Bacteroides</i> spp. <i>Prevotella</i> spp. <i>Fusobacterium necrophorum</i> <i>Clostridium perfringens</i> <i>Cutibacterium acnes</i>

Antimicrobial agents

Jan 2022	June 2023
<ul style="list-style-type: none">• Benzylpenicillin¹• Piperacillin-tazobactam• Meropenem• Vancomycin²• Clindamycin• Metronidazole³	<ul style="list-style-type: none">• Ampicillin¹• Ampicillin-sulbactam• Amoxicillin¹• Amoxicillin-clavulanic acid• Cefotaxime⁴• Ceftriaxone⁴• Ertapenem• Imipenem• Linezolid⁴

1. Not for *Bacteroides* spp.; 2. Gram-pos organisms only; 3. Not for *C. acnes*; 4. *C. acnes* only

Evaluation of EUCAST disk diffusion method for ten additional anaerobic species

Additional species

- *Clostridium innocuum*
- *Clostridium ramosum* (*Thomasclavelia ramosa*)
- *Clostridium septicum*
- *Clostridium tertium*
- *Fusobacterium nucleatum*
- *Cutibacterium avidum*
- *Finegoldia magna*
- *Parvimonas micra*
- *Peptostreptococcus anaerobius*
- *Peptoniphilus* spp.

} Gram-Positive Anaerobic Cocci (GPAC)

Study design

- 500 isolates (50 per species) from five international laboratories.
- Disk diffusion according to EUCAST method
 - Media from two manufacturers (Neogen and E&O Labs) in parallel.
 - Incubation: Anoxomat gas system and gas-generating sachets (AnaeroGen, Thermo Scientific).
- MICs with agar dilution (AD) or gradient strips (GS) were provided by the respective laboratory for 85 % of the isolates (not for all agents).
- For each species and agent, zone diameter distributions were produced and the correlation between zones and available MIC values analysed.

Antimicrobial agents

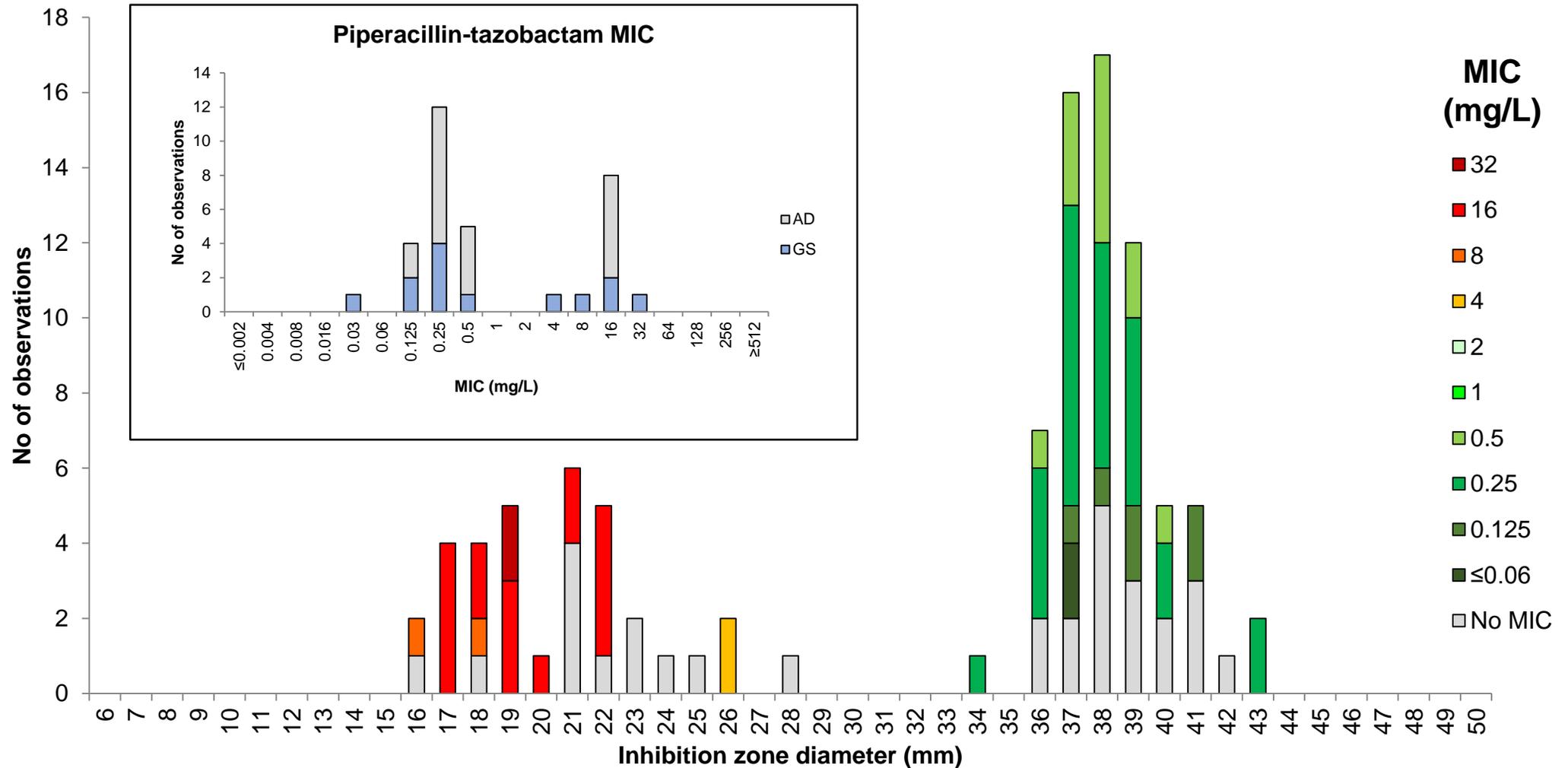
Antimicrobial disks	
Benzylopenicillin 1unit	Imipenem 10 µg
Ampicillin 2 µg	Meropenem 10 µg
Ampicillin-sulbactam 10-10 µg	Vancomycin 5 µg ²
Amoxicillin-clavulanic acid 2-1 µg	Erythromycin 15 µg ³
Piperacillin-tazobactam 30-6 µg	Clindamycin 2 µg
Cefotaxime 5 µg ¹	Linezolid 10 µg ¹
Ceftriaxone 30 µg ¹	Metronidazole 5 µg ⁴
Ertapenem 10 µg	

1. *C. avidum* only; 2. Gram-pos organisms only; 3. Gram-pos cocci only; 4. Not *C. avidum*.

