

# EUCAST disk diffusion testing of anaerobic bacteria – Expanding the range of antimicrobial agents

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## Introduction

In January 2022, EUCAST published a disk diffusion method for five frequently isolated genera/species of anaerobic bacteria. The method is based on Fastidious Anaerobe Agar with 5% mechanically defibrinated horse blood (FAA-HB) and 16-20 hours incubation in anaerobic environment. Initially, breakpoints were developed for six antimicrobial agents. Simultaneously, EUCAST revised the MIC breakpoints and defined species-specific breakpoints for the five genera/species.

## Objectives

The objective of this study was to produce MIC-zone diameter correlates for additional agents for EUCAST to expand the range of agents with clinical breakpoints for anaerobic bacteria.

## Methods

Antimicrobial susceptibility testing was performed on a collection of *Bacteroides* spp. (n=53), *Prevotella* spp. (n=49), *Fusobacterium necrophorum* (n=50), *Clostridium perfringens* (n=58) and *Cutibacterium acnes* (n=54), previously used for developing breakpoints for the initial six agents. Disk diffusion was performed at the EUCAST Development Laboratory (Växjö, Sweden) according to EUCAST methodology on FAA-HB based on FAA from two manufacturers; Neogen and E&O Laboratories. Agar dilution MICs were performed at the UK Anaerobe Reference Unit (Cardiff, UK), following CLSI methodology but on FAA-HB (recommended by EUCAST). The agents tested are listed in **Table 1**. Quality control was performed with *B. fragilis* ATCC 25285 and *C. perfringens* ATCC 13124 (45 tests each).

Table 1. Antimicrobial agents and species of anaerobic bacteria included in the study.

Antimicrobial agent	Disk content (µg)	<i>Bacteroides</i> spp. <sup>A</sup>	<i>Prevotella</i> spp.	<i>Fusobacterium necrophorum</i>	<i>Clostridium perfringens</i>	<i>Cutibacterium acnes</i>
Ampicillin	2		•	•	•	•
Amoxicillin (MIC only)	-		•	•	•	•
Amoxicillin-clavulanic acid <sup>B</sup>	2-1	•	•	•	•	•
Ampicillin-sulbactam <sup>C</sup>	10-10	•	•	•	•	•
Cefotaxime (disk diffusion only)	5					•
Ceftriaxone	30					•
Ertapenem	10	•	•	•	•	•
Imipenem	10	•	•	•	•	•
Linezolid	10					•

A. Including *Parabacteroides* spp. and *Phocaeicola dorei/vulgatus*.

B. For MIC testing, the concentration of clavulanic acid was fixed at 2 mg/L.

C. For MIC testing, the concentration of sulbactam was fixed at 4 mg/L.

## Conclusions

This study demonstrates that the EUCAST disk diffusion method for anaerobic bacteria is suitable for an extended range of clinically important agents. Based on these results, EUCAST has recently completed a consultation on proposed MIC breakpoints and is prepared to soon publish species-specific clinical breakpoints for these agents as an addendum to the Breakpoint Tables during 2023. Offering breakpoints for more agents will be important in an era of increasing resistance among anaerobic bacteria.

## Results

Overall, the correlation between MIC and zone diameters for the agents and species tested was good (Examples in **Figure 1**). The ampicillin 2-µg disk was a good predictor of amoxicillin susceptibility. For *B. fragilis* (n=35), all *cfiA* positive isolates had ertapenem zone diameters <26 mm. For *C. perfringens*, isolates resistant to benzylpenicillin were clearly non-wild-type for ampicillin, amoxicillin, ampicillin-sulbactam and amoxicillin-clavulanic acid. Results for the quality control strains were reproducible and zone diameter distributions ranged from 4-6 mm.

