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Original article

Combatting carbapenem-resistant Gram-negative bacteria: *In vitro* synergistic potential of ceftazidime-avibactam and aztreonam

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ABSTRACT

Background: Metallo-β-lactamase (MBL)-producing Gram-negative bacteria, particularly those co-expressing serine β-lactamases, pose a major global health threat due to limited treatment options. In Egypt, $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$ are prevalent in clinical settings. While ceftazidime-avibactam (CZA) is the only available β-lactam/β-lactamase inhibitor (BL/BLI) combination, its limited efficacy against locally prevalent carbapenem-resistant isolates necessitates alternative strategies. **Aim:** To evaluate the activity of the CZA-aztreonam (ATM) combination against carbapenem-resistant Gram-negative (CR-GN) isolates, particularly MBL producers, either alone or co-producing serine carbapenemases. **Methods:** Thirty-four non-duplicate CR-GN isolates were collected from hospitalized patients in Egypt. Carbapenemase genes were identified via multiplex PCR, and Susceptibility to CZA and ATM combination was assessed using the CLSI-endorsed broth disc elution method. Fisher's exact test was used for statistical comparisons. **Results:** *Klebsiella pneumoniae* (52.9%) was the most common isolate, followed by *Acinetobacter spp*. (29.4%), *Pseudomonas aeruginosa (P. aeruginosa)* (11.8%), *E. coli* and *Proteus spp*. (2.9% each). The bla_{NDM} gene was detected in 91.2% of isolates, $bla_{\text{OXA-48}}$ in 64.7%, with 52.9% co-harboring both. Overall, 76.5% of isolates were susceptible to the CZA-ATM combination, including 69.2% of CZA-resistant isolates. Among CZA-resistant isolates, susceptibility to CZA+ATM was higher among *Enterobacterales* (82.4%), compared to *Acinetobacter spp.* (50%) and *P. aeruginosa* (0%). **Conclusions:** The ATM-CZA combination demonstrates promising *in vitro* efficacy against MBL-producing *Enterobacterales* but shows limited activity against *P. aeruginosa* and *Acinetobacter spp*. Key limitations include the small sample size and the study's *in vitro* design. Further clinical studies and resistance monitoring are necessary to guide treatment decisions and optimize therapeutic strategies.

Introduction

Carbapenem-resistant Gram-negative (CR-GN) bacterial infections present a serious threat to world health driven by their resistance mechanisms and scarcity of available treatments [1]. The primary driver of carbapenem resistance is carbapenemase production, which includes class A *Klebsiella pneumoniae* carbapenemase (KPC), class B metallo-β-lactamases (MBLs), and class D oxacillinase (OXA)-48 [2]. Among these, MBLs are especially worrisome because they can render practically all β-

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lactams inactive, with the exception of aztreonam (ATM). However, co-production of other β-lactamases, such as extended-spectrum β-lactamases (ESBLs) or AmpC enzymes, often compromises ATM's effectiveness as monotherapy [3].

MBLs are the most genetically diverse type of carbapenemases, found in a wide variety of GN organisms such as *Enterobacterales*, *Pseudomonas spp*., and *Acinetobacter spp*. The global prevalence of MBL-producing bacteria, especially in regions like Asia and the Middle East, has risen significantly, resulting in poor clinical results, including longer hospital stays, more ICU admissions, and greater death rates [4]. In Egypt, NDM-1 and OXA-48 carbapenemases are prevailing and often co-produced, further complicating treatment strategies [5]. Although ceftazidime-avibactam (CZA), a new β-lactam/β-lactamase inhibitor (BL/BLI) combination, offers a capability to inhibit class A, class C, and some class D β-lactamases, its inability to inhibit MBLs limits its utility against MBL-producing bacteria [6].

The combination of ATM and avibactam (AVI) represents a promising strategy for overcoming MBL-mediated resistance. Although ATM-AVI (AZA) is not yet commercially available, studies have shown that the therapeutic benefits of AZA can be replicated by combining ATM with CZA [7]. This combination has gained support from organizations such as the Infectious Diseases Society of America (IDSA) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) for empiric use against carbapenem-resistant *Enterobacterales* (CRE) co-producing NDM and KPC [8,9]. Nonetheless, *in vitro* susceptibility testing should ideally direct its clinical use [10].

