

Toxigenic and non-toxigenic *Vibrio cholerae* can share the same MIC breakpoints

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Introduction

Vibrio cholerae is the causative agent of the acute diarrhoeal disease cholera. Although often mild and self-limiting, one in ten people with cholera develop severe symptoms which requires antimicrobial treatment. Prior to this study, standardised antimicrobial susceptibility testing guidelines with EUCAST methodology were lacking for *Vibrio* spp., including *V. cholerae*.

Objective

As part of EUCAST's recent efforts to set clinical breakpoints for *Vibrio* spp., we investigated if toxigenic and non-toxigenic *V. cholerae* isolates could share the same MIC breakpoints.

Methods

An international collection comprising 163 *V. cholerae* isolates was included. MIC determination was performed with broth microdilution (BMD) in accordance with ISO 20776-1 using un-supplemented Mueller-Hinton broth on freeze-dried Sensititre panels (Thermo Scientific, UK) against 11 antimicrobial agents from seven antimicrobial classes (Table 1). The non-toxigenic isolates ($n = 116$) were tested at the EUCAST Development Laboratory (EDL, Växjö, Sweden), whereas the toxigenic isolates ($n = 47$) were tested at the Centers for Disease Control and Prevention (CDC, Atlanta, USA) where they were originally isolated to avoid shipping of a potential biothreat. All isolates were tested using the same batch of BMD panels and following the same reading instructions. Quality control (QC) of the BMD panels was performed at the EDL before they were shipped to the CDC where QC was repeated before testing of study isolates. *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 were used for the QC of BMD.

Table 1. Antimicrobial agents included in broth microdilution (BMD) testing of *Vibrio cholerae*

Antimicrobial agent	Concentration range in BMD panel (mg/L)
Piperacillin-tazobactam ¹	0.06-16
Cefotaxime	0.016-4
Ceftazidime	0.06-4
Meropenem	0.002-2
Ciprofloxacin	0.001-1
Levofloxacin	0.002-2
Azithromycin	0.06-8
Erythromycin	0.5-16
Doxycycline	0.06-4
Tetracycline	0.125-8
Trimethoprim-sulfamethoxazole ²	0.008-2

¹ Fixed concentration of tazobactam at 4 mg/L.

² Trimethoprim-sulfamethoxazole in the ratio 1:19, concentrations are expressed for the trimethoprim component.

Results

MIC distributions were generated using BMD results obtained for 163 *V. cholerae* isolates. The analysis of aggregated MIC data revealed that the toxigenic and non-toxigenic isolates mostly behave uniformly and that the putative wild-type isolates exhibit symmetrical, mono-modal MIC distributions covering 3 to 4 dilutions (Figure 1). Resistant isolates were observed for each of the agents tested. Ciprofloxacin and levofloxacin distributions demonstrated low- and high-level resistant populations with the latter being clearly above PK/PD breakpoints. The EUCAST Steering Committee established MIC breakpoints for nine of the 11 tested agents (Table 2). Based on the results of this study, the committee determined that the toxigenic and non-toxigenic *Vibrio cholerae* could share the same MIC clinical breakpoints.

Table 2. EUCAST MIC clinical breakpoints for *Vibrio* spp., including toxigenic and non-toxigenic *V. cholerae* (EUCAST breakpoint tables v. 13.0, 2023)

Antimicrobial agent	MIC breakpoints (mg/L)	
	S ≤	R >
Piperacillin-tazobactam ¹	1	1
Cefotaxime	0.25	0.25
Ceftazidime	1	1
Meropenem	0.5	0.5
Ciprofloxacin	0.25	0.25
Levofloxacin	0.25	0.25
Azithromycin	4	4
Doxycycline	0.5	0.5
Trimethoprim-sulfamethoxazole ²	0.25	0.25

¹ Fixed concentration of tazobactam at 4 mg/L.

² Trimethoprim-sulfamethoxazole in the ratio 1:19, breakpoints are expressed for the trimethoprim component.

Conclusions

In this study, we have demonstrated that for 11 antimicrobial agents from seven different classes, MIC distributions are similar for toxigenic and non-toxigenic *V. cholerae*, and these can therefore share the same clinical breakpoints.

With the MIC distributions generated in this study, EUCAST was able to determine clinical MIC breakpoints for *Vibrio* spp., including toxigenic and non-toxigenic *V. cholerae* (EUCAST breakpoint tables v. 13.0, 2023).

