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Determination of disk diffusion susceptibility testing interpretive criteria using model-based analysis: development and implementation

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ABSTRACT

The determination of diffusion test breakpoints has become a challenging issue due to the increasing resistance of microorganisms to antibiotics. Currently, the most commonly-used method for determining these breakpoints is the modified error-rate bounded method. Its use has remained widespread despite the introduction of several model-based methods that have been shown superior in terms of precision and accuracy. However, the computational complexities associated with these new approaches has been a significant barrier for clinicians. To remedy this, we developed and examine the utility of a free online software package designed for the determination of diffusion test breakpoints: dBETS (**d**iffusion **B**reakpoint **E**stimation **T**esting **S**oftware). This software package allows clinicians to easily analyze data from susceptibility experiments through visualization, error-rate bounded, and model-based approaches. We analyze four publicly available data sets from the Clinical and Laboratory Standards Institute using dBETS.

1. Introduction

Multiple agencies have issued serious warnings regarding the future impact of antibiotic resistance (Andersson and Hughes, 2010; BBC, 2013; Davies and Davies, 2010; Hawser, 2012; World Health Organization, 2014). A contributor in helping detect and control this resistance is the appropriate selection of disk diffusion correlates (breakpoints) that are compared to minimum inhibitory concentration breakpoints (Turnidge and Paterson, 2007).

The disk diffusion procedure is a long-established diagnostic laboratory method for determining susceptibility to antimicrobial agents, even preceding the routine use of dilution procedures (Acar and Goldstein, 1991). With the evolution of susceptibility testing, and particularly following the International Collaborative Study (Ericsson and Sherris, 1971), the focus shifted to the use of the minimum inhibitory concentration (MIC) as a means of determining antimicrobial susceptibility. The MIC assay, in the broth micro dilution format, is now the reference standard to which all other methods must be compared (International Organization for Standardization (ISO), 2006). The MIC assay determines the lowest concentration of an antimicrobial agent that completely or near completely inhibits the visible growth of a microorganism. The MICs are compared to previously established interpretive criteria, or breakpoints, to categorize the strains as "susceptible", "intermediate", or "resistant" (Turnidge and Paterson, 2007). This concentration is compared to the agent-specific MIC breakpoints to determine if the agent is likely to be clinically effective for treatment.

The diffusion test reports results in terms of the diameter (DIA) of complete growth inhibition (the clear zone) around an antimicrobial disk of known potency. Unfortunately, there is not a straightforward conversion from diameter to MIC; as a consequence, a range of methods have been developed over the years for correlating disk diffusion zone diameters with MICs, starting with linear regression (Ericsson and Sherris, 1971).

The first departure from the use of linear regression to estimate the disk diffusion breakpoints was that of Metzler and DeHaan in 1974 (Metzler and DeHaan, 1974). This became known as the error-rate bounded method (ERB), as zone diameter selection was made using predetermined error percentages for "very major", "major", and "minor" errors of categorization. Two modifications of the error-rate bounded method emerged subsequently, that of Brunden et al. and that published by the Clinical and Laboratory Standards Institute (CLSI; called the National Committee for Clinical Laboratory Standards at the time) (Brunden et al., 1992; NCCLS document M23-A2, 2001). While relatively simple and intuitive, these approaches lack precision and accuracy because they do not fully account for the assay characteristics, such as rounding and inherent within-lab variability (Annis and Craig, 2005; Craig, 2000).

Building off linear regression, more sophisticated model-based approaches have recently been proposed (Craig, 2000; DePalma, 2013;

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Table 1
Description of the data sets used in this paper. For MIC breakpoints (mg/L), the first value is the ≤ susceptible breakpoint and the second value is the ≥ resistant breakpoint. For DIA breakpoints (mm), the first value is the ≤ resistant breakpoint and the second value is the ≥ susceptible breakpoint.

