

# *Corynebacterium diphtheriae* and *C. ulcerans* – Development of clinical breakpoints using EUCAST methodology

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## Introduction

*Corynebacterium diphtheriae* and *C. ulcerans* can infect the nasopharynx and the skin. Toxigenic strains may cause diphtheria, a severe infection that can lead to breathing problems, paralysis, extensive organ damage and death. EUCAST provides clinical breakpoints for *Corynebacterium* spp., but these were developed for species other than *C. diphtheriae* and *C. ulcerans*.

## Objective

The objective of this study was to produce MIC-zone diameter correlates for *C. diphtheriae* and *C. ulcerans* to allow EUCAST to determine clinical breakpoints and disk diffusion correlates for these species.

## Methods

Antimicrobial susceptibility testing (AST) was performed at the National Reference Center for Diphtheria, Institut Pasteur (France) and the National Consiliary Laboratory for Diphtheria (Germany) on collections of 100 *C. diphtheriae* and 100 *C. ulcerans* isolates per site. Toxigenic and non-toxigenic strains with and without previous reported resistance were included. Broth microdilution was performed according to ISO 20776-1 on custom Sensititer panels (Thermo Scientific) in MH-F broth against 12 antimicrobial agents. Disk diffusion was performed according to EUCAST methodology on local MH-F media. The study design, quality control of materials and data analysis was performed by the EUCAST Development Laboratory.

## Results

The correlation between MIC and zone diameters was excellent for all agents tested (Examples in **Figure 1 a-d**). MIC distributions for *C. diphtheriae* and *C. ulcerans* were similar for all agents, except for clindamycin, where MICs were considerably higher for *C. ulcerans*. The benzylpenicillin 1-unit disk could detect benzylpenicillin, amoxicillin, cefotaxime and meropenem non-wild type isolates. MIC distributions were consistent between the two sites and for disk diffusion only minor differences were observed. The aggregated data from both sites suggests that *C. diphtheriae* and *C. ulcerans* can share clinical breakpoints.

