



Follow-up on anaerobes

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Background

- MIC breakpoints only
 - Gram-positive anaerobes
 - Gram-negative anaerobes
- MIC determination:
 - Agar dilution
 - Gradient tests
- A disk diffusion method was lacking
- MIC breakpoints for anaerobes were reviewed by EUCAST → species-specific breakpoints!

EUCAST disk diffusion method for anaerobic bacteria

Medium	FAA-HB: Fastidious Anaerobe Agar with 5% mechanically defibrinated horse blood
Inoculum	McF 1.0
Incubation	Anaerobic environment 35-37°C 16-20 h

Species and antimicrobial agents

Species	<ul style="list-style-type: none">• <i>Bacteroides</i> spp.<ul style="list-style-type: none">• Including <i>Parabacteroides</i> spp. and <i>Phocaeicola dorei/vulgatus</i>• <i>Prevotella</i> spp.• <i>Fusobacterium necrophorum</i>• <i>Clostridium perfringens</i>• <i>Cutibacterium acnes</i>
Agents	<ul style="list-style-type: none">• Benzylpenicillin• Piperacillin-tazobactam• Meropenem• Vancomycin• Clindamycin• Metronidazole

Method development

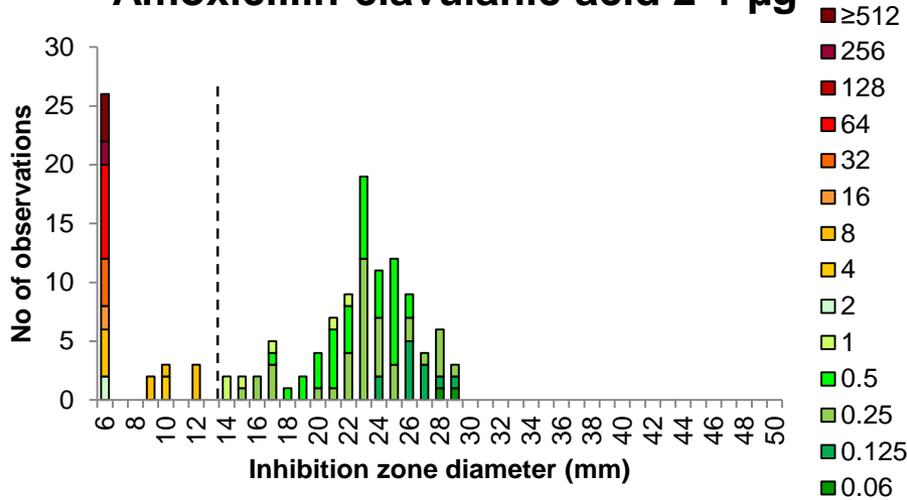
- Disk diffusion:
 - Media from several manufacturers
 - In-house and commercial
 - Different anaerobic incubations:
 - Workstation
 - Gas-generating envelopes
 - Anoxomat
- Reference Agar dilution MICs
 - UK Anaerobe Reference Unit, Cardiff
- External validation: Field trial 16 laboratories

Additional agents

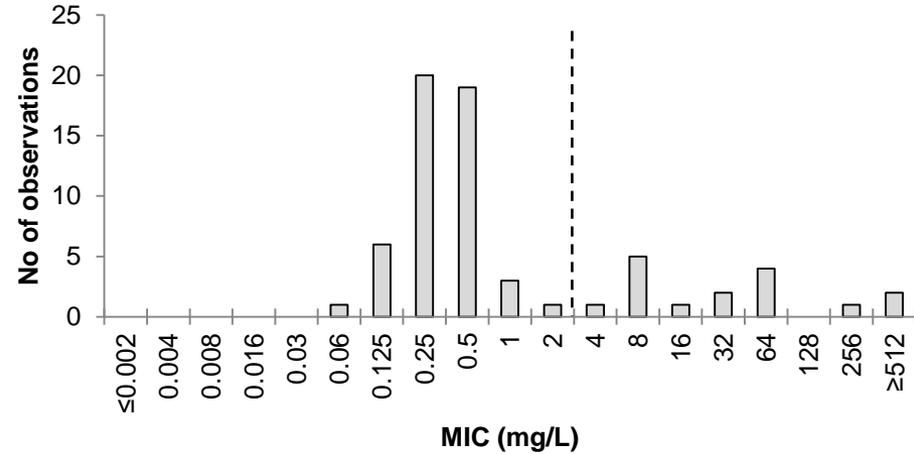
Antimicrobial agent	<i>Bacteroides</i> spp.	<i>Prevotella</i> spp.	<i>F. necrophorum</i>	<i>C. perfringens</i>	<i>C. acnes</i>
Ampicillin 2 µg		•	•	•	•
Ampicillin-sulbactam 10-10 µg	•	•	•	•	•
Amoxicillin (MIC only)		•	•	•	•
Amoxicillin-clavulanic acid 2-1 µg	•	•	•	•	•
Cefotaxime 5 µg					•
Ceftriaxone 30 µg					•
Ertapenem 10 µg	•	•	•	•	•
Imipenem 10 µg	•	•	•	•	•
Linezolid 10 µg					•

Bacteroides spp.

