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Introduction

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recently established clinical MIC breakpoints for *Corynebacterium* spp. (available in the EUCAST Breakpoint Table v. 4.0) and a disk diffusion method based on the EUCAST fastidious medium (MH-F).

Objectives

The objective of this study was to produce MIC and zone diameter correlations using EUCAST methods and to establish zone diameter breakpoints for *Corynebacterium* spp.

Methods

Antimicrobial susceptibility testing was performed on a collection of 258 clinical isolates of *Corynebacterium* spp. of different geographical origin: Kronoberg county, Sweden; Santander, Spain and isolates from the worldwide SENTRY Antimicrobial Surveillance Program (provided by JMI Laboratories, USA). Species identification was performed with MALDI-TOF MS. Disk diffusion was performed according to EUCAST methodology for fastidious organisms on Mueller-Hinton (MH) agar with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) using in-house prepared plates with agar from two manufacturers (BBL/BD and Oxoid/Thermo Fischer Scientific). MIC determination was performed with broth microdilution (BMD) according to ISO standard 20776-1 using EUCAST media for fastidious organisms (cation-adjusted MH broth with 5% lysed horse blood and 20 mg/L β-NAD). Sealed BMD plates were incubated in ambient air at 35°C. If standard incubation for 16-20 h did not result in sufficient growth, incubation was prolonged to a total time of 40-44 h, both for disk diffusion and BMD. Antimicrobial agents tested are listed in **Table 1**. Very major, major and minor errors (VME, ME and mE) for disk diffusion with the proposed zone diameter breakpoints were calculated according to ISO standard 20776-2 with BMD as reference.

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Results

The collection of *Corynebacterium* spp. (n=258) comprised 20 different species, with *C. striatum* (n=78), *C. amycolatum* (n=63), *C. jeikeium* (n=35), *C. pseudodiphtheriticum* (n=20) and *C. urealyticum* (n=18) dominating. Prolonged incubation (totally 40-44 h) was needed to achieve sufficient growth for 11% (disk diffusion) and 16% (BMD) of the isolates. This was mainly for *C. jeikeium*, *C. urealyticum* and *C. pseudodiphtheriticum*.

The correlation between MIC and zone diameters for *Corynebacterium* spp. was excellent, despite the high number of different species represented. It was apparent that MICs varied between species, but it did not affect the MIC-zone diameter correlation per se (**Figure 1**). Zone diameter breakpoints were established to minimize the occurrence of VME, resulting in overall error rates as follows: VME 0.13, ME 0.99, and mE 0.04 %, see **Table 1**. The corresponding values after excluding vancomycin and linezolid because of the lack of resistant isolates are: VME 0.17, ME 1.27 and mE 0.06 %.

MIC-zone diameter correlations for *Corynebacterium* spp. based on the results in this study are available on the EUCAST website:

http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation/.

Conclusions

The results from this study show that antimicrobial susceptibility testing of *Corynebacterium* spp. can be reliably performed using EUCAST methodology. Based on these data, EUCAST has established zone diameter breakpoints for *Corynebacterium* spp. calibrated to the clinical MIC breakpoints. These are available in the EUCAST Breakpoint Table v. 4.0, January 2014.