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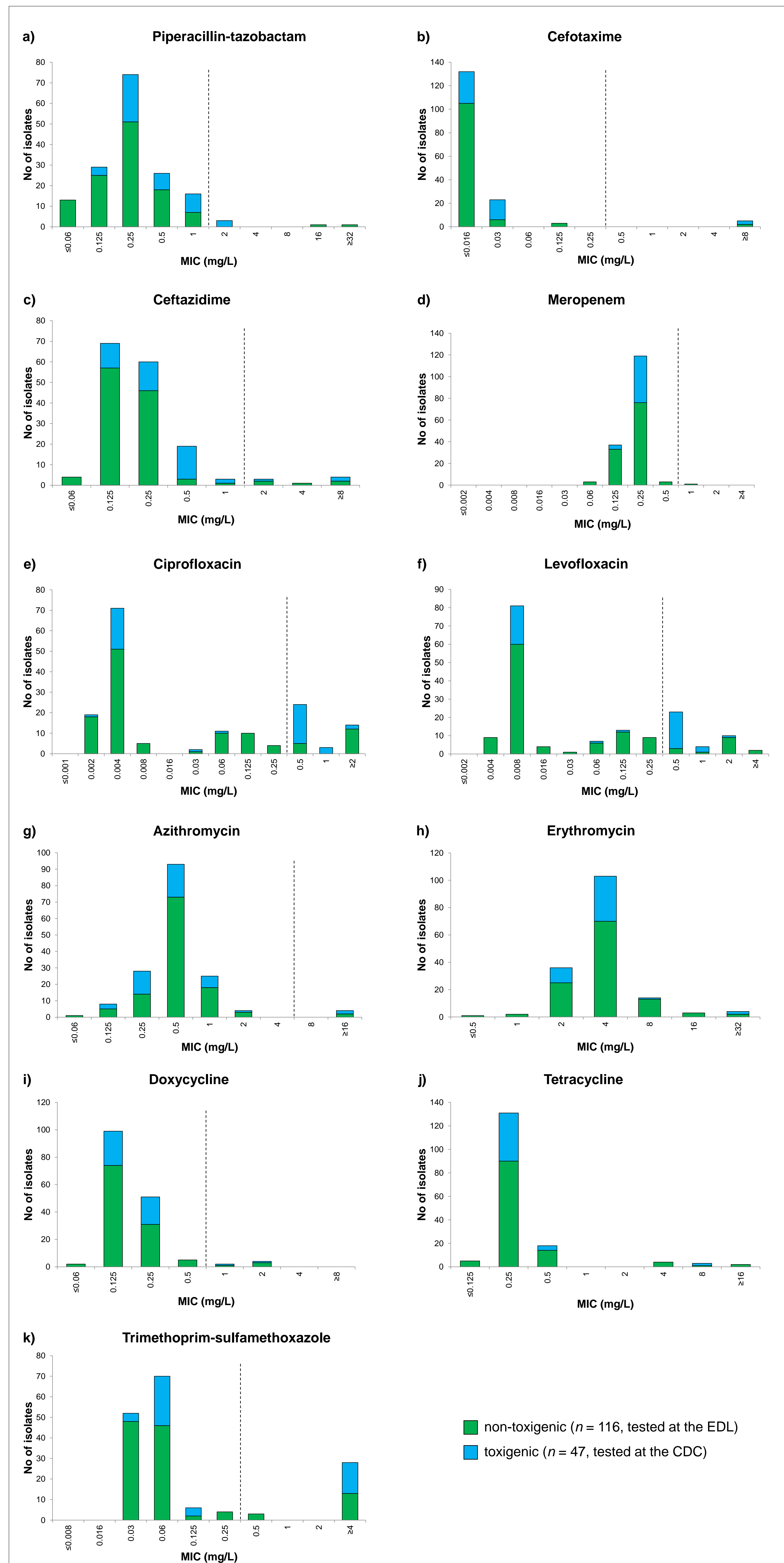


Figure 1. MIC distribution histograms for *Vibrio cholerae* isolates ($n = 163$; aggregated data for non-toxigenic and toxigenic isolates). Non-toxigenic ($n = 116$) and toxigenic ($n = 47$) *V. cholerae* isolates are indicated by the colours green and blue, respectively. The scale of the x-axis corresponds exactly to concentrations used in broth microdilution panels for each antibiotic. Where available, EUCAST MIC clinical breakpoints for *Vibrio* spp., including toxigenic and non-toxigenic *V. cholerae* (v. 13.0) are shown as dotted lines.

Acknowledgements

We thank Centers for Disease Control and Prevention (CDC), Atlanta, USA for testing the toxigenic *V. cholerae* isolates. We would like to express our gratitude to below listed institutions for their contribution of study isolates: **Finland:** Finnish Institute for Health and Welfare, Helsinki; **India:** ICMR-National Institute of Cholera & Enteric Diseases, Kolkata; **Norway:** Norwegian Institute of Public Health, Oslo; **Spain:** University of Cádiz, Cádiz; **Sweden:** Public Health Agency of Sweden, Solna; **United Kingdom:** UK Health Security Agency, London; **United States:** Centers for Disease Control and Prevention, Atlanta.