A Practical Phenotypic Approach for Detecting Ceftazidime-Avibactam/Aztreonam Synergism Among Stenotrophomonas maltophilia

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Introduction: *Stenotrophomonas maltophilia* is an important nosocomial pathogen recognized by its intrinsic multidrug resistance, restricting therapeutic options. The latest guideline of the Brazilian Society of Infectious Diseases recommends ceftazidime-avibactam (CZA) combined with aztreonam (ATM) for severe *S. maltophilia* infections, as aztreonam-avibactam (AZA) remains unavailable in Brazil. However, simple phenotypic methods for assessing synergism between these agents are still poorly explored for this pathogen.

Objective: To evaluate the susceptibility profile of *S. maltophilia* to CZA, ATM-CZA, and AZA, and propose a phenotypic method for assessing synergism between CZA and ATM based on disk diffusion.

Materials and Methods: Fifty isolates prospectively recovered were included. Identification was performed by MALDI-TOF MS (Bruker Daltonics, Germany). Minimum inhibitory concentrations (MICs) for CZA, ATM-CZA, and AZA were determined by broth microdilution according to ISO 20776-1. Antimicrobial concentrations ranged from 0.5 to 256 µg/mL. Avibactam was fixed at 4 µg/mL and CZA concentration in ATM-CZA at 8/4 µg/mL. Results were interpreted according to EUCAST epidemiological cut-off values (ECOFFs). For CZA, the ECOFF established for ceftazidime (8 µg/mL) was applied. For ATM-CZA and AZA, the AZA ECOFF (8 µg/mL) was used. Phenotypic synergism testing included disks containing CZA (10/4 µg), ATM (30 µg), and disks supplemented with 10 µL of ATM at 3000 µg/mL. Synergism was defined as an increase of ≥5 mm in inhibition zone diameter between CZA alone and CZA combined with ATM. Molecular diversity was assessed by IR Biotyper (Bruker Daltonics, Germany).

Results: $MIC_{50/90}$ values were 32/128 µg/mL for CZA, ≤0.5/4 µg/mL for ATM-CZA, and 2/4 µg/mL for AZA. ATM-CZA promoted marked MIC reductions compared with CZA alone, demonstrating synergistic in vitro activity. One isolate resistant to AZA (MIC = 16 µg/mL) remained susceptible to ATM-CZA (MIC = 8 µg/mL). Compared with ATM alone, avibactam addition resulted in MIC reductions ranging from 0 to 9 two-fold dilutions, whereas ATM-CZA achieved reductions from 2 to ≥10 dilutions. Overall, 24/50 (48%) isolates demonstrated synergism by disk diffusion. Inhibition zone increases ranged from 5 to 23 mm, with larger

increments mainly observed among isolates with elevated CZA MICs. In isolates with absent inhibition zones for CZA alone, the CZA+ATM combination produced inhibition zones up to 23 mm.

Discussion: These findings demonstrate consistent synergistic activity between CZA and ATM against *S. maltophilia*. Although MIC reductions with ATM-CZA were consistently observed, not all isolates showed proportional increases in inhibition zone diameters. Compared with AZA, ATM-CZA consistently promoted greater MIC reductions, which may indicate a contribution of ceftazidime to the overall activity of the combination despite its limited intrinsic activity against *S. maltophilia*. Additionally, the proposed phenotypic method was simple, reproducible, and easily applicable in routine clinical microbiology laboratories.

Conclusion: The disk diffusion synergism test identified synergistic activity in a substantial proportion of isolates and may provide a simple and accessible tool for screening ATM-CZA activity in routine laboratories, particularly while AZA remains unavailable in Brazil.

Agradecimentos: