A Review of Polymyxin Disc Testing from a CDS Perspective

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Background

Polymyxin B is a lipopeptide antibiotic isolated from *Bacillus polymyxa* and is active against Gram negative bacilli. *Pseudomonas aeruginosa*, and *Acinetobacter* species are inherently susceptible along with *Escherichia coli*, *Enterobacter* spp., *Citrobacter* spp., *Salmonella* spp., *Shigella* spp., and *Klebsiella* spp. *Proteus* spp., *Providencia* spp., *Morganella morganii* and *Serratia marcescens* are intrinsically resistant. Polymyxin B is active against species of *Aeromonas*.

Polymyxins were discovered in 1947. However, their use was reconsidered in the 1970's because of their toxicity. They were then replaced by novel, more active and less toxic antibiotics such as aminoglycosides, quinolones and β lactams.

With the emergence of multi-drug resistant organisms, polymyxin B and colistin (polymyxin E) are being re-evaluated as a last resort treatment option. A perceived need to retain the efficacy of the polymyxins has also prompted a re-evaluation of its use in veterinary medicine where it is often used as a growth promoter.

Despite decades of use, the optimal method of polymyxin susceptibility testing still remains undefined.[1]

The poor and slow diffusion of polymyxin B was recognised by Bell et al in 1975 when the Calibrated Dichotomous Sensitivity (CDS) method was first published in Pathology.[2] Due to the poor diffusion of the antibiotic in agar it produces zones smaller than the standard 6mm annular radii of other agents. Interpretation on this antibiotic is based on an annular radius of 4mm or more for susceptibility, equivalent to a minimal inhibitory concentration of less than or equal to 1.0 ug/mL.

In the CDS method polymyxin B was originally tested against pseudomonas only but was later expanded to a broader range of Enterobacteriaceae. The CDS Reference Laboratory has undertaken a review of disc diffusion testing using the CDS methods. The initial results are presented.

Method

Isolates

A large number of Gram negative bacilli were recovered from the frozen collection in the CDS Reference laboratory, and sourced from external quality assurance programs and the American Type Culture Collection (ATCC).

Agar Dilution

The minimum inhibitory concentration (MIC) of the strains was determined by the agar dilution method. Inocula containing 10^6 and 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.[3]