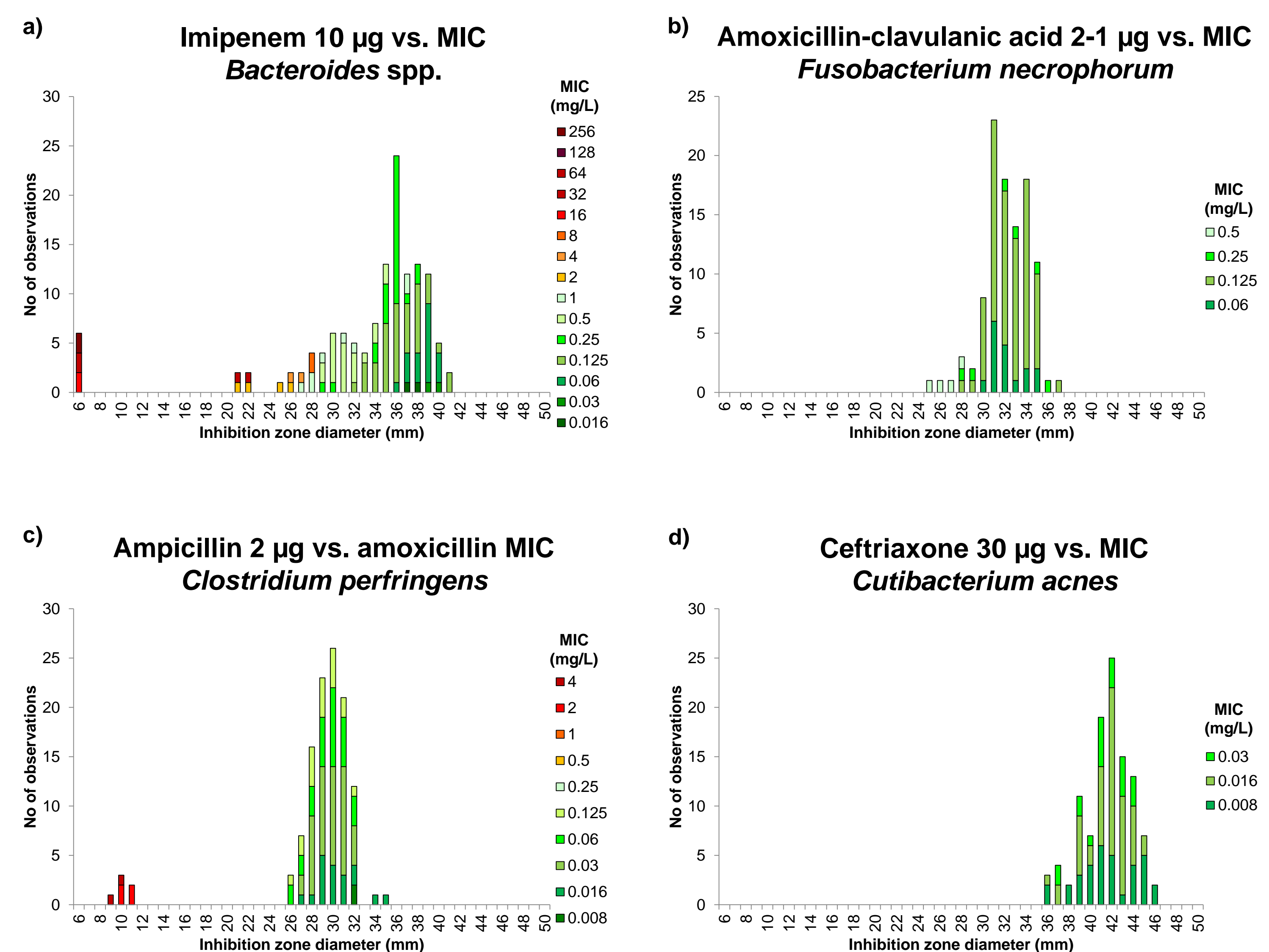


Figure 1. Zone diameter distributions for a) *Bacteroides* spp. (n=53) with imipenem, b) *F. necrophorum* (n=50) with amoxicillin-clavulanic acid, c) *C. perfringens* (n=58) with ampicillin zone vs. amoxicillin MIC and d) *C. acnes* (n=54) with ceftriaxone. All isolates were tested with FAA media from two manufacturers. MIC values are shown as coloured bars.