Beyond CZA-ATM, cefiderocol is recommended by IDSA as an alternative treatment option for MBL-producing *Enterobacterales* and MBL-producing *P. aeruginosa* [11]. Additionally, novel BL/BLI combinations are under development, including boronate derivatives such as xeruborbactam, which directly inhibit MBLs, and agents like zidebactam and nacubactam, which enhance β-lactam activity despite lacking direct MBL inhibition. While these emerging therapies represent significant advancements, their limited availability in many regions underscores the need for further evaluation of CZA-ATM as a more accessible therapeutic option [4].

While the broth microdilution (BMD) method is the gold standard for antimicrobial susceptibility testing, its labor-intensive and time-consuming nature limits its routine use in clinical laboratories [12]. To address this, the Clinical and Laboratory Standards Institute (CLSI) recently endorsed broth disc elution methods, which provide a practical alternative for assessing CZA-ATM susceptibility [13]. These methods involve eluting antibiotics from paper discs into broth, followed by the addition of bacterial inoculum. Growth after incubation indicates resistance, offering a streamlined approach for routine testing [14].

The clinical application of ATM combined with CZA should ideally be guided by *in vitro* susceptibility testing of locally prevalent isolates. Given the limited efficacy of CZA against NDM-1 and OXA-48-producing isolates in Egypt, there is a pressing need to assess the performance of the CZA-ATM combination [15]. This study aims to evaluate the activity of the CZA-ATM combination against carbapenem-resistant Gram-negative (CR-GN) isolates, particularly MBL producers, either alone or co-producing serine carbapenemases.

Methods:

Study design and setting

This cross-sectional study was carried out in the Medical Microbiology and Immunology Department of Cairo University's Faculty of Medicine in Egypt from July to November 2024. The study was approved by the Institutional Review Board of Cairo University's Faculty of Medicine (permission Code: N-152-2024) and was conducted in accordance with the Declaration of Helsinki's ethical principles.

Bacterial isolation and identification:

A convenience sampling method was used to select the 34 Gram-negative bacterial isolates from clinical samples obtained from hospitalized patients. The samples were cultured on MacConkey agar and blood agar (Oxoid, UK) and incubated aerobically at 37°C for 48 hours. Standard microbiological methods, such as colony morphology evaluation, Gram staining, and biochemical testing, were used to identify the bacteria [16]. Resistance to carbapenems was determined by evaluating the susceptibility of the isolates to meropenem (10 µg), imipenem (10 µg), and ertapenem (10 µg) antibiotic discs (Oxoid, UK)

using the disc diffusion method, following standardized microbiological procedures. Isolates were classified as CR-GN if they exhibited intermediate or resistant profiles to one or more carbapenems, as per CLSI guidelines [13].

Characterization of carbapenemase genes

Carbapenemase genes (*bla*$_{OXA-48}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{KPC}$, and *bla*$_{IMP}$) were identified using multiplex PCR. DNA was extracted by the thermal method [17]. GoTaq®G2 Hot Start Green Master Mix, gene-specific primers, and DNA template were used in 25 µL reactions for amplification. Initial denaturation took place at 95°C for 2 minutes, and then there were 35 cycles of denaturation (95°C for 1 minute), annealing (60°C for 1 minute), and extension (72°C for 2 minutes), culminating in a final extension at 72°C for 10 minutes. After being separated on agarose gels stained with ethidium bromide, amplicons were seen under a UV lamp.

