TITLE: The combined disk pre-diffusion is a simple test to predict aztreonam-avibactam *in vitro* activity against NDM-producing *Klebsiella pneumoniae* group

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ABSTRACT:

Ceftazidime-avibactam (CAZ-AVI) has been largely used to treat infections caused by KPC-producing *Klebsiella*. However, infections caused by strains that produce metallo-β-lactamases (MBLs), such as NDMs, or those co-producing NDM and KPC, have become a great therapeutic challenge, as they are usually resistant to CAZ-AVI and can be resistant to polymyxins. In Brazil we are facing the rapid emergence of these strains in healthcare associated infections. A promising alternative for the treatment of CAZ-AVI-resistant *Klebsiella*, but not yet approved for clinical use, is the aztreonam-avibactam combination (ATM-AVI). However, there are no commercially available disks, gradient strips, or microdilution panels for evaluating the *in vitro* activity of this compound. In this context, the aim of this study was to describe an easy-to-perform and low-cost test to predict the *in vitro* activity of the ATM-AVI combination based on a modification of the disk pre-diffusion assay. A total of 113 unrepeated NDM-producing *Klebsiella* isolates were submitted to species identification by multiplex PCR. Minimal inhibitory concentrations (MICs) for ATM and ATM-AVI were determined by broth microdilution. In the combined disk pre-diffusion method, a regular 14µg CAZ-AVI disk of was applied to the surface of a uninoculated Mueller-Hinton agar plate. After incubation for two hours at 36°C, the disk was removed, the bacterial suspension was applied and a 30µg ATM disk was placed in precisely the same position where the CAZ-AVI disk was. After incubation for

16 to 20 hours, the inhibition zone diameters were measured and plotted against ATM-AVI MICs. The species distribution among the 113 isolates tested was: 75.2% (n=85) *K.pneumoniae*, 16.8% (n=19) *K. quasipneumoniae* and 8.0% (n=9) *K. variicola*. A total of 99 isolates had only the *bla*$_{NDM}$ gene and 14 had the *bla*$_{NDM}$ and *bla*$_{KPC}$ genes. A fraction of 38.4% of the isolates, positive for *bla*$_{NDM}$, were susceptible to ATM and 7.1% were susceptible increased exposure, according to BrCAST/EUCAST. All isolates had an ATM-AVI MIC ≤ 1 mg/L and the smallest inhibition zone diameter observed was 23 mm. In conclusion, combined disk pre-diffusion is a simple and reliable test that can be easily implemented in the routine of any clinical microbiology laboratory for screening ATM-AVI activity against *Klebsiella*, while disks, gradient strips or microdilution panels with this compound are not commercially available.

Keywords: ceftazidime-avibactam, aztreonam-avibactam, NDM, KPC, combined disk pre-diffusion

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