Results

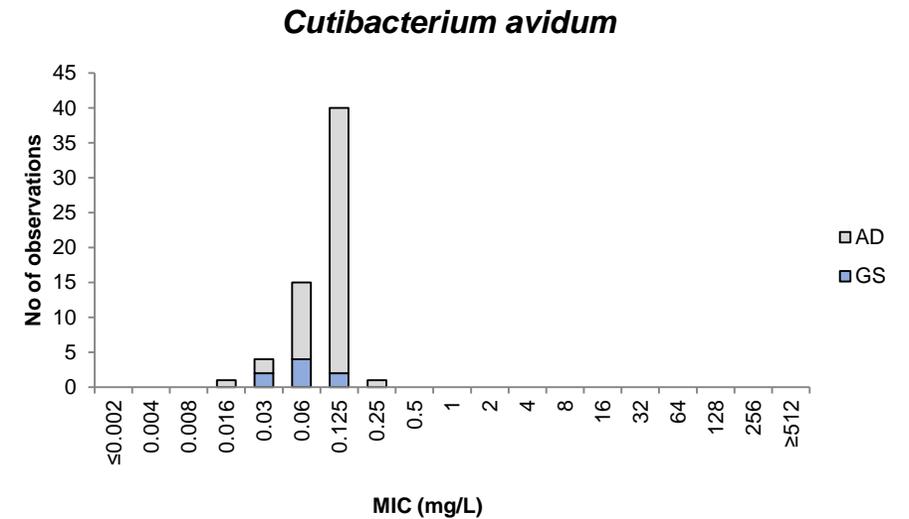
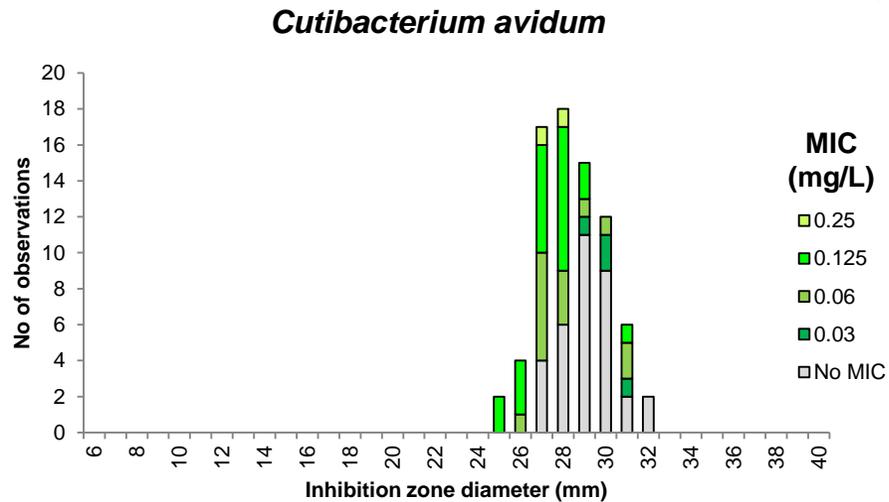
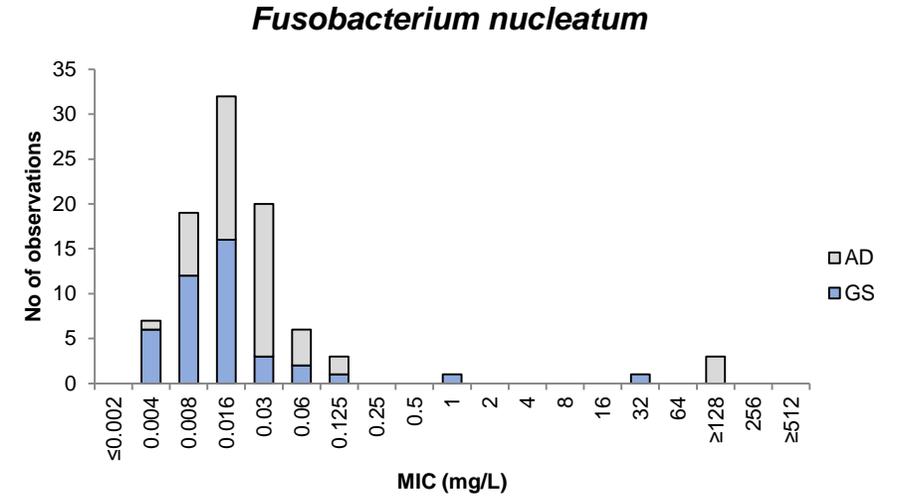
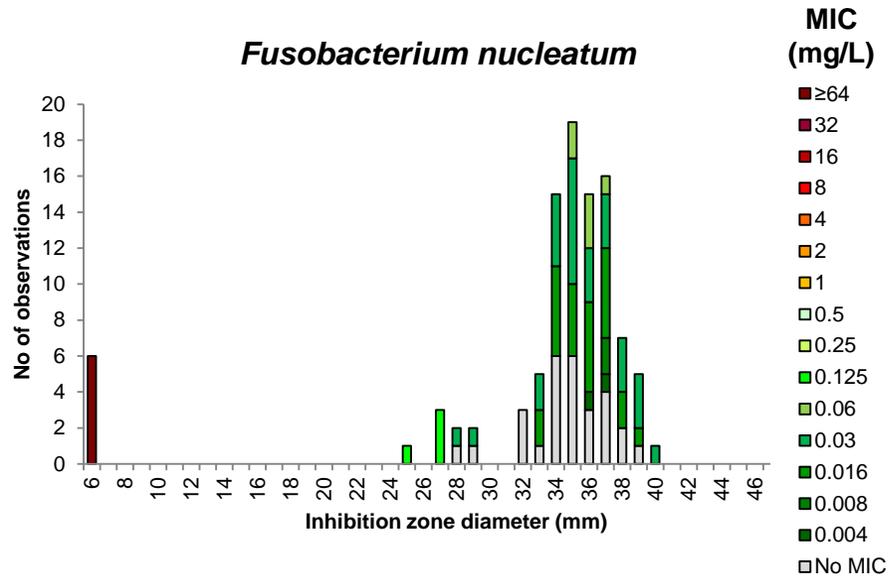
- Confluent growth after 16-20 h incubation for all isolates.
- Good correlation between MIC values and zone diameters.
- Disk diffusion testing was able to separate wild-type from non-wild type isolates.
 - MICs were interpreted using cut-off values in EUCAST Guidance document “When there are no breakpoints”.
- Inducible clindamycin resistance was phenotypically detected in 7/50 *F. magna*, 22/50 *P. anaerobius* and 14/50 *Peptoniphilus* spp.

Piperacillin-tazobactam vs. *P. anaerobius*



The colours of the bars correspond to cut-off values for anaerobic bacteria lacking EUCAST breakpoints.