***In vitro* activity testing Ceftazidime-avibactam and aztreonam combination**

The *in vitro* activity of combining ATM with CZA was evaluated using the CLSI-endorsed "Broth Disc Elution Method." [13,14]. Briefly, four tubes containing 5 mL of cation-adjusted Mueller-Hinton broth (CA-MHB) were prepared. To each tube, one of the following was added: a 30-µg ATM disc, a 30/20-µg CZA disc, both discs combined, or no disc (growth control). A standardized bacterial inoculum (0.5 McFarland, 25 µL) was added to each tube, achieving a final concentration of approximately 7.5×10^5 CFU/mL. After overnight incubation, turbidity indicated resistance, while the absence of turbidity signified susceptibility. For any ambiguous results, the test was repeated as recommended per CLSI.

Statistical analysis

SPSS software version 20.0 was used to analyze the data. Numbers and percentages were used to represent categorical variables. Differences in the distribution of categorical variables were evaluated using the Fisher's exact test; a p-value of less than 0.05 was deemed statistically significant.

Results:

A total of 34 Gram-negative Bacterial isolates came from a variety of clinical samples. Most isolates were identified as *Klebsiella pneumoniae (K. pneumoniae)* (18/34, 52.9%), followed by *Acinetobacter spp.* (10/34, 29.4%), *Pseudomonas aeruginosa* (4/34, 11.8%), with *E. coli* and *Proteus spp.* each representing a single isolate (2.9%).

Carbapenemase Genes:

PCR analysis revealed that the *bla*$_{NDM}$ gene was the most frequently detected carbapenemase (31/34, 91.2%), followed by *bla*$_{OXA-48}$ (22/34, 64.7%). Co-existence of *bla*$_{NDM}$ and *bla*$_{OXA-48}$ was identified in 18 isolates (52.9%), while one isolate (2.9%) harbored *bla*$_{NDM}$, *bla*$_{OXA-48}$, and *bla*$_{KPC}$.

CZA and ATM susceptibility Profiles:

Out of 34 carbapenem-resistant isolates, 76.5% (26/34) were resistant to CZA, and 97% (33/34) were resistant to ATM.

The overall susceptibility rate to CZA was 23.5%, with the highest susceptibility observed among OXA-48-producing isolates (66.7%), followed by MBL producers (19.3%), with breakdowns of 16.6% for MBL-only producers and 21.1% for those co-producing MBLs and other carbapenemases, (p=0.486) (Table 1).

Susceptibility to CZA and ATM combination:

Using the disc elution method to evaluate the combined activity of CZA and ATM, the overall susceptibility to ATM+CZA was 76.5% (26/34) of all the study isolates and 69.2% (18/26) among CZA-resistant isolates.

Species-Specific Analysis:

- Overall susceptibility to the CZA+ATM combination varied by species: 85% for *Enterobacterales*, 75% for *P. aeruginosa*, and 60% for *Acinetobacter spp.* (p=0.313) **(Figure 1)**.
- Among CZA-resistant isolates, susceptibility to CZA+ATM was significant in *Enterobacterales* (82.4%), compared to *P. aeruginosa* (0%) and *Acinetobacter spp.* (50%) (p=0.082).

Gene-Specific Analysis:

- Overall susceptibility to CZA+ATM among carbapenemase genes was 66.7% for *bla*$_{NDM}$ alone, 78.9% for *bla*$_{NDM}$ + *bla*$_{OXA-48}$, and 100% for *bla*$_{OXA-48}$ alone (p=0.625) **(Figure 2)**.
- For CZA-resistant isolates, susceptibility to CZA+ATM was observed in 60% of *bla*$_{NDM}$ -only isolates, 73.3% of *bla*$_{NDM}$ + *bla*$_{OXA-48}$ isolates, and 100% of *bla*$_{OXA-48}$ -only isolates (p=0.618).

