Calibrated Dichotomous Susceptibility Disc Testing of Polymyxin B: A Review

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Introduction

The CDS test uses agar dilution to calibrate antimicrobial agents for use in disc diffusion. In light of the indications against agar-based phenotypic testing, the CDS Reference Laboratory undertook a review of polymyxin B calibrations for *Pseudomonas* spp and Enterobacteriaceae using three methods – agar dilution, disc diffusion broth microdilution, using a susceptibility break point of ≤ 1 ug/mL.

Method

Isolates

A large number of Gram negative bacilli were recovered from the frozen collection in the CDS Reference Laboratory, Monash University and from isolates referred to the Australian Group on Antimicrobial Resistance (AGAR).

Agar Dilution

The minimum inhibitory concentration (MIC) of the strains was determined by the agar dilution method. Inocula containing 10^4 cfu were delivered by a Steer's replicator onto the surface of freshly prepared agar plates containing varying concentrations of polymyxin B. Full details of the method are described in the CDS manual for medical and veterinary laboratories.[1]

Broth Microdilution

Wells of a microtitre tray containing identical volumes of antimicrobial agent solutions in incrementally increasing concentrations were inoculated with a 10^5 cfu solution of microorganisms according to ISO 20776-1.[2]

Disc Diffusion

Polymyxin B 300 u paper discs were applied to the surface of a Sensitest agar plate after inoculation with a standard CDS bacterial suspension. Susceptible strains have an annular radius of ≥ 4mm.[1]

Calibration

Calibration consists of plotting the zone sizes observed with the test strains against the log MIC of polymyxin B. The zone size is directly proportional to the diffusion constant and the log of the disc potency and inversely proportional to the log of the MIC. [1,3]

Correlation of Results

Categorical agreement - % of isolates producing the same category result (S/R) when a breakpoint of ≤ 1 ug/mL is applied.

Essential agreement - % of isolates producing MICs within ± 1 doubling dilution.

Results

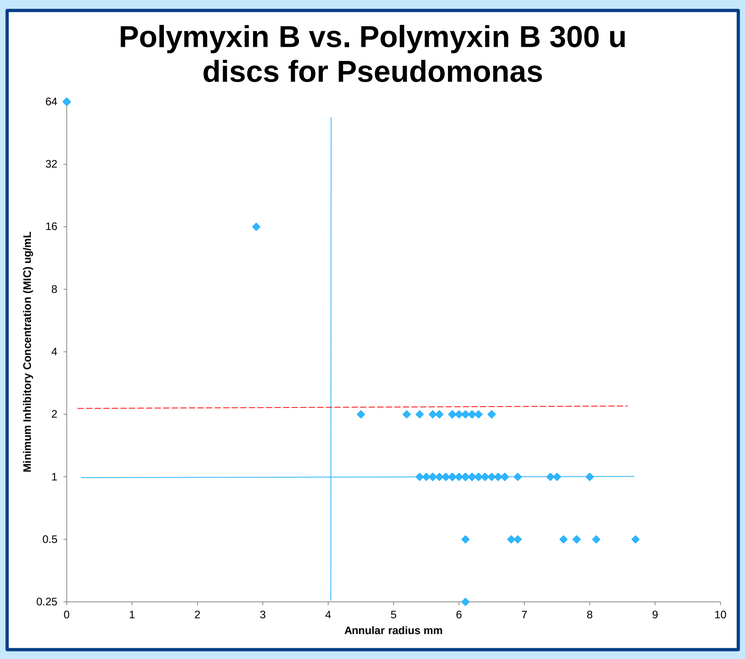

Figure 1.The pseudomonads exhibited bimodal distribution when the recorded annular radius was plotted against MIC. We observed that the current break point of ≤ 1 ug/mL may need to be revised to ≤ 2 ug/mL. Categorical agreement between disc diffusion and agar dilution was 79% with the break point of ≤ 1 ug/mL (blue) but increased to 100% if the breakpoint was review to ≤ 2 ug/mL as shown by the red line.