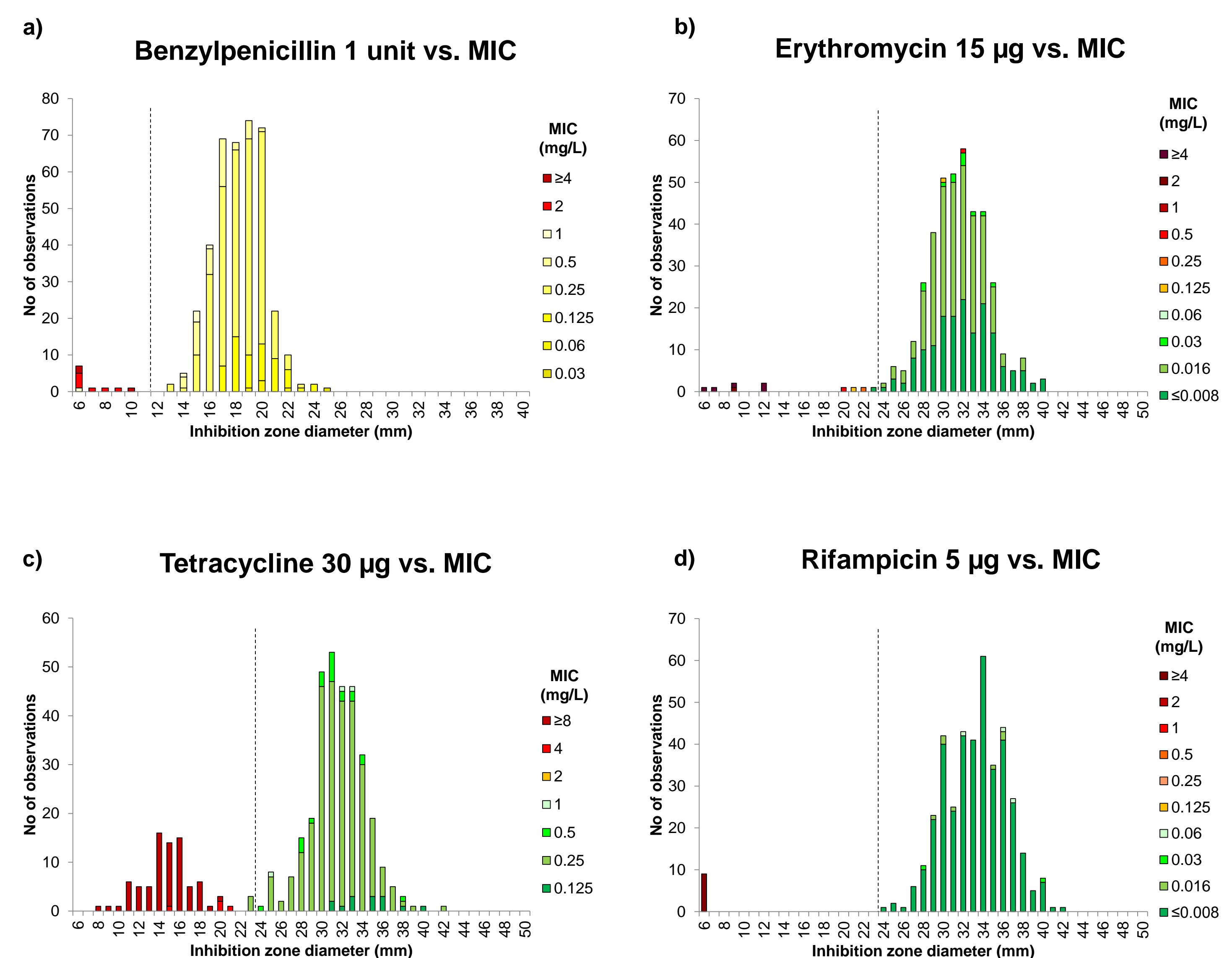


Figure 1. Zone diameter distributions based on aggregated data for *C. diphtheriae* (n=200) and *C. ulcerans* (n=200). MIC values are presented in different colours according to MIC breakpoints; susceptible standard dosing, S (green), susceptible increased exposure, I (yellow) and resistant, R (orange-red). EUCAST disk diffusion breakpoints are indicated by the dotted lines.

Table 1. EUCAST breakpoints for *Corynebacterium diphtheriae* and *C. ulcerans*.

Antimicrobial agent	MIC breakpoints (mg/L)		Disk content (µg)	Zone diameter breakpoints (mm)	
	S ≤	R >		S ≥	R <
Benzylpenicillin	0.001	1	1 unit	50	12
Amoxicillin	1 <sup>A</sup>	1 <sup>A</sup>		Note <sup>A</sup>	Note <sup>A</sup>
Cefotaxime	0.001 <sup>B</sup>	2 <sup>B</sup>	5	50 <sup>B</sup>	15 <sup>B</sup>
Meropenem	0.25 <sup>A</sup>	0.25 <sup>A</sup>	10	24 <sup>A</sup>	24 <sup>A</sup>
Ciprofloxacin	0.001	0.5	5	50	24
Erythromycin	0.06	0.06	15	24	24
Clindamycin, <i>C. diphtheriae</i>	0.5	0.5	2	15	15
Doxycycline	0.5	0.5		Note <sup>C</sup>	Note <sup>C</sup>
Tetracycline	1	1	30	24	24
Linezolid	2	2	10	25	25
Rifampicin	0.06	0.06	5	24	24
Trimethoprim-sulfamethoxazole	0.5	0.5	1.25-23.75	23	23

Note A. Isolates "susceptible, increased exposure" (I) to benzylpenicillin can be reported S to amoxicillin and meropenem.

Isolates resistant to benzylpenicillin should be tested for susceptibility to the individual agent or reported R.

Note B. Susceptibility to cefotaxime can be inferred from benzylpenicillin.

Note C. Susceptibility to doxycycline can be inferred from tetracycline.

## Conclusions

EUCAST methodology for AST of *Corynebacterium* spp. can also be used for *C. diphtheriae* and *C. ulcerans*. However, these species require separate clinical breakpoints. Based on the results of this study EUCAST has established clinical MIC and zone diameter breakpoints for *C. diphtheriae* and *C. ulcerans* (**Table 1**). These were published in the EUCAST Breakpoint Tables v.13.0, 2023.