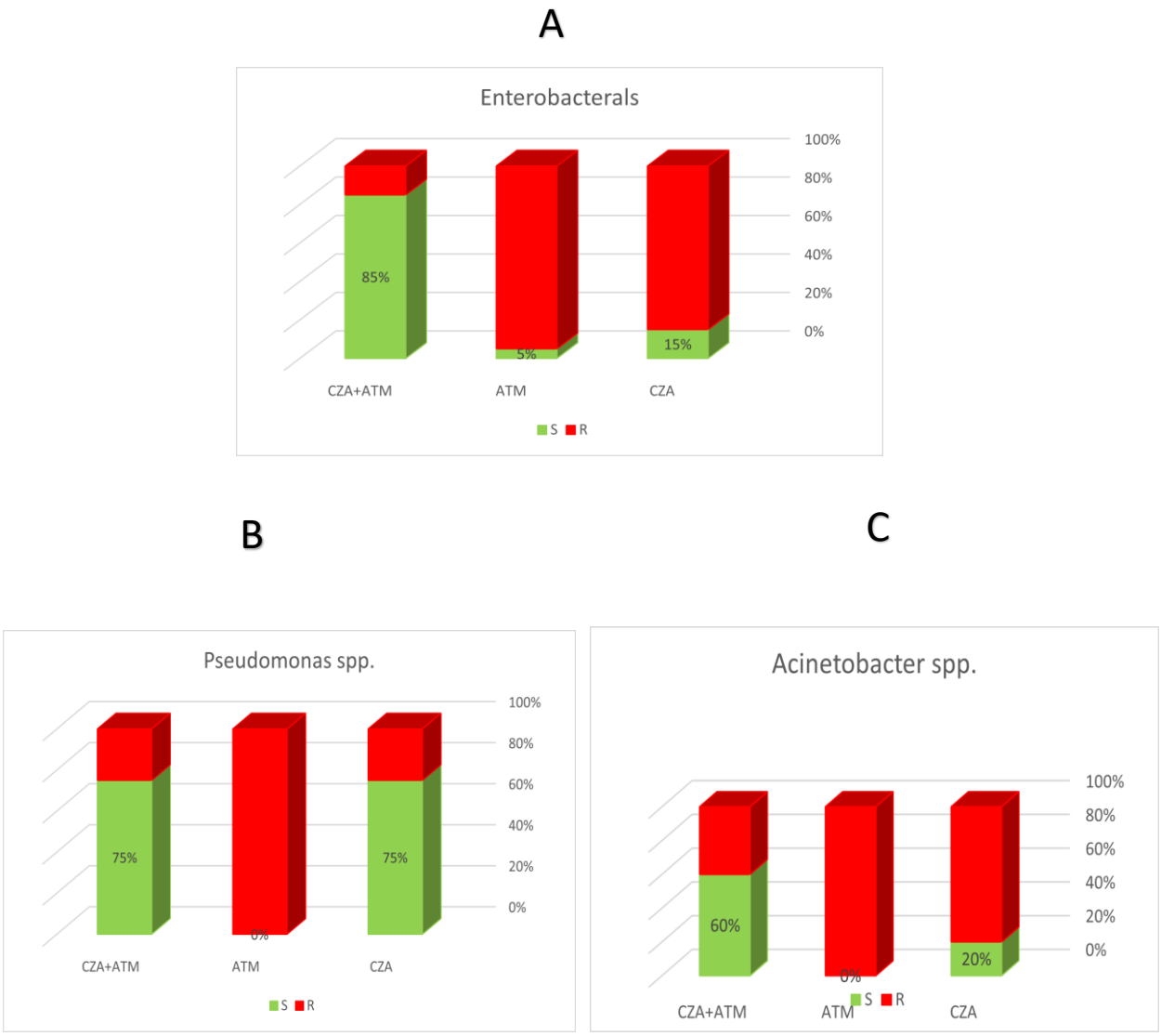
MBL vs on MBL- producers:

Among MBL producers, 74.2% (23/31) demonstrated susceptibility to CZA+ATM, with breakdowns of 66.7% for MBL-only producers and 78.9% for those co-producing MBLs and other carbapenemases. Notably, all non-MBL producers (3/3) were susceptible to the CZA+ATM combination, although 2 of these isolates were already susceptible to CZA alone (p=0.652) **(Table 1)**.

Table 1. Susceptibility profiles of CR-GN isolates to CZA, ATM, and their combination (CZA+ATM), stratified by species and carbapenemase gene profiles.