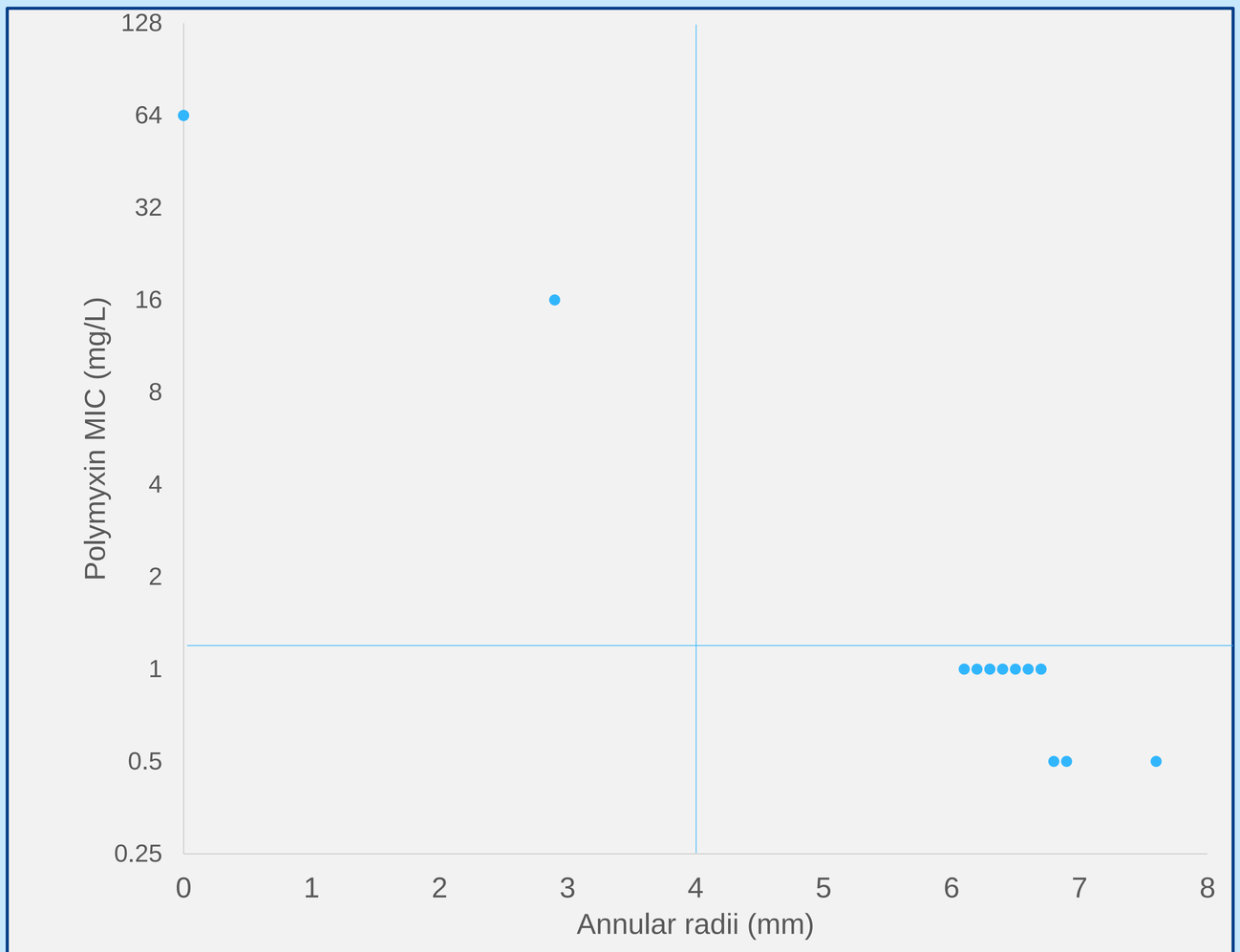
Disc Diffusion

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.

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. [3,4]

Results



The graph above shows the MIC and annular radii of pseudomonas and pseudomonas-like species tested. There was reliable correlation for this group.

The results for the broader Enterobacteriaceae group predominantly showed reasonably good association. Of 98 isolates tested, two strains (2%) have not been resolved. We are undertaking further investigation is needed to determine the cause of the discrepancies.

Discussion

Susceptibility testing whether by agar dilution, broth microdilution or double disc diffusion, is challenging with polymyxins. The difficulties in testing susceptibility to polymyxins include poor diffusion of the agent into agar, adsorption to the surface of micro titre plates, heteroresistance of organisms to polymyxins, composition of the antibiotic powder used in testing, and the effect of surfactants. [1,5,6] Furthermore, the mechanisms underlying resistance to polymyxins have not all been explained.

Studies have compared the results of broth microdilution and disc diffusion on Mueller-Hinton using colistin 10 µg (recommended by CLSI) and 50 µg (recommended by EUCAST). The results have shown that disc diffusion using these methods is nonreliable, giving a high rate of false susceptibility.[1] None of the studies have investigated the use of polymyxin 300 u discs on Sensitest agar. Our results indicate that the CDS method is reliable for the Pseudomonas and pseudomonas-like group but further work is needed to fully assess the Enterobacteriaceae.

Conclusions

So far the only satisfactory correlation is with the Pseudomonas and pseudomonas-like group and reporting should be restricted to this group. If polymyxin susceptibilities are required for multidrug resistant organisms we recommend users refer the isolate to the CDS laboratory for full investigation.

References

1. Poirel L., Jayol A., Nordmann P *Clinical Mircobiology Reviews 30(2): 557-596, 2017*
2. Bell, S.M. 1975 *The CDS disc method of Antibiotic Sensitivity Testing (Calibrated Dichotomous Sensitivity Test)* Pathology 7, No 4 Suppl. 1-18
3. Bell S.M., Pham J.N., Rafferty D.I., Allerton J.K., *Antibiotic Susceptibility Testing by the CDS Method – A Manual for Medical and Veterinary Laboratories Ninth Edition, 2018*
4. Humphrey J.H., & Lightbown J.W., *Journal of General Microbiology 7, 129-43*
5. Bakthavatchalam Y.D., Pragasam A.K., Biswas I., Veeraraghavan B. *Journal of Global Antimicrobial Resistance 12: 124-136, 2018*
6. Humphries R.M., *Pharmacotherapy 35(1) 22-27, 2015*

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