Organism	Agent	Abbreviation	Date	N	*Breakpoints*			
					MIC		DIA	
Acinetobacter	Doripenem	DOR ACIN	06/2011	77	2	8	16	18
Staphylococcus	Doripenem	DOR STAP	06/2011	395	0.5	.	.	30
Enterobacteriaceae	Ertapenem	ERT EB	06/2011	948	0.5	2	18	22
Escherichia coli	Pradofloxacin	PRA ECOL	06/2013	312	0.25	2	19	24

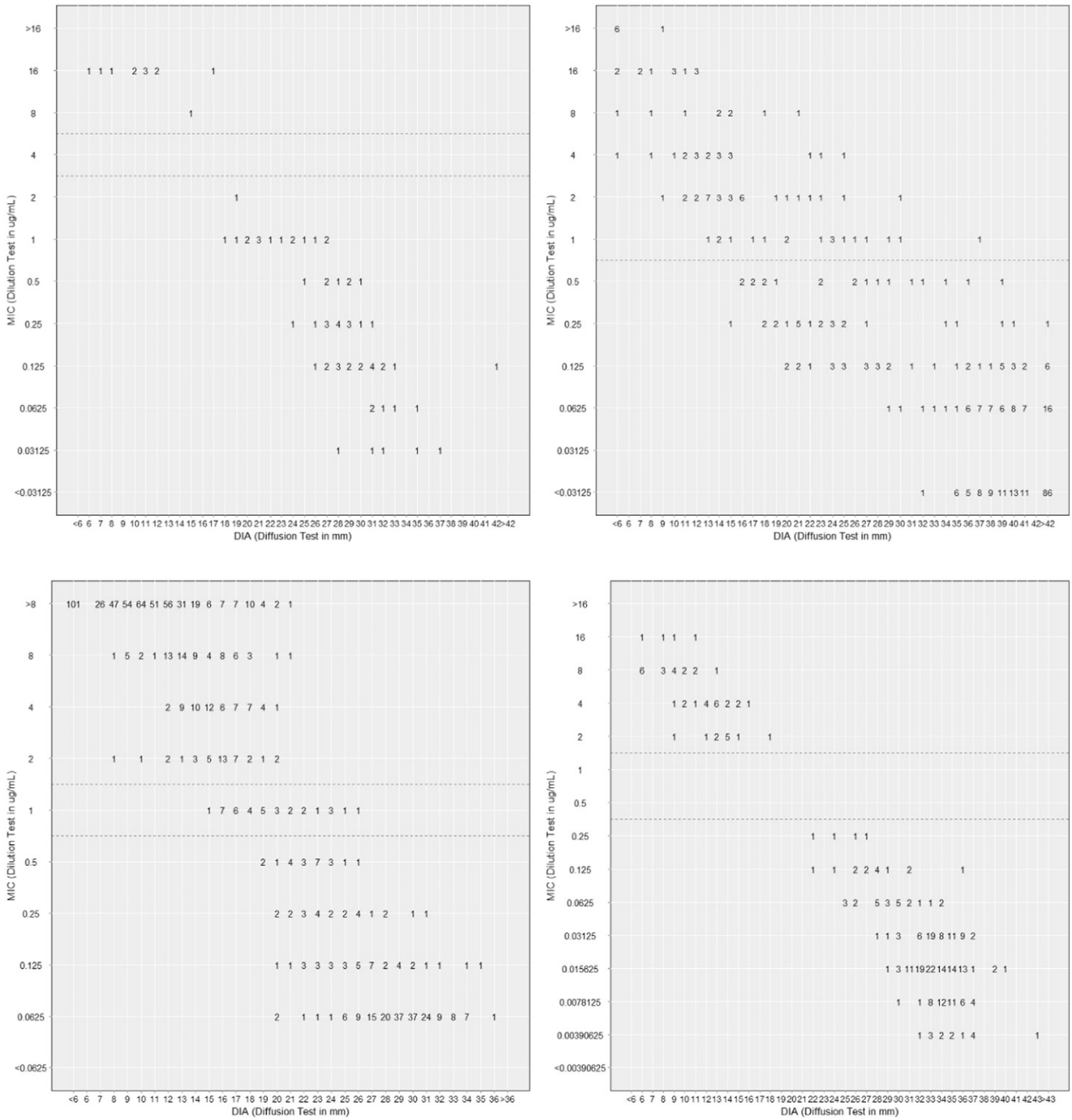

Fig. 1. Scatterplots of the data sets. The dashed lines represent the MIC breakpoints. Starting from top left and moving clockwise: DOR ACIN, DOR STAP, PRA ECOL, and ERT EB.