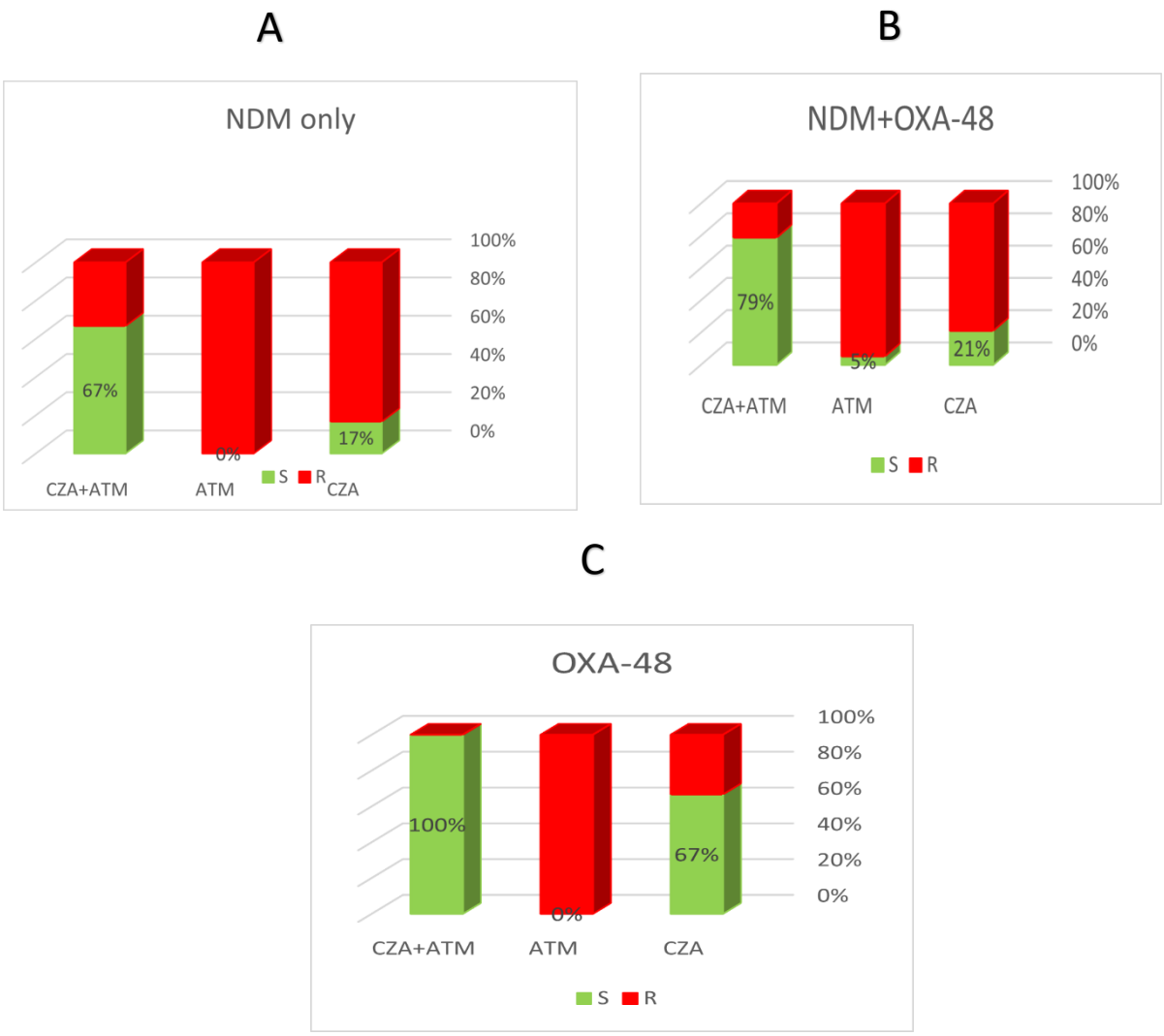
Carbapenemase gene	Isolate	CZA		ATM		CZA+ATM	
		S	R	S	R	S	R
NDM	*K. pneumonia (3)*	0	3	0	3	3	0
	Acinetobacter spp. (7)	1	6	0	7	4	3
	P. aeruginosa (1)	1	0	0	1	1	0
	E. coli (1)	0	1	0	1	0	1
	Total (12)	2(16.7%)	10(83.3%)	0 (0%)	12 (100%)	8(66.7%)	4(33.3%)
NDM+OXA-48	*K. pneumonia (13)*	2	11	1	12	11	2
	Acinetobacter spp. (3)	1	2	0	3	2	1
	P. aeruginosa (2)	1	1	0	2	1	1
	Total (18)	4 (22.2%)	14 (77.8%)	1 (5.6%)	17 (94.4%)	14 (77.8%)	4 (22.2%)
NDM+OXA-48+KPC	*Proteus (1)*	0	1	0	1	1	0
MBL producers	*K. pneumonia (16)*	2	14	1	15	14	2
	Acinetobacter (10)	2	8	0	10	6	4
	P. aeruginosa (3)	2	1	0	3	2	1
	E. coli (1)	0	1	0	1	0	1
	Proteus (1)	0	1	0	1	1	0
	Total (31)	6(19.3%)	25 (80.6%)	1(3.2%)	30(96.8%)	23 (74.2%)	8 (25.8%)
OXA-48 (MBL non producers)	*K. pneumonia (2)*	1	1	0	2	2	0
	P. aeruginosa (1)	1	0	0	1	1	0
	Total (3)	2(66.7%)	1(33.3%)	0 (0%)	3 (100%)	3 (100%)	0 (0%)
Total isolates	*K. pneumonia (18)*	3 (16.7%)	15(83.3%)	1(5.9%)	17 (94.4%)	16(88.9%)	2(11.1%)
	Acinetobacter (10)	2 (20%)	8 (80%)	0 (0%)	10 (100%)	6 (60%)	4 (40%)
	P. aeruginosa (4)	3 (75%)	1 (25%)	0 (0%)	4 (100%)	3 (75%)	1 (25%)
	Proteus (1)	0 (0%)	1 (100%)	0 (0%)	1(100%)	1 (100%)	0(0%)
	E. coli (1)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
	Total (34)	8 (23.5%)	26(76.5%)	1 (3%)	33 (97%)	26(76.5%)	8(23.5%)