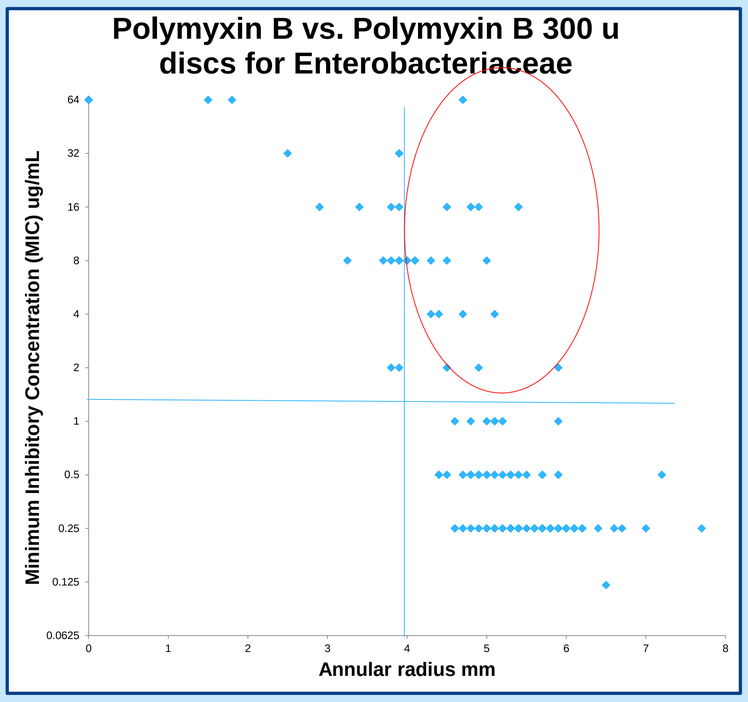

Figure 2. The Enterobacteriaceae group revealed a continuous distribution. An unacceptable number of tested strains gave 'susceptible' annular radii (≤ 4mm) with high MICs as shown in the circle. Categorical agreement between the measured annular radius and MIC was 86% with agar dilution and 78% with BMD.

		Agar dilution/ Annular radius	BMD/ Annular radius	Agar dilution/ BMD
Pseudomonas & pseud-like spp	Essential agreement	N/A	N/A	100%
	Categorical agreement	79% (100%)	100%	100%
Enterobacteriaceae	Essential agreement	N/A	N/A	75%
	Categorical agreement	86%	78%	93%

Figure 3. Agar and BMD demonstrated 100% correlation for both categorical and essential agreement among the pseudomonad group.
Correlation between agar dilution and BMD for the Enterobacteriaceae was only 75% but categorical agreement was 93%.

Discussion

The results indicate a reliable correlation for the pseudomonas group. CDS users can continue to test and report polymyxin results for these isolates. However, users should not trust disc testing with polymyxin B 300 u for Enterobacteriaceae as an unacceptable number of false susceptibilities were observed. These results reflect the literature published for colistin, the representative polymyxin favoured by other phenotypic methods.
While the World Health Organisation (WHO) and other standard setting groups such as CLSI have all recommended BMD as the accepted reference method, there appears to be a number of unanswered problems with this type of testing and phenotypic testing in general. A few include "skip wells," perhaps representing heteroresistance of the isolates, adhesion of polymyxins to plastic surfaces and the question of resistance due to combined resistance mechanisms. Even the impact of subculture on expression of resistance has come under scrutiny.
The issue of an appropriate break point also came into question. The current break point of ≤ 1 ug/mL for the CDS test will be revised. CLSI currently have no break point for Enterobacteriaceae and a break point of ≤ 2 ug/mL for *P. aeruginosa* and *Acinetobacter* spp. EUCAST has published a breakpoint of ≤ 2 ug/mL for all Gram-negative bacteria, including the Enterobacterales.
A compounding problem with setting a suitable break point is the lack of supporting evidence by way of published studies on clinical response, accepted tissue levels or extrapolation from experience.
An optimal alternative for determining an isolate's susceptibility is yet to be established. Poirel et al published a comprehensive evaluation of available tests in 2017.[4] No single test was completely free of complications. It is likely laboratories will need to use a combination of information to infer any given isolate's level of susceptibility. CDS users can refer complicated organisms to the Reference Laboratory for further investigations.

Contact Us

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References

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