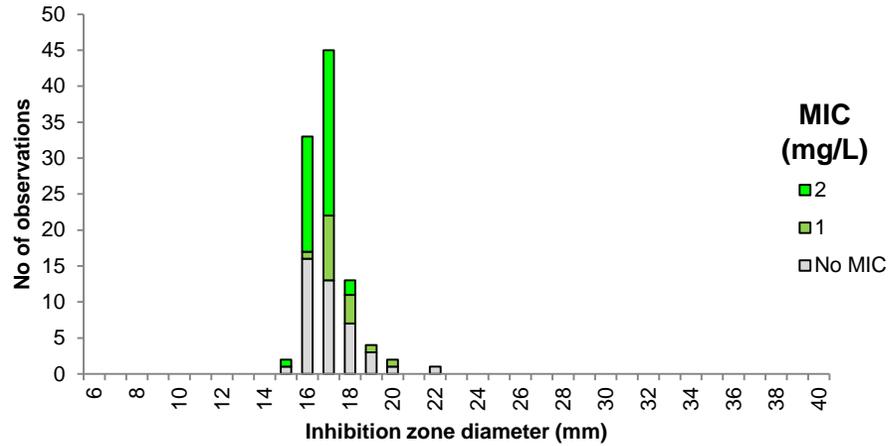
Benzylpenicillin



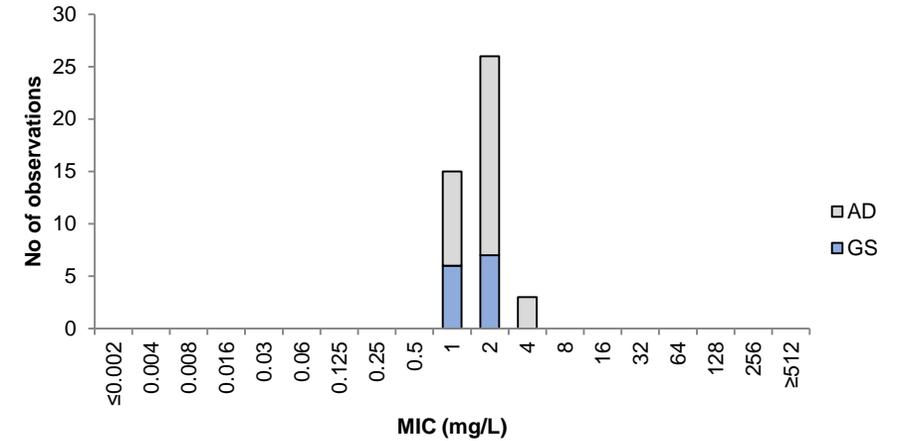
The colours of the bars correspond to cut-off values for anaerobic bacteria lacking EUCAST breakpoints.

