Efficacy of Aztreonam Combined with Novel Diazabicyclooctane Zidebactam Against Multidrug-Resistant *Klebsiella pneumoniae*

Farah Al-Marzooq*, Maitha Alzaabi, Noora Alhaddad, Noor Al Ali, Nourah Alhefeiti, Aisha Alnaqbi, Fatima Alnuaimi, Akela Ghazawai

Department of Medical Microbiology and Immunology & Zayed Center for Health Sciences
College of Medicine and Health Sciences, United Arab Emirates University, UAE

Background:

- ✓ Carbapenem resistance represents a critical health threat, particularly among isolates producing metallo-β-lactamases (MBLs).
- ✓ Among species exhibiting high resistance rates, *Klebsiella pneumoniae* is a major concern.
- ✓ Most currently available β-lactam/β-lactamase inhibitor (BL/BLI) combinations lack activity against MBL-producing isolates; thus, new combinations are needed [1-2].
- ✓ This study aimed to:
  - ❖ evaluate the *in vitro* activity of aztreonam (AZT) combined with the novel diazabicyclooctane (DBO) β-lactam enhancer zidebactam (ZID)
  - ❖ compare the efficacy of an equal-ratio AZT+ZID combination with AZT combined with a fixed ZID concentration (4 µg/mL) against AZT monotherapy.
- ✓ The concept of the study and main objectives are shown in Figure 1

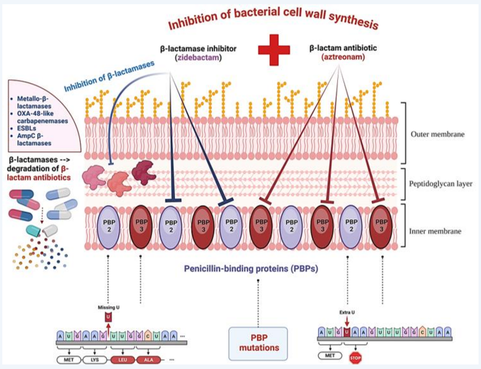

Figure 1. Concept of the Study and Main Objectives. ZID targets the β-lactamase enzymes and the penicillin-binding protein 2 (PBP2), while AZT targets penicillin-binding protein 3 (PBP3).
We hypothesize that combining ZID +AZT can protect the latter from β-lactamase-mediated degradation, leading to more efficient inhibition of cell wall synthesis and ultimately resulting in bacterial cell death.

Methods:

- ✓ Minimum inhibitory concentrations (MICs) were determined for 130 clinical *K. pneumoniae* isolates [3] and MICs of BL/BLI combinations were compared.
- ✓ Carbapenemase genes were detected by PCR, and isolates were classified as MBL producers (NDM, VIM) or serine carbapenemase producers (KPC, OXA-48-like).

Results:

- ✓ 91.5% (n=119) of strains were resistant to ≥1 drug class, with 61.5% (n=80) classified as MDR and 24.6% (n=32) as XDR (MICs are shown in Table 1)
- ✓ Strains were classified into multiple groups according to carbapenemase production and resistance patterns (Figure 2).
- ✓ Both AZT+ZID formulations demonstrated statistically significant reductions in MICs compared with AZT monotherapy.
- ✓ Equal-ratio combination resulted in a 128-fold median MIC reduction, whereas AZT combined with ZID fixed at 4 µg/mL achieved >1,000-fold median MIC reduction.

Table 1. Antibiotic susceptibility testing results for 130 *K. pneumoniae* strains.

Antimicrobial agents	MIC (µg/ml)		
	Range	MIC_{50}	MIC_{90}
Tigecycline	0.125 - 4	0.5	2
Colistin	≤0.125 - >8	0.5	>8
Ciprofloxacin	≤0.125 - >64	32	>64
Gentamicin	≤0.5 - >64	4	>64
Meropenem	≤0.0312 - >32	16	>32
Ertapenem	≤0.015 - >32	32	>32
Imipenem	≤0.0625 - >32	4	>32
Ceftazidime	≤0.25 - >128	>128	>128
Cefepime	≤0.25 - >128	>128	>128
Aztreonam	≤0.25 - >128	>128	>128
Aztreonam + Avibactam (4 µg/ml)	≤0.0312 - 64	0.5	1
Ceftazidime + Avibactam	≤0.25 - >128	2	>128
Zidebactam	≤0.0625 - >128	2	>128
Cefepime + Zidebactam (equal ratio)	0.0312 - 16	0.5	2
Aztreonam + Zidebactam (4 µg/ml)	0.015 - 64	0.031	0.25
Aztreonam + Zidebactam (equal ratio)	0.0312 - 64	0.5	1