Figure 1. Overall susceptibility profiles of isolates to CZA, ATM, and their combination across bacterial species. (A) *Enterobacterales* isolates: susceptibility rates to CZA, ATM, CZA+ATM were 15%, 5% and 85% (B) *Pseudomonas spp.* isolates: susceptibility rates to CZA, ATM, CZA+ATM were 75%, 0% and 75% (C) *Acinetobacter spp.* isolates: susceptibility rates to CZA, ATM, CZA+ATM were 20%, 0% and 60%. No significant difference was observed in susceptibility to the combination among species (p = 0.313) or among CZA-resistant isolates (p = 0.082).



**Abbreviations*: S = Susceptible, R = Resistant.

Figure 2. Overall susceptibility profiles of isolates to CZA, ATM, and their combination across carbapenemase genes. (A) NDM only isolates: susceptibility rates to CZA, ATM, CZA+ATM were 16.7%, 0% and 66.7% (B) NDM+OXA-48 isolates: susceptibility rates to CZA, ATM, CZA+ATM were 21.1%, 5.3% and 78.9% (C) OXA-48 only isolates: susceptibility rates to CZA, ATM, CZA+ATM were 66.7%, 0% and 100%. No significant difference was observed among genes either overall (p = 0.625), or among CZA-resistant isolates (p = 0.618).