Vancomycin

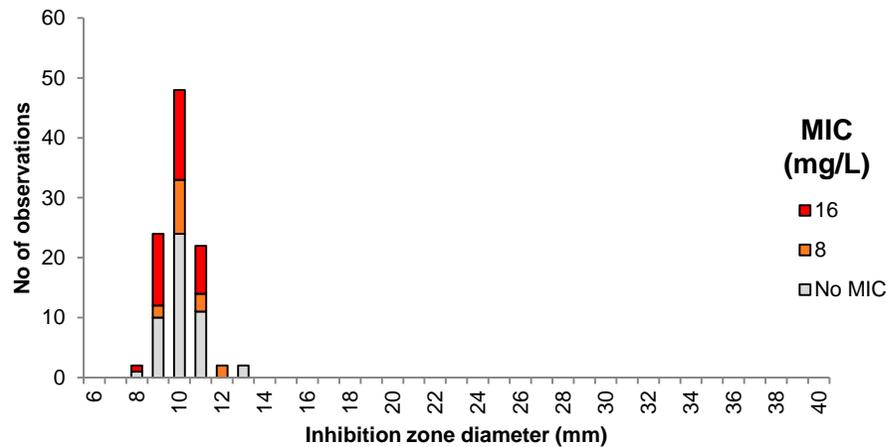
Clostridium tertium



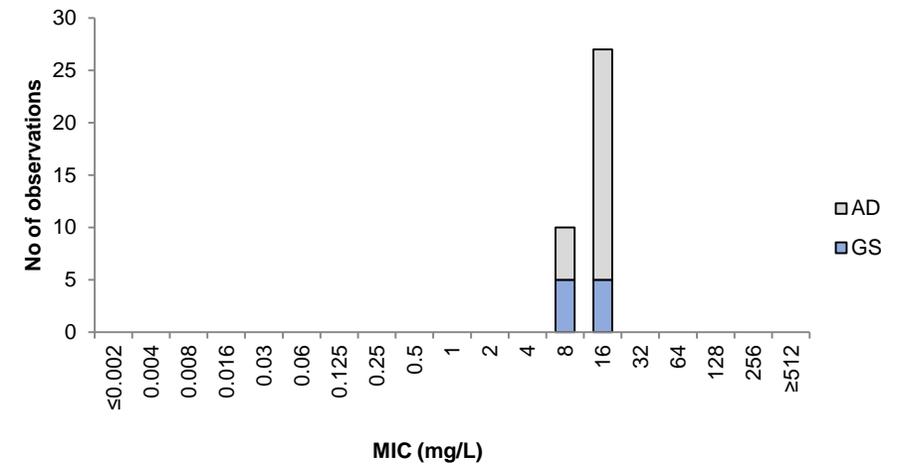
Clostridium tertium



Clostridium innocuum

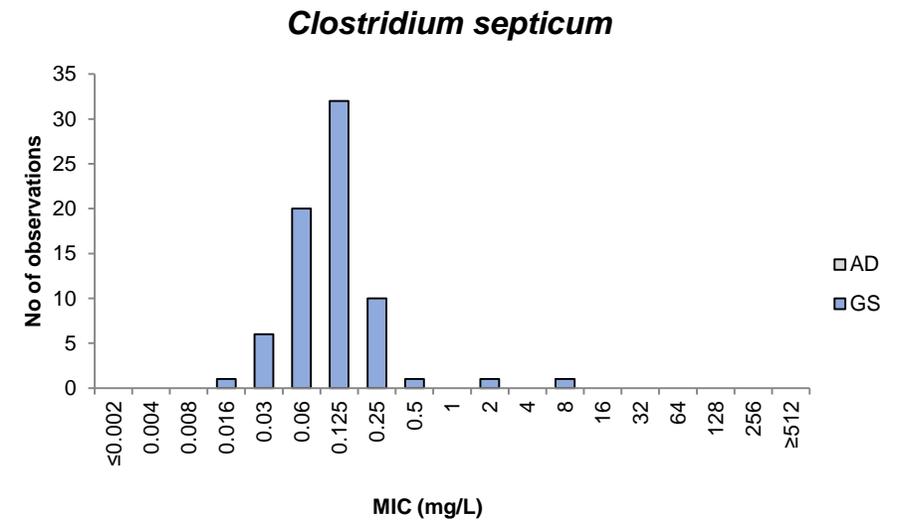
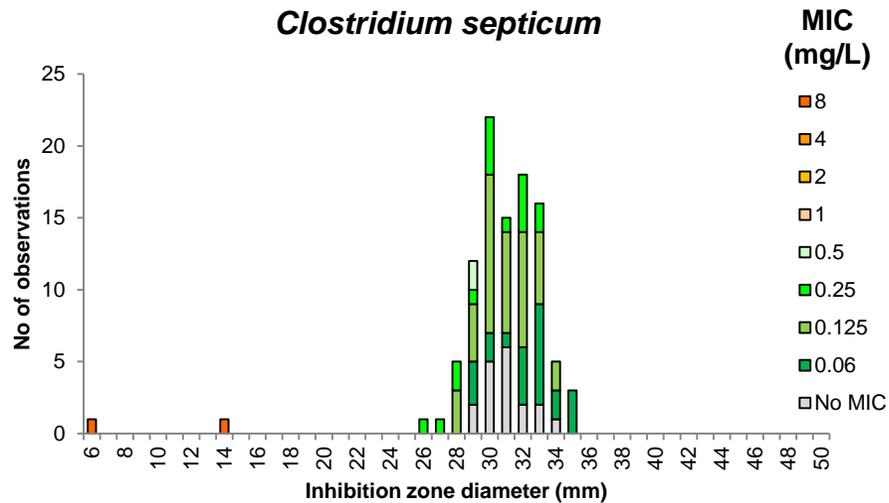
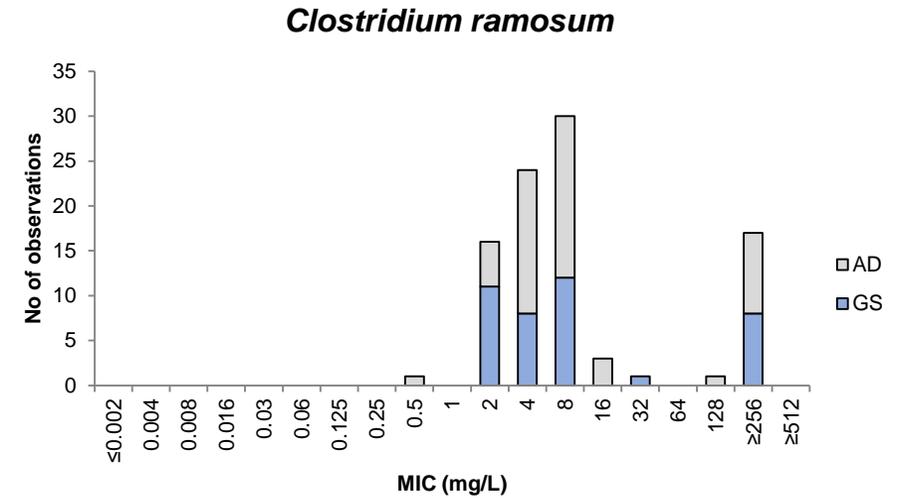
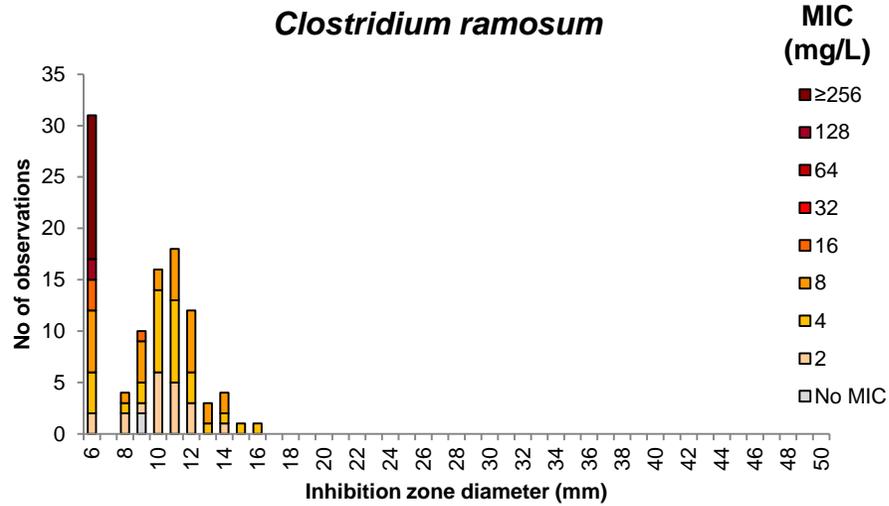


Clostridium innocuum



The colours of the bars correspond to cut-off values for anaerobic bacteria lacking EUCAST breakpoints.

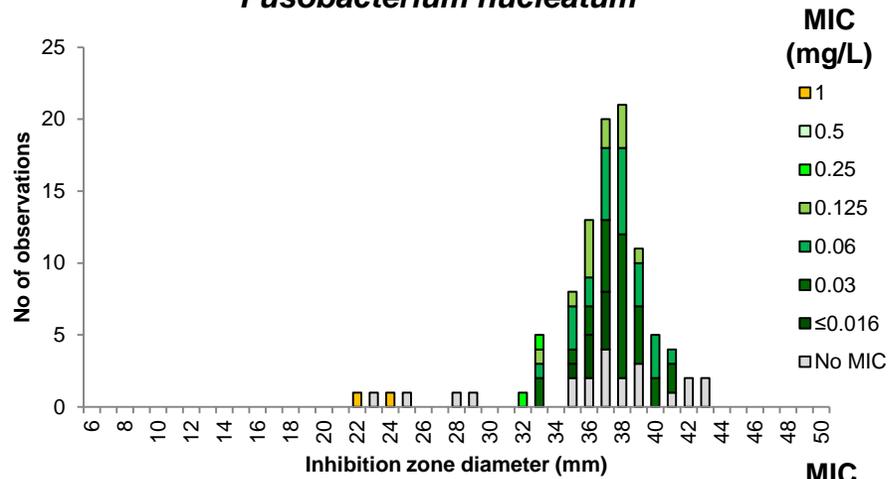
Clindamycin



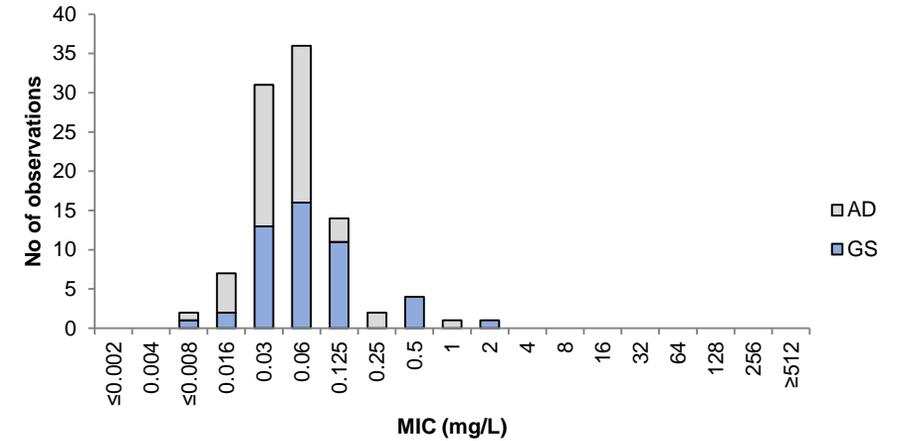
The colours of the bars correspond to cut-off values for anaerobic bacteria lacking EUCAST breakpoints.