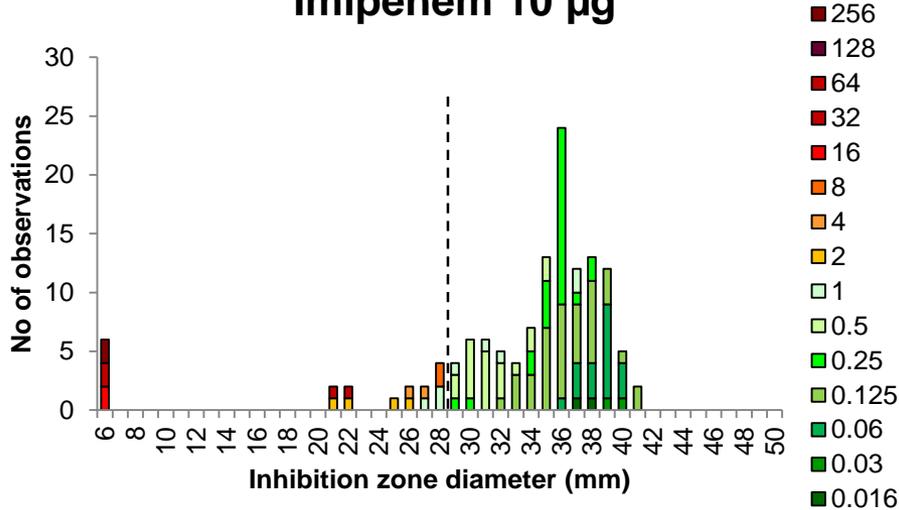
Amoxicillin-clavulanic acid 2-1 μ g



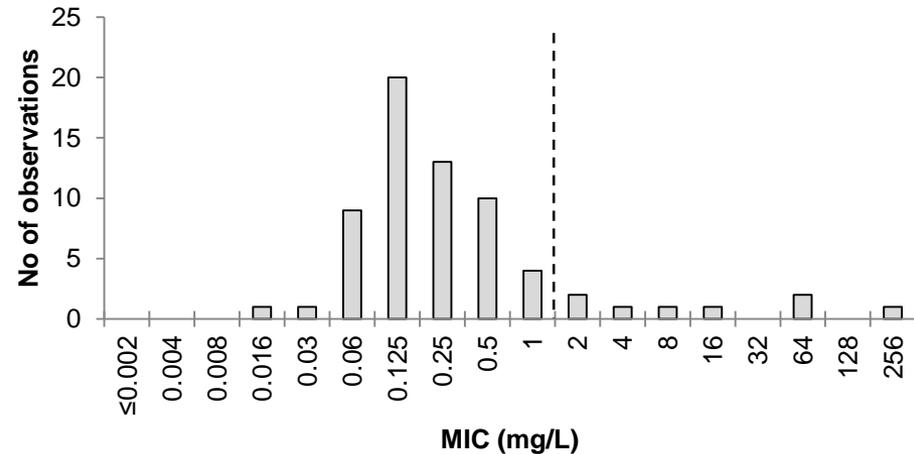
Amoxicillin-clavulanic acid MIC



Imipenem 10 μ g



Imipenem MIC



Testing of beta-lactam agents

New recommendation for *Prevotella* spp.,
F. necrophorum, *C. perfringens* and *C. acnes*:

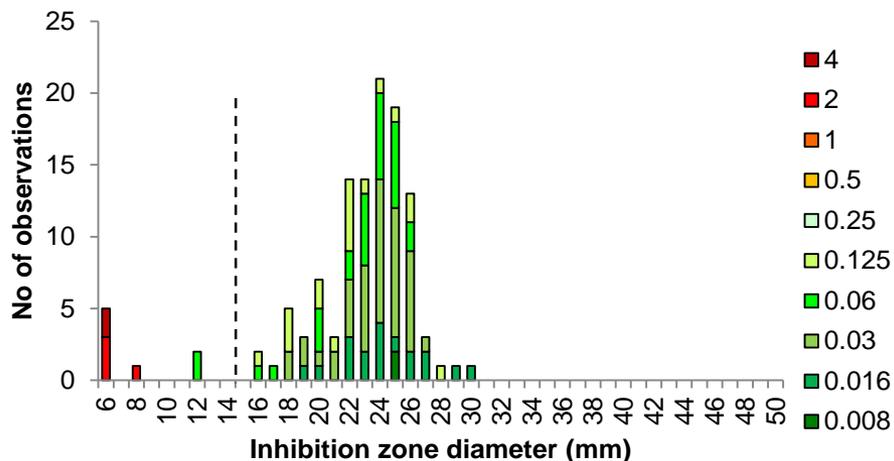
- Isolates susceptible to benzylpenicillin (zone or MIC) can be reported susceptible to all beta-lactam agents with breakpoints (including those with Note)* without further testing.

