Calibration of Ceftolozane-tazobactam for the CDS test

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Introduction

Ceftolozane-tazobactam is a cephalosporin/β-lactamase inhibitor combination with activity against Gram-negative pathogens (including most extended-spectrum β-lactamase producing Enterobacteriaceae and *Pseudomonas aeruginosa* (including drug resistant strains).

Before any new antimicrobial agent can be included for testing using the CDS test it is necessary to confirm that there is a correlation between zone size and minimal inhibitory concentration (MIC). The CDS test where possible using a uniform zone size of 6mm. This standardised annular radius represents that point of the diffusion sigmoid curve that enable the greatest discrimination between susceptible and resistant isolates for the majority of antibiotics. [1]

A selection of current and historical isolates were tested against ceftolozane in the presence of 4mg/L tazobactam to establish the breakpoint and determine whether a uniform zone size could be applied for use with the CDS disc diffusion test.

Method

Isolates

114 Enterobacteriaceae (*E coli* 49, *Klebsiella* spp 36, *Enterobacter* spp. 21, and other 8) and 82 *Pseudomonas* aeruginosa isolates were recovered from the frozen collection in the CDS Reference laboratory, 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 twofold dilutions of ceftolozane in the presence of 4mg tazobactam. A full description of the method is described in the CDS manual for medical and veterinary laboratories.[1]

Disc Diffusion

Ceftolozane-tazobactam 30-10ug paper discs (MAST) were applied to the surface of a Sensitest agar plate after inoculation with a standard 10^7 cfu CDS bacterial suspension.

Calibration

Calibration consists of plotting the zone sizes observed with the test strains against the log MIC of ceftolozane in the presence of 4mg/L tazobactam. 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. [2]

Quality Assurance Reference ranges

The acceptable range (95% confidence limit) is the mean ± 2 standard deviations. Reference ranges E coli ATCC 25922 and Ps aeruginosa ATCC 27853 were established. The mean of ceftolozane-tazobactam 30-10ug discs was calculated using >120 measurements with different operators, batch numbers of both agar and discs and measuring devices.

Results

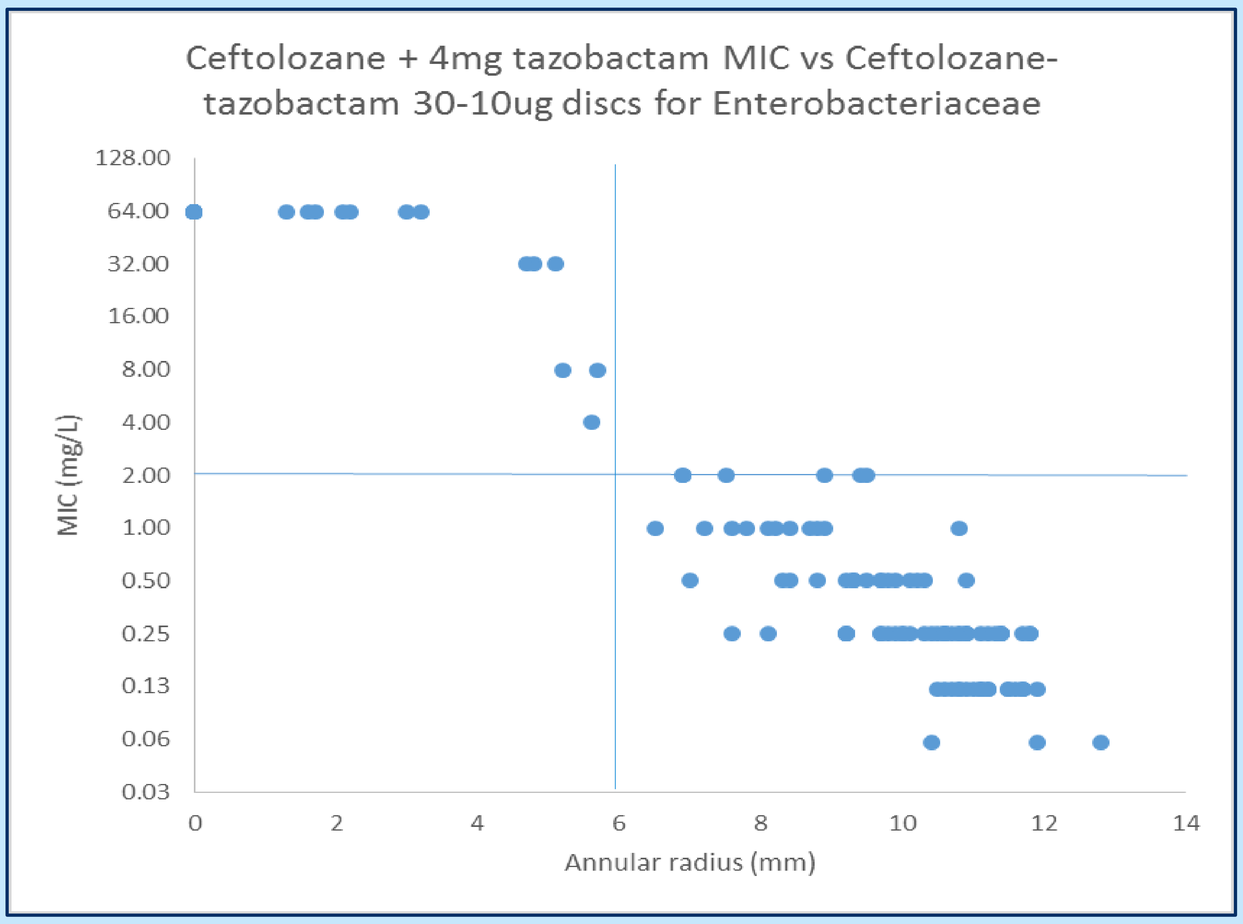

Figure 1: MIC and annular radii of Enterobacteriaceae tested. MIC breakpoint shown as ≤ 2.0 mg/L with a zone size of ≥ 6mm.