Clindamycin

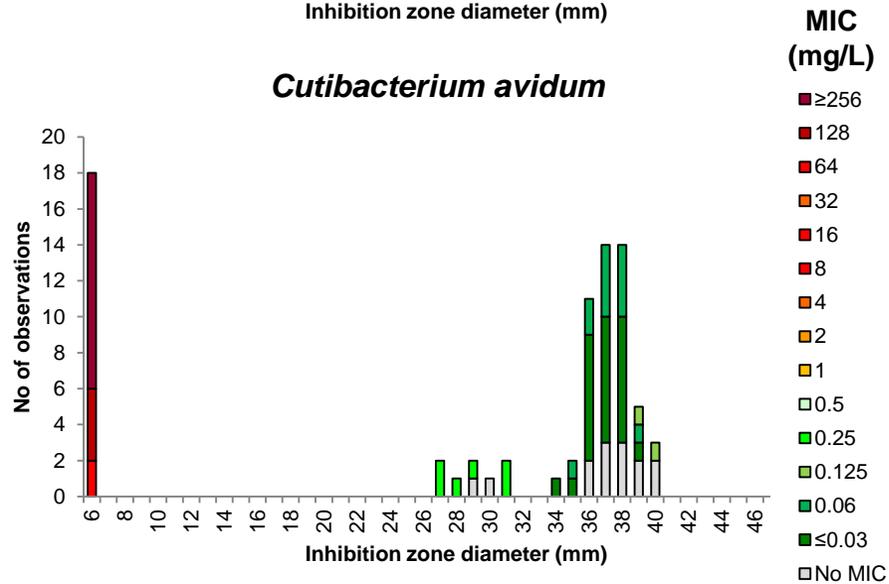
Fusobacterium nucleatum



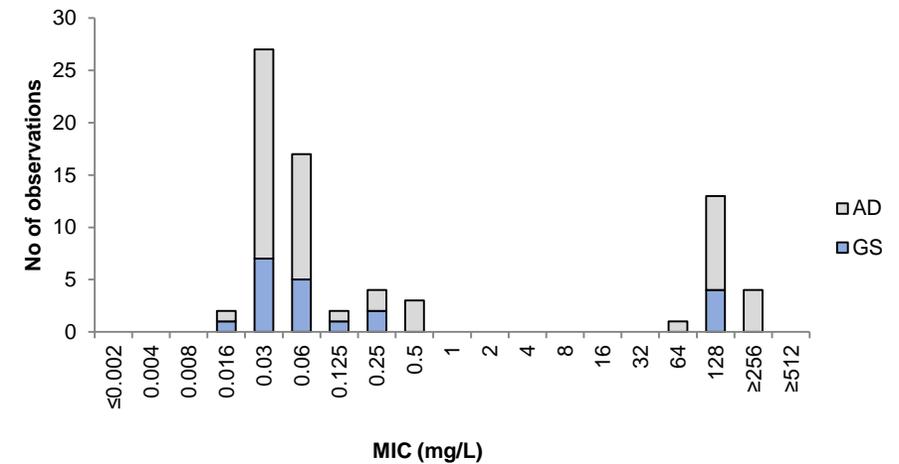
Fusobacterium nucleatum



Cutibacterium avidum



Cutibacterium avidum



The colours of the bars correspond to cut-off values for anaerobic bacteria lacking EUCAST breakpoints.

Conclusions

- EUCAST disk diffusion method for anaerobic bacteria is suitable for ten additional species of anaerobes.
- Additional MIC data is needed for EUCAST to establish species-specific MIC breakpoints for these species.
- Based on data from this study EUCAST will establish corresponding zone diameter breakpoints.
- New recommendations expected 2025/2026.

Species without breakpoints

- Perform an MIC test, either by agar dilution or gradient tests (according to manufacturer's instructions).
- Consult EUCAST Guidance document "When there are no breakpoints" (<https://www.eucast.org/eucastguidancedocuments>)
 - If the MIC is above the value in the table: advise against the use of the agent (alternatively report resistant).
 - If the MIC is equal to or below the value in the table: the agent may be considered for use, interpret with caution. Do not report susceptible.

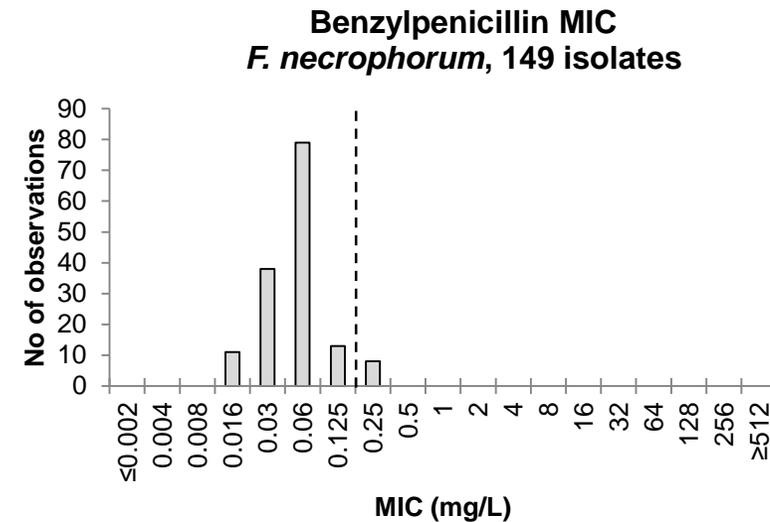
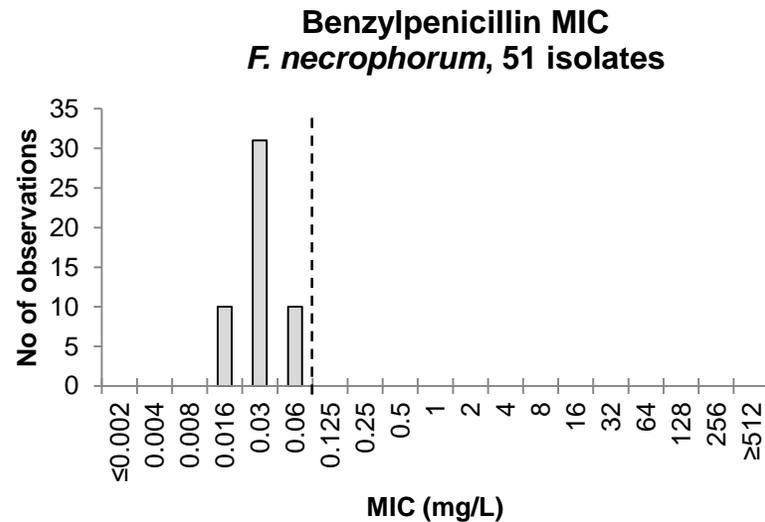
Table 2. Antimicrobial agents relevant for the treatment of anaerobic bacteria with guidance for bacteria lacking breakpoints in standard EUCAST breakpoint tables.