*Ampicillin, ampicillin-sulbactam, amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, ertapenem, imipenem and meropenem.

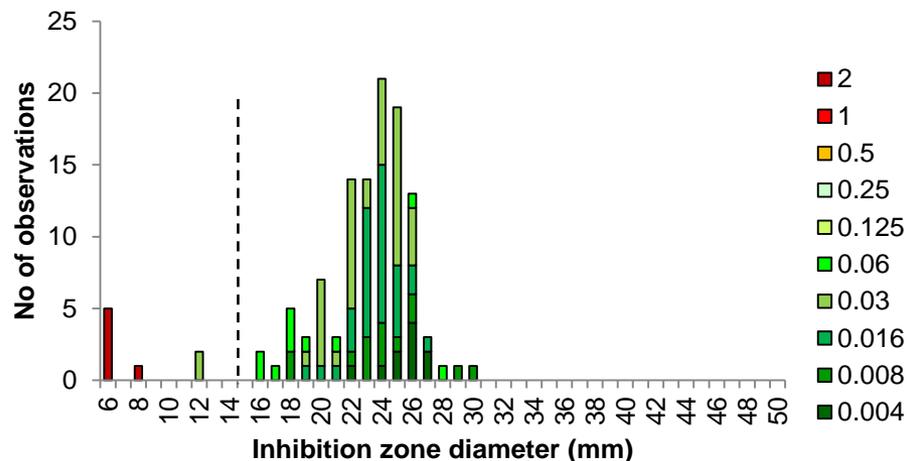
- Isolates resistant to benzylpenicillin should be tested to individual agents.

Clostridium perfringens

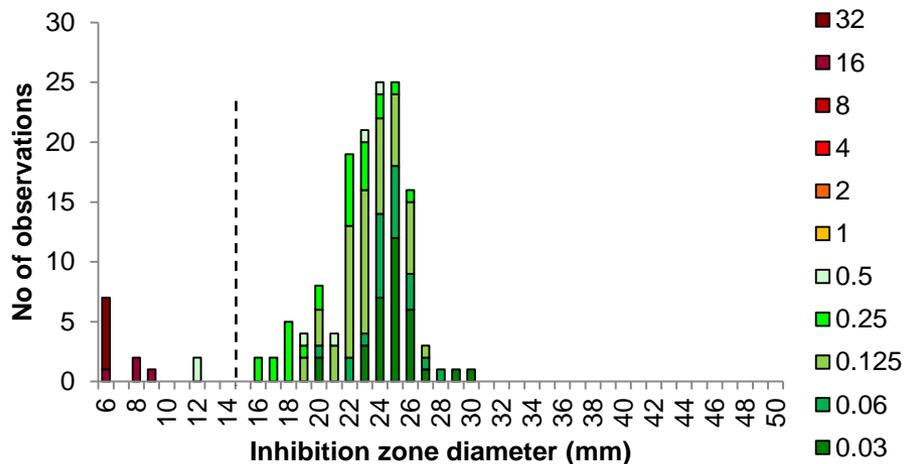
Benzylopenicillin 1 unit vs. Amoxicillin MIC



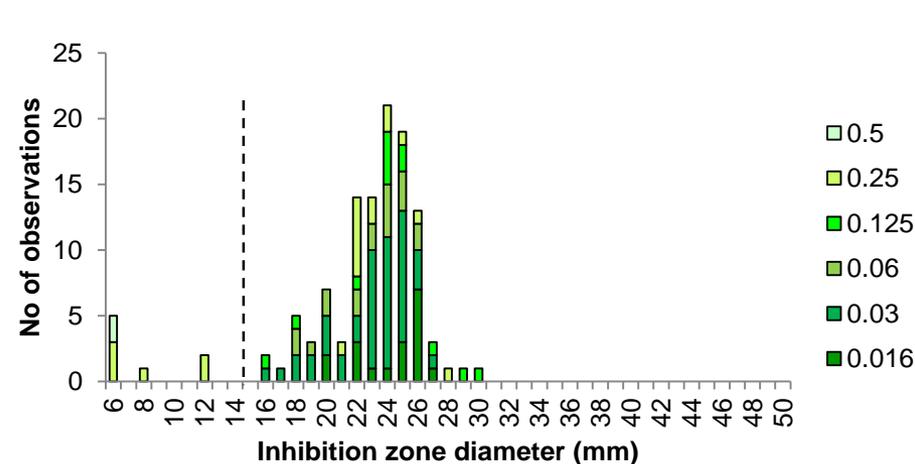
Benzylopenicillin 1 unit vs. Amox-clav MIC



Benzylopenicillin 1 unit vs. Pip-tazo MIC



Benzylopenicillin 1 unit vs. Imipenem MIC



Frequently asked questions

How can we confirm that our disk diffusion testing of anaerobic bacteria performs as expected?

