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Adjusting EUCAST zone diameter breakpoints for *Haemophilus influenzae* on the Mueller-Hinton Fastidious medium.

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Introduction

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has published clinical MIC and zone diameter breakpoints for *Haemophilus influenzae* (HI). Zone diameter breakpoints were developed for the new Mueller-Hinton Fastidious medium (MH-F) and have been tentative since 2010.

Objective

The objective of this study was to optimise EUCAST zone diameter breakpoints for HI using broth microdilution (BMD) as reference.

Methods

A total of 150 clinical isolates of HI were selected from the SENTRY collection (JMI Laboratories, USA). The collection was intentionally biased towards beta-lactam-resistant strains, including beta-lactamase positive strains and those with PBP mutations (*i.e.* BLNAR). Disk diffusion was performed at the EUCAST Laboratory according to EUCAST methodology on Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F), using agar from two manufacturers. MIC values were determined by BMD, in Mueller-Hinton broth with 5% lysed horse blood and 20 mg/L β-NAD (MH-F broth), at JMI Laboratories. BMD was performed on custom panels (TREK Diagnostics/Thermo Fisher Scientific) and the horse blood was lysed by repeated freezing and thawing. The presence of PBP mutations was determined by PCR. Data were analysed by EUCAST for antimicrobial agents with both MIC and zone diameter breakpoints in EUCAST tables. Very major, major and minor errors (VME, ME and mE) were calculated.

Table 1. EUCAST clinical breakpoints for *Haemophilus influenzae*.
EUCAST Clinical Breakpoint Table v. 2.0, January 2012.
New breakpoints highlighted in yellow, with old breakpoints in parenthesis.

Antimicrobial agent	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Ampicillin	1	1	**2**	16	16
Amoxicillin-clavulanate	2 (1)	2 (1)	**2-1**	17 (20*)	17 (20*)
Cefepime	0.25	0.25	**30**	27 (25)	27 (25)
Cefixime	0.12	0.12	**5**	25 (22)	25 (22)
Cefotaxime	0.12	0.12	**5**	26 (22)	26 (22)
Cefpodoxime	0.25	0.5	**10**	26 (24)	23 (21)
Ceftibuten	1	1	**30**	25 (24)	25 (24)
Ceftriaxone	0.12	0.12	**30**	30 (27)	30 (27)
Cefuroxime	1	2	**30**	26 (25)	25 (22)
Cefuroxime axetil	0.12	1	**30**	50	26 (25)
Doripenem	1	1	**10**	20	20
Ertapenem	0.5	0.5	**10**	20	20
Imipenem	2	2	**10**	20 (16)	20 (16)
Meropenem (non-meningitis)	2	2	**10**	20	20
Ciprofloxacin	0.5	0.5	**5**	26 (23)	26 (23)
Levofloxacin	1	1	**5**	26 (21)	26 (21)
Moxifloxacin	0.5	0.5	**5**	25 (23)	25 (23)
Ofloxacin	0.5	0.5	**5**	23 (21)	23 (21)
Erythromycin	0.5	16	**15**	50	10 (12)
Telithromycin	0.12	8	**15**	50	12 (14)
Minocycline	1	2	**30**	24 (25)	21 (22)
Tetracycline	1	2	**30**	25 (24)	22 (21)
Chloramphenicol	2 (1)	2	**30**	28	28 (25)
Rifampicin	1 (0.5)	1 (0.5)	**5**	18	18
Trimethoprim-sulfamethoxazole	0.5	1	**1.25-23.75**	23	20

* Old zone diameter breakpoint for amoxicillin-clavulanate 20-10 µg.

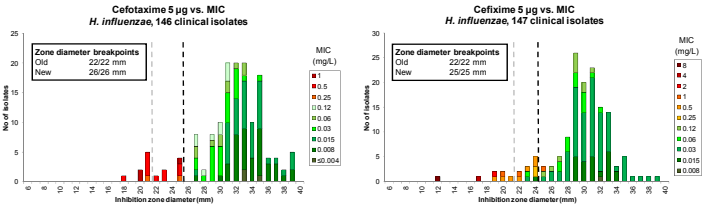

Figure 1
Inhibition zone distributions for *H. influenzae* with cefotaxime to the left and cefixime to the right, with MIC values shown as coloured bars. All isolates resistant by MIC were positive for PBP mutations.

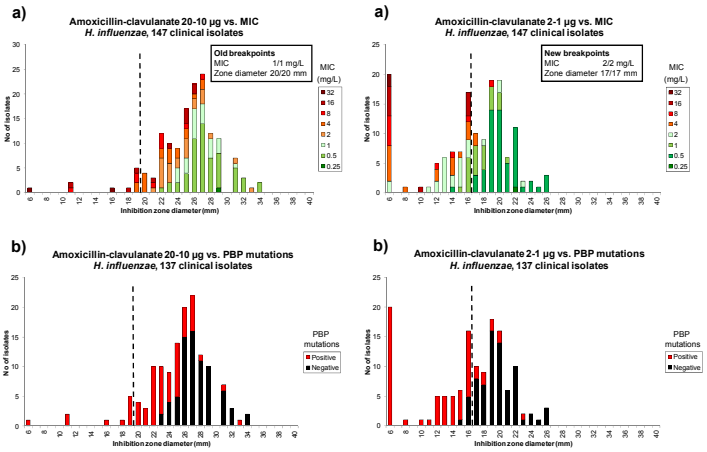

Figure 2
Inhibition zone distributions for *H. influenzae* with amoxicillin-clavulanate 20-10 µg to the left and 2-1 µg to the right, showing **a)** MIC values as coloured bars **b)** positive (red) and negative (black) for PBP mutations.

Results & Discussion

Correlation of MIC values and inhibition zones resulted in an adjustment of breakpoints for HI as presented in **Table 1**. Several zone diameter breakpoints were increased (most often 1-3 mm) to reduce VMEs (examples in **Figure 1**). For amoxicillin-clavulanate, chloramphenicol and rifampicin the MIC breakpoints were adjusted by the EUCAST. New breakpoints resulted in overall error rates (%) as follows (rates for old breakpoints in parenthesis): VME 1.5 (4.0), ME 2.1 (0.8) and mE 0.8 (1.4). For amoxicillin-clavulanate, a change in MIC breakpoints and disk potency (from 20-10 µg to 2-1 µg), lowered the VME for this antibiotic from 36.7 to 2.0 % (**Figure 2a**). Furthermore, correlation to PBP mutations was much better with the 2-1 µg disk (**Figure 2b**). The bias towards BLNAR isolates in this collection resulted in high error rates for some beta-lactam antibiotics, but the occurrence of such strains is rare among clinical isolates.

Conclusion

An analysis of the MIC/zone diameter correlates for *Haemophilus influenzae* led to adjustment of zone diameter breakpoints for several antimicrobials. The MIC breakpoints were adjusted for three antimicrobial agents. Revised breakpoints for HI are published in the EUCAST Clinical Breakpoint Table v. 2.0 and valid from January 2012.

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