Agents and notes for anaerobic bacteria	MIC-values above which therapy with the agent should be discouraged	
Benzympenicillin	0.5	Breakpoints for anaerobic bacteria in the breakpoint table are 0.06 – 0.5 mg/L. If a beta-lactamase is detected, report resistant without further testing.
Amoxicillin	0.5	Breakpoints for anaerobic bacteria in the breakpoint table are 0.25 – 0.5 mg/L. If a beta-lactamase is detected, report resistant without further testing.
Amoxicillin-clavulanic acid	0.5	Breakpoints for anaerobic bacteria in the breakpoint table are 0.25 – 0.5 mg/L.
Ampicillin-sulbactam	0.5	Breakpoints for anaerobic bacteria in the breakpoint table are 0.25 – 0.5 mg/L.
Piperacillin-tazobactam	2	Breakpoints for anaerobic bacteria in the breakpoint table are 0.5 – 2 mg/L.
Meropenem	1	Breakpoints for anaerobic bacteria in the breakpoint table are 0.03 – 1 mg/L.
Imipenem	1	Breakpoints for anaerobic bacteria in the breakpoint table are 0.03 – 1 mg/L.
Ertapenem	0.25	Breakpoints for anaerobic bacteria in the breakpoint table are 0.06 – 0.5 mg/L.
Clindamycin	0.5	Breakpoints for anaerobic bacteria in the breakpoint table are 0.25 mg/L.
Metronidazole	4	Breakpoints for anaerobic bacteria in the breakpoint table are 0.5 - 4 mg/L.
Vancomycin (Gram-positive)	2	Only relevant for a few gram-positive anaerobic bacteria. A breakpoint of 2 mg/L is common for targeted species.
Rifampicin (Gram-positive)	0.125	Breakpoints for species already in the EUCAST breakpoint tables are 0.06 – 0.125 mg/L.
Linezolid (mixed infections)	Pending	Linezolid has been used in the treatment of mixed infections where anaerobic bacteria were considered causative, but rarely for targeted therapy of anaerobic infections.
Moxifloxacin (mixed infections)	Pending	Moxifloxacin has been used in the treatment of mixed infections where anaerobic bacteria were considered causative, but rarely for targeted therapy of anaerobic infections.

Changes in Breakpoint Tables and QC Tables for anaerobic bacteria 2025

Revised MIC breakpoints

- Benzylpenicillin MIC breakpoints for *Fusobacterium necrophorum* have been changed from 0.06/0.06 mg/L → 0.125/0.125 mg/L.
 - No changes in zone diameter breakpoints



Revision of QC criteria

- Validation study at the EDL:
 - Media from 3 manufacturers
 - Anaerobic work station, Anoxomat, gas-generating envelopes
- Validation study at 5 external laboratories
- Tendency towards zones in the lower part of the range or below the lower limit for some strain-agent combinations.
 - Some QC ranges for the anaerobic QC strains have been revised in EUCAST QC Tables, January 2025.

Revised QC criteria

***Bacteroides fragilis* ATCC 25285**

Antimicrobial agent	QC target	QC range
Clindamycin 2 µg	25	22-28
Ertapenem 10 µg	36	33-39

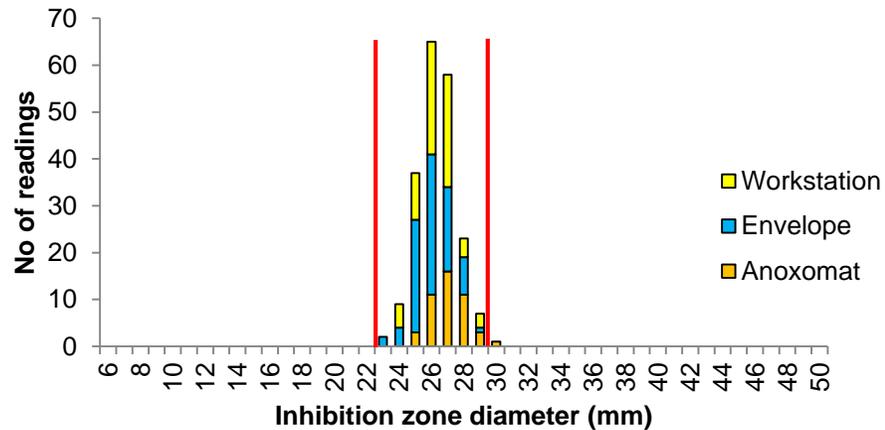
***Clostridium perfringens* ATCC 13124**

Antimicrobial agent	QC target	QC range
Benzylpenicillin 1 unit	25	22-28
Imipenem 10 µg	34	31-37
Linezolid 10 µg	24	21-27
Meropenem 10 µg	36	33-39
Piperacillin-tazobactam 30-6 µg	32	29-35

B. fragilis ATCC 25285 and clindamycin

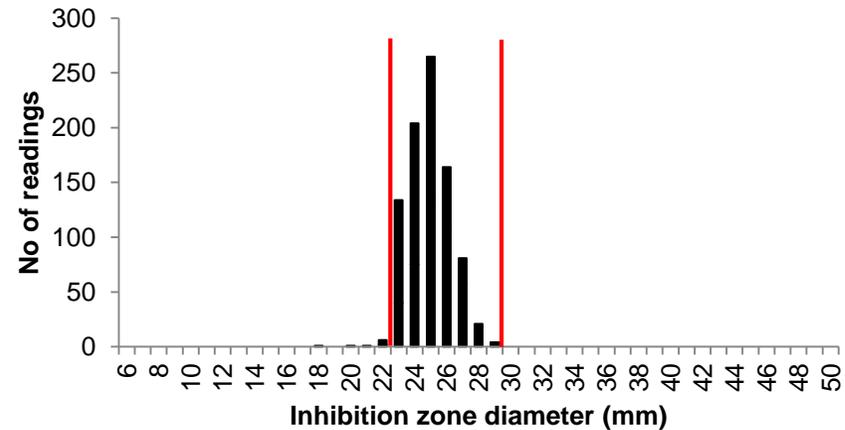
Original data

Clindamycin 2 µg
B. fragilis ATCC 25285 (n=202)