Quality control

- To monitor test performance:
 - *B. fragilis* ATCC 25285
 - *C. perfringens* ATCC 13124
- To monitor the anaerobic atmosphere:
 - *C. perfringens* DSM 25589 (CCUG 75076, NCTC 14679) with metronidazole 5 µg.
- Follow up (4-6 months after implementation)
 - Review routine QC data
 - Compare zone diameter distributions for clinical isolates to reference distributions.

Where can we buy FAA-HB media?

FAA media

- **FAA powder** is available from several manufacturers: Neogen, E&O, Bioconnections *etc.*
- **Pre-poured FAA-HB plates** are available from SSI Diagnostica, Liofilchem, Xebios, Thermo Fisher *etc.*

- No additives, other than 5% horse blood.
- Agar depth 4.0 mm.
- Comply with EUCAST QC criteria for anaerobic QC strains.

**Can we perform susceptibility testing
from cultures on selective media?**

Inoculum preparation

- It is a general recommendation to avoid picking colonies from selective media for susceptibility testing.
 - Same as for aerobic bacteria
- As with all standardized methods, any modification to the method must be validated by the laboratory!

If growth is insufficient after 16-20 hours, can we incubate susceptibility plates another 24 hours?

Incubation time

- No, the breakpoints are only valid for 16-20 h incubation!
- Prolonged incubation beyond 20 hours affect zone sizes significantly.
- If growth is insufficient after 16-20 hours incubation:
 - Repeat the disk diffusion test or
 - Perform and MIC test

Testing of *Cutibacterium acnes* can be challenging. How can we improve testing of *C. acnes*?

Cutibacterium acnes

- Growth is usually pale with very small colonies on FAA-HB media.
- Zone diameters for beta-lactam agents are usually large.
- Streak plates extra carefully to ensure there are no gaps between streaks.
- Limit the number of disks per plate to allow good growth and avoid overlapping of zones.
 - Optimally 2 disks per plate!

We get too small zones for the anaerobic QC strains with several agents. How can we improve this?

Inoculation and media

- For *B. fragilis* ATCC 25285 and *C. perfringens* ATCC 13124, remove excess fluid by turning the swab against the inside of the tube to avoid over-inoculation.
- Humidity in FAA-HB media may result in fuzzy zone edges, swarming or haze within zones.
- FAA-HB plates must be dried at 20-25°C overnight or at 35°C for 15 min with the lid removed.

Reading of results

- Hold the plate about 30 cm from the eye at a 45-degree angle to the work bench when reading.
- If haze within the zone occurs, read the most obvious zone edge.
 - Tilt the plate towards you to better define the obvious zone edge.

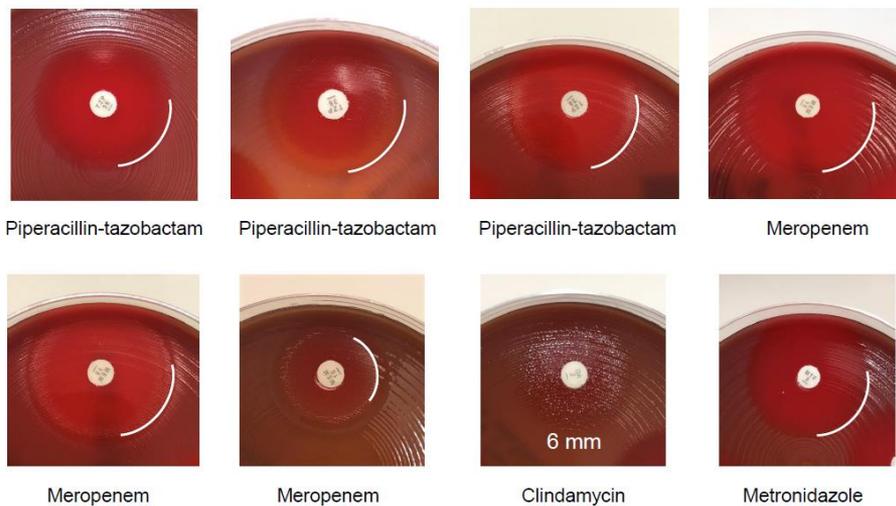


Reading guide

EUCAST disk diffusion for selected rapidly growing anaerobic bacteria on Fastidious Anaerobe Agar with 5% horse blood (FAA-HB)

Version 2.0
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Bacteroides spp.



Fusobacterium necrophorum



Clostridium perfringens



Thank you for your attention!



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