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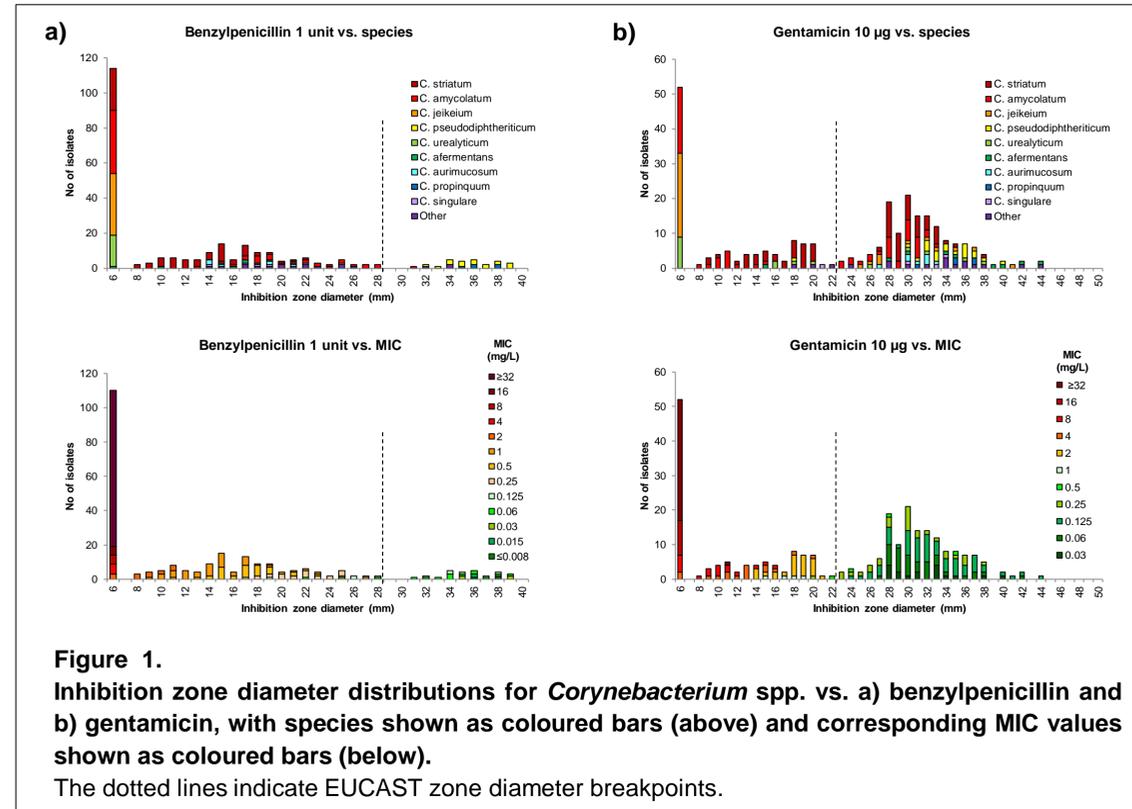


Figure 1. Inhibition zone diameter distributions for *Corynebacterium* spp. vs. a) benzylpenicillin and b) gentamicin, with species shown as coloured bars (above) and corresponding MIC values shown as coloured bars (below). The dotted lines indicate EUCAST zone diameter breakpoints.

Table 1. EUCAST breakpoints for *Corynebacterium* spp. with categorical errors for disk diffusion.

Antimicrobial agent	MIC breakpoints (mg/L)		Zone diameter breakpoints (mm)			Categorical errors					
	S ≤	R >	Disk content (µg)	S ≥	R <	VME	VME (%)	ME	ME (%)	mE	mE (%)
Benzylpenicillin	0.12	0.12	1 unit	29	29	0	0	8	3.1	-	-
Ciprofloxacin	1	1	5	25	25	0	0	2	0.8	-	-
Moxifloxacin	0.5	0.5	5	25	25	0	0	5	1.9	-	-
Gentamicin	1	1	10	23	23	0	0	6	2.3	-	-
Vancomycin	2	2	5	17	17	0*	0*	0*	0*	-	-
Clindamycin	0.5	0.5	2	20	20	0	0	2	0.8	-	-
Tetracycline	2	2	30	24	24	3	1.2	0	0	-	-
Linezolid	2	2	10	25	25	0*	0*	0*	0*	-	-
Rifampicin	0.06	0.5	5	30	25	0	0	0	0	1	0.4

* For vancomycin and linezolid, only susceptible isolates were included.
S = Susceptible, R = Resistant, VME = Very Major Error, ME = Major Error, mE = minor Error