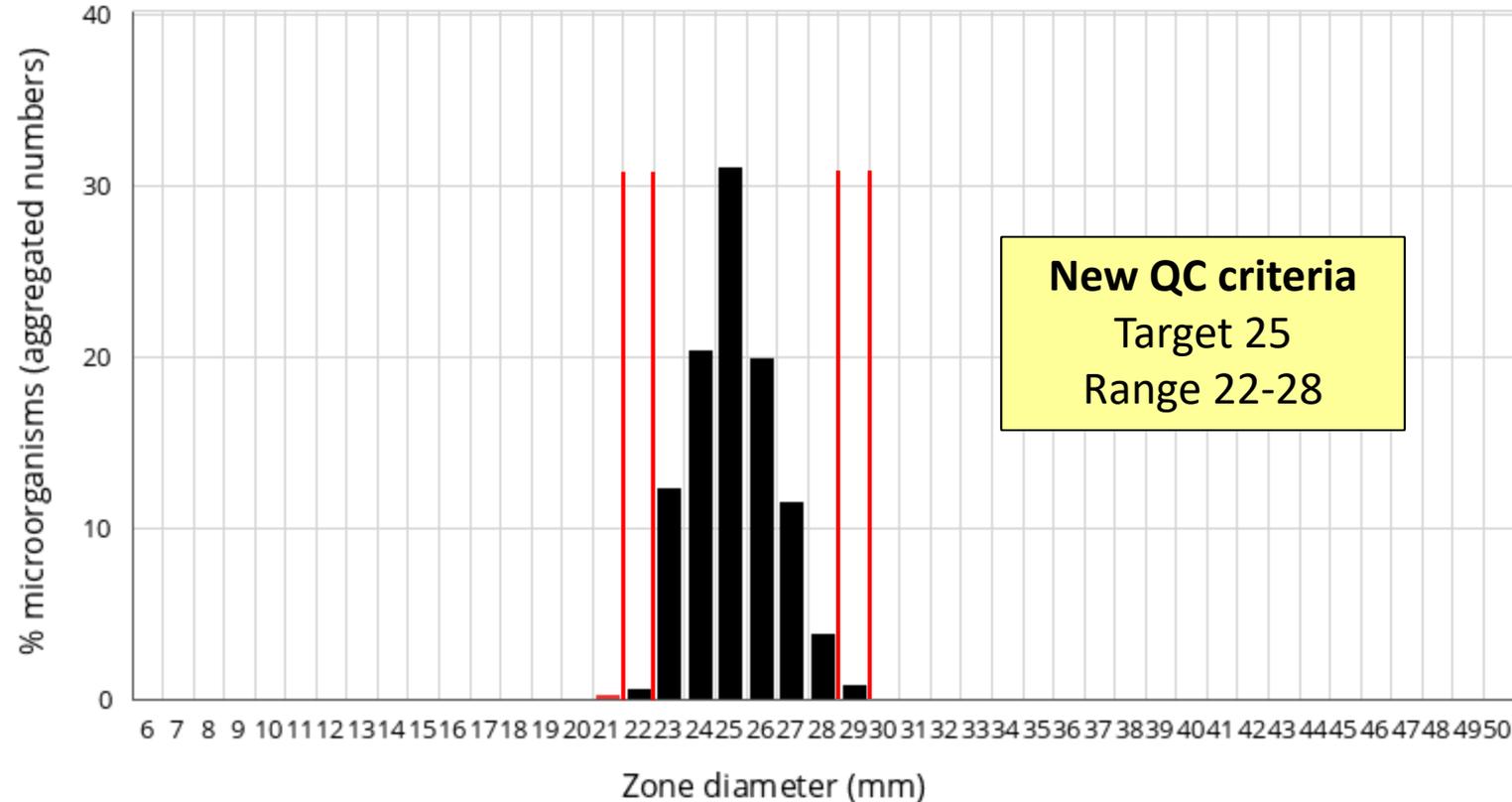
Additional data

Clindamycin 2 µg
B. fragilis ATCC 25285 (n=882)



Target 26
Range 23-29

B. fragilis ATCC 25285 and clindamycin

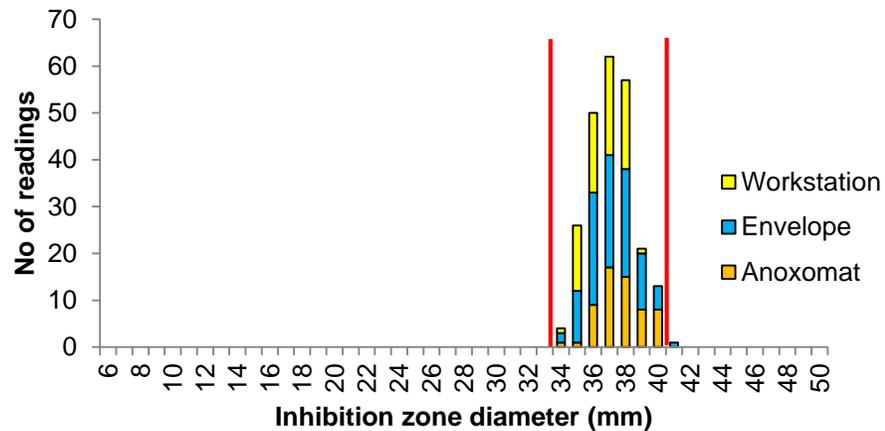


	Range	Target	Within range	On lower limit	On upper limit	On target	On target ± 1
Old QC criteria	23-29	26	99%	12%	1%	20%	62%
New QC criteria	22-28	25	99%	0%	1%	31%	71%

C. perfringens ATCC 13124 and meropenem

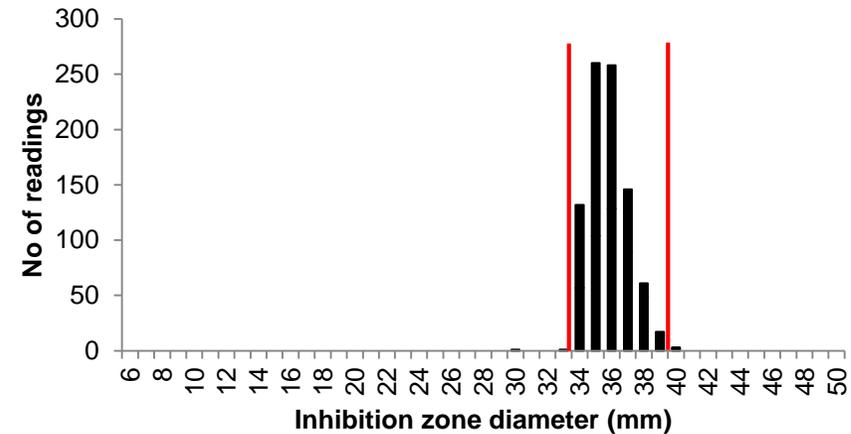
Original data

Meropenem 10 µg
C. perfringens ATCC 13124 (n=234)



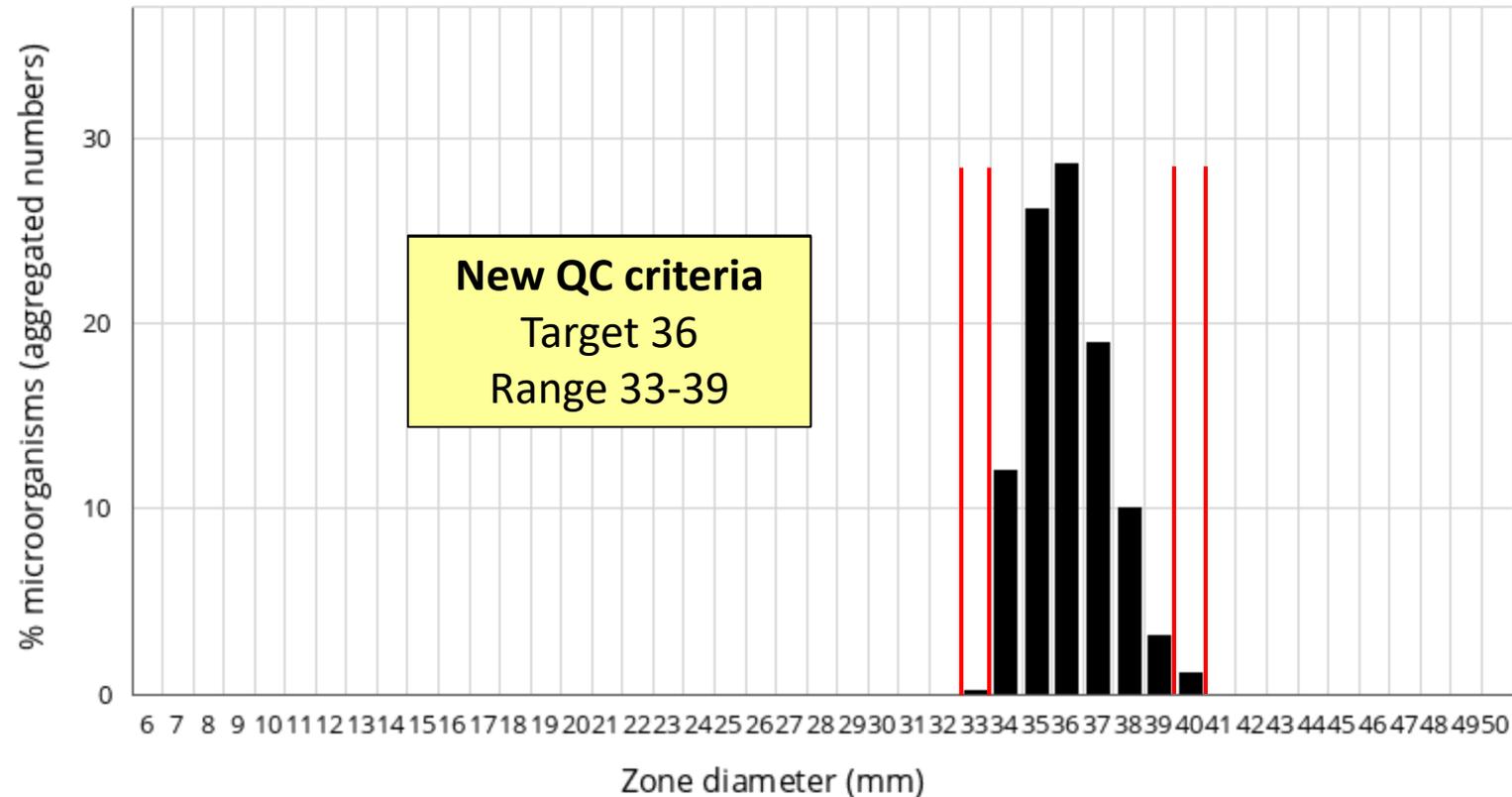
Additional data

Meropenem 10 µg
C. perfringens ATCC 13124 (n=879)



Target 37
Range 34-40

C. perfringens ATCC 13124 and meropenem



	Range	Target	Within range	On lower limit	On upper limit	On target	On target ± 1
Old QC criteria	34-40	37	100%	12%	1%	19%	58%
New QC criteria	33-39	36	99%	0%	3%	29%	74%

The anaerobic atmosphere

- Strict anaerobic conditions throughout incubation is essential for disk diffusion of anaerobic bacteria.
 - Growth of anaerobic bacteria and the activity of some antimicrobial agents may be affected even by the presence of very low concentrations of oxygen.
 - Regardless of which anaerobic system is used, the atmosphere need to be monitored.
 - Workstations and gas-generating systems (Anoxomat) provide immediate anaerobic conditions, whereas gas-generating envelopes take a couple of hours to reach anaerobiosis.
 - For workstations, operating procedures (loading/unloading plates) may affect temperature and oxygen levels during the work day.

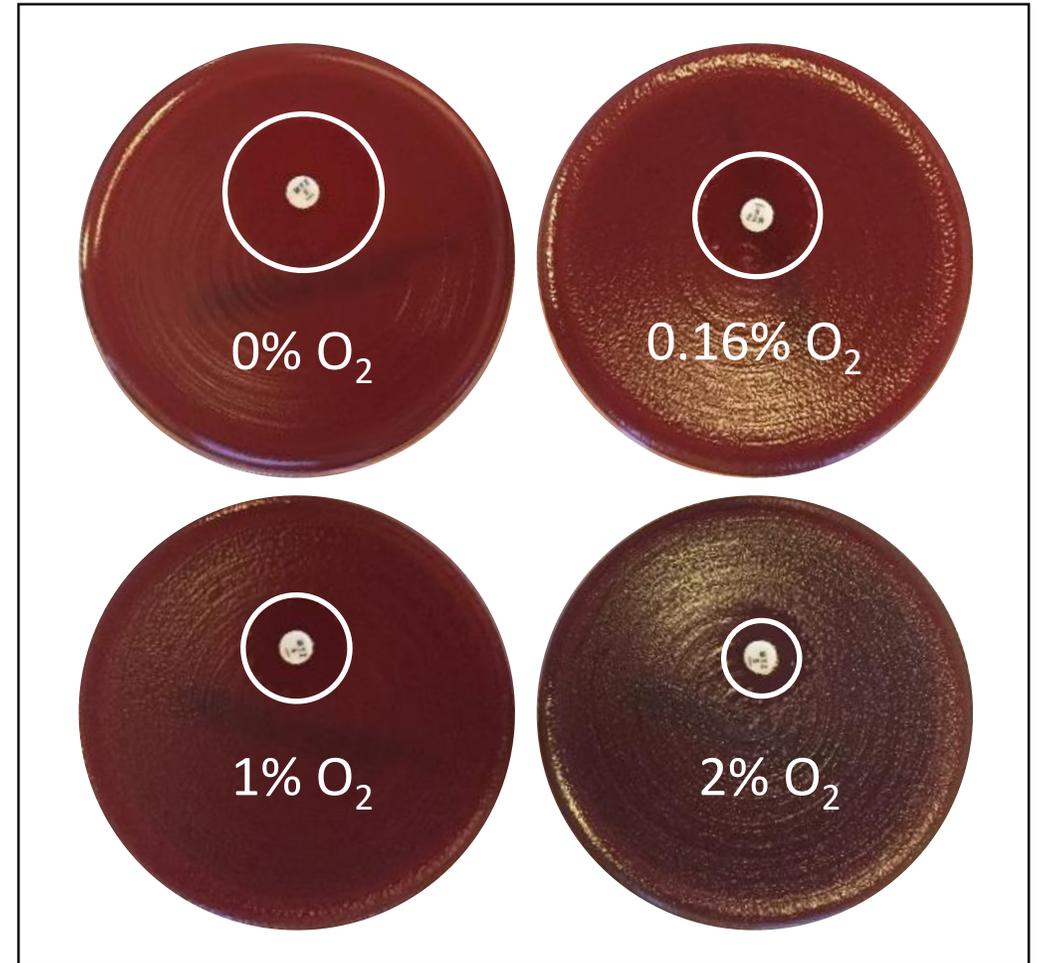
Monitoring the anaerobic atmosphere

- Anaerobic indicator (methylene blue or resazurin)
 - Produces a colour change in anaerobic conditions.
 - Does not reflect conditions over time, only the current status.
 - Colour change can occur even with low levels of oxygen.
- *C. perfringens* DSM 25589 with metronidazole 5 µg
 - Aerotolerant strain for which the metronidazole zone will decrease with increasing levels of oxygen.
 - + Sensitive to very low levels of oxygen.
 - + Reflects the conditions over the full incubation time.

C. perfringens DSM 25589 (CCUG 75076)

- Aerotolerant strain, will grow at 4% oxygen and even at higher levels.
- Extremely sensitive test:
Metronidazole zone will decrease significantly (30 → 22 mm) when oxygen levels increase from 0% to 0.16%.

Metronidazole zone ≥ 25 mm
indicates sufficient anaerobicity!



Acknowledgements

- Laboratories providing isolates of additional anaerobic species:
 - Dept of Microbiology, Haukeland University hospital, Bergen, Norway
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 - Clinical Microbiology Kalmar, Karlskrona and Örebro, Sweden
- Staff at EUCAST Development Laboratory