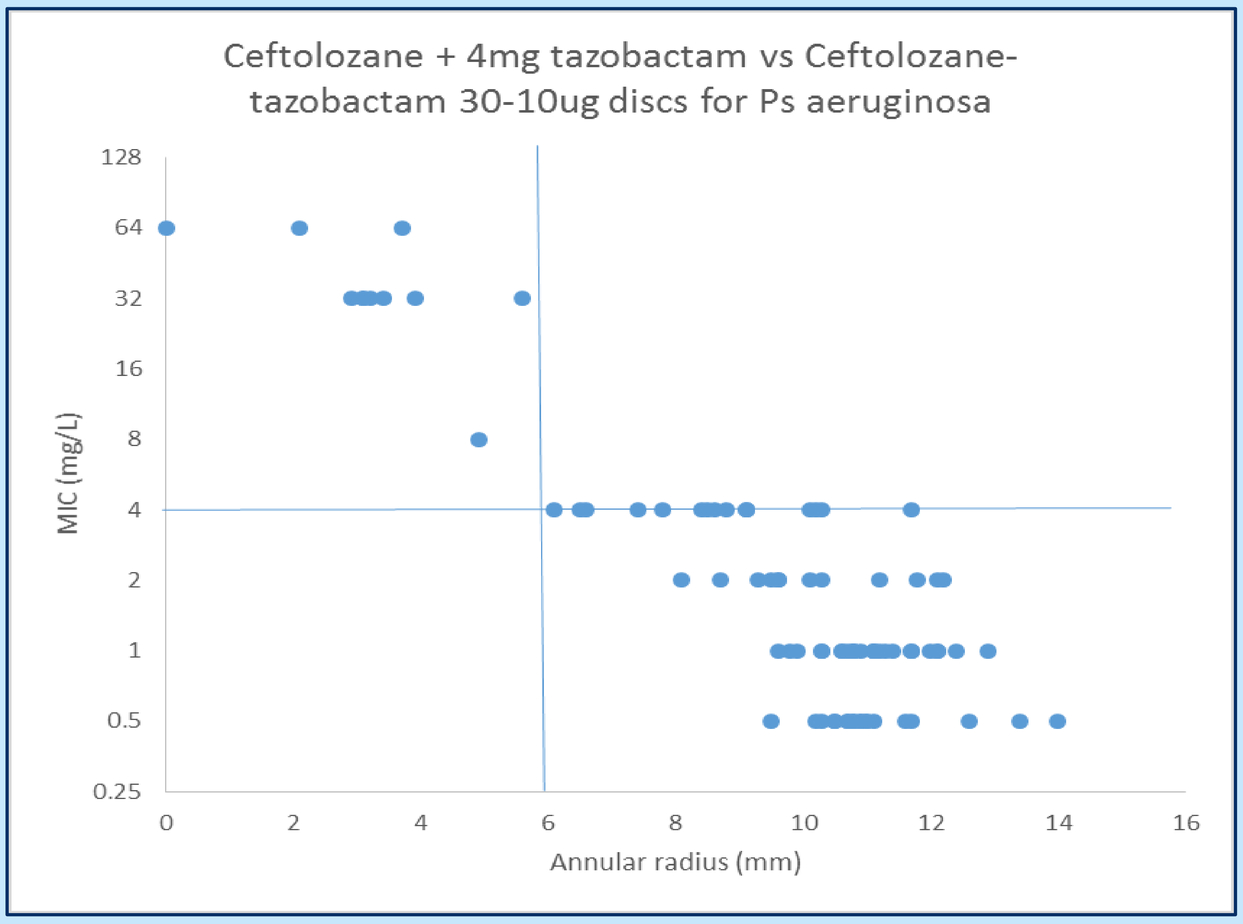

Figure 2: MIC and annular radii of *Pseudomonas aeruginosa* tested. MIC breakpoint shown as ≤ 4.0 mg/L with a zone size of ≥ 6mm.

Conclusion

Ceftolozane-tazobactam 10-30ug discs have been calibrated for use with the CDS test. Excellent correlation between a zone of inhibition of > 6mm and break points of MIC of ≤ 2.0 mg/L and ≤ 4.0 mg/L for Enterobacteriaceae and *Pseudomonas aeruginosa* respectively were demonstrated. Quality assurance recommended reference ranges are *E coli* ATCC 25922 8.9 - 11.7mm and *Ps aeruginosa* ATCC 27853 8.4 - 11.7mm.

Acknowledgement

Ceftolozane and tazobactam pure substances supplied by Merck Sharp & Dohme, Australia.

References

1. Bell S.M., Pham J.N., Rafferty D.L., Allerton J.K., *Antibiotic Susceptibility Testing by the CDS Method – A Manual for Medical and Veterinary Laboratories Ninth Edition, 2018*
2. Humphrey J.H., & Lightbown J.W., *Journal of General Microbiology 7, 129-43*

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