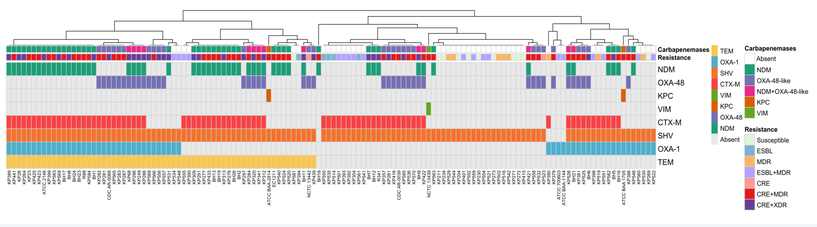

Figure 2. Classification of 130 *K. pneumoniae* strains based on phenotype and genotype. The legend shows the beta-lactamase genes detected in the strains by PCR, with resistance groups based on MIC results.

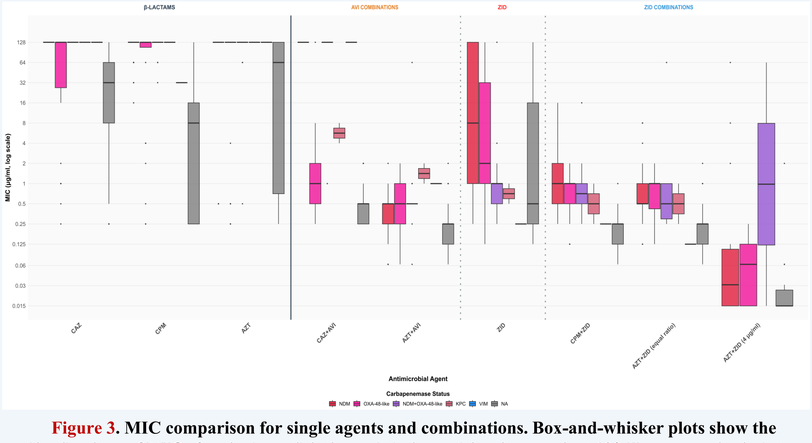

Figure 3. MIC comparison for single agents and combinations. Box-and-whisker plots show the distributions of MICs for single antibiotics and their combinations against 130 *K. pneumoniae* isolates.

- ✓ AZT+ZID formulations were compared to other BL/BLI combinations (Figure 3).
  - ❖ Aztreonam+avibactam showed improved activity compared with AZT alone but was less potent than ZID-based combinations.
  - ❖ Cefepime+zidebactam also demonstrated substantial MIC reductions across carbapenemase classes.
  - ❖ In contrast, ceftazidime+avibactam showed activity primarily against serine carbapenemase producers and no significant effect against MBL producers.
- ✓ MICs were compared by genotype to assess the impact of carbapenemase genes on susceptibility (Figure 3).
  - ❖ NDM-producing isolates showed the highest MICs (MIC_{50}: 8 µg/ml, MIC_{90}: 128 µg/ml), while OXA-48-like producers exhibited intermediate susceptibility (MIC_{50}: 2 µg/ml) and non-carbapenemase isolates the greatest susceptibility (MIC_{50}: 0.5 µg/ml).
  - ❖ NDM+OXA-48-like co-producers showed unexpectedly lower MICs (MIC_{50}: 1 µg/ml) compared with either enzyme class alone.
- ✓ High ZID MICs (≥8 µg/ml) were most frequent in NDM carriers (47.6%; 20/42) and OXA-48-like producers (42.9%; 12/28) but were also observed in 32.6% (14/43) of non-carbapenemase isolates, indicating that factors beyond carbapenemase genotype contribute to ZID susceptibility variability in some isolates.

Conclusion:

- ✓ Overall, zidebactam-based combinations consistently outperformed standard BL/BLI regimens, including aztreonam+avibactam, in MBL-producing isolates.
- ✓ These findings support further preclinical and clinical investigations of aztreonam+zidebactam for the treatment of infections caused by highly resistant bacteria.

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f.almarzooq@uaeu.ac.ae

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