**Abbreviations*: S = Susceptible, R = Resistant.

Discussion

Multidrug resistance in GN bacteria has become an increasingly significant issue, especially during the COVID-19 pandemic, where a lot of antibiotics were used for preventative therapy contributed to the acceleration of antimicrobial resistance. The effective management of multidrug-resistant (MDR) bacteria demands precise and optimal use of antibiotics to combat these resistant pathogens[18]. This study aimed to evaluate the susceptibility of carbapenem-resistant Gram-negative isolates to the combination of ATM and CZA, specifically targeting MBL-producing isolates, either alone or in combination with serine carbapenemases, using the newly approved CLSI broth disc elution method

Among the 34 Gram-negative bacterial isolates from various clinical samples, 52.9% were identified as *K. pneumoniae* (18/34), 29.4% as *Acinetobacter* species (10/34), 11.8% as *P. aeruginosa* (4/34), and only 2.9% as *E. coli* and *Proteus spp.* (one isolate each). PCR results revealed that the most common carbapenemase gene was bla_{NDM} (31, 91.2%), followed by bla_{OXA-48} (22, 64.7%). In recent years, MBL-producing isolates

have become increasingly prevalent both globally and locally. In the Middle East, including Egypt, multiple studies have identified *bla*$_{NDM}$ as the most dominant carbapenemase gene, followed by *bla*$_{OXA-48}$, with widespread dissemination across various Egyptian hospitals [19,20].

The present study also found that 52.9% of Gram-negative isolates co-harbored *bla*$_{NDM}$ and *bla*$_{OXA-48}$ genes, with one isolate (2.9%) carrying a combination of *bla*$_{NDM}$, *bla*$_{OXA-48}$, and *bla*$_{KPC}$. The co-occurrence of NDM-1 and OXA-48 has been extensively reported in Egypt [5,21]. emphasizing the growing prevalence of multiple carbapenemase genes. This highlights the urgent need for better detection of mobile carbapenemase genes to control the rising resistance and enforce stricter infection prevention measures to protect patient health [22].

The clinical challenges posed by MBL-producing GN bacterial infections are substantial because of their strong resistance mechanisms and the shortage of viable treatments. Recent studies in Egypt, including this one, have shown low susceptibility rates of carbapenem-resistant Gram-negative bacteria to CZA, a promising treatment option [3]. In our study, the susceptibility rate overall to CZA was 23.5%, with the highest susceptibility observed among OXA-48-producing isolates (66.67%), followed by MBL producers (19.3%). These findings are in line with earlier studies carried out in the same region**.** Elfeky *et al.* reported a 31.4% susceptibility rate among CRE isolated from two Egyptian tertiary care hospitals [15]. Ahmed *et al.* observed comparable CZA susceptibility rates (30%) among CR-GN strains from a pediatric hospital in Cairo [23]. Likewise, a study in Zagazig reported a susceptibility rate of 23.5% among CRE isolates from ICU patients [24].

ATM is an older antibiotic that received approval from the US FDA in 1986. In the past, it was mostly used to treat septicemia, lower respiratory tract infections, intra-abdominal infections, and urinary tract infections caused by aerobic GN organisms [25]. In this study, 97% of the tested isolates were resistant to ATM. Similar findings were reported in a study from North India, where 100% of MBL-producing GN bacilli were resistant to aztreonam [18]. In another study evaluating the susceptibility of MBL-producing MDR *Enterobacterales* and *Pseudomonas* species to ATM and newer β-lactamase inhibitor combinations, 83% of the isolates were found to be resistant to ATM [26]. A study from Spain also found a nearly 82% resistance rate to ATM among 55 *Enterobacterales* isolates [26]. The resistance to aztreonam is attributed to the frequent co-production of class A β-lactamases, ESBLs, or AmpC-type β-lactamases, which limit the clinical effectiveness of ATM, even though MBLs themselves do not hydrolyze ATM.

With avibactam's ability to deactivate other β-lactamases, CZA and ATM present an appealing alternative for treating pathogens that produce MBL. However, the susceptibility of MBL-producing bacteria to this combination is often unpredictable, influenced by other resistance mechanisms present in these pathogens [3]. This study's main objective was to evaluate the antimicrobial susceptibility of GN-CR isolates to the CZA-ATM combination using the newly CLSI-approved broth disc elution method. In our study, the overall susceptibility rate to the CZA-ATM combination was 76.5%, with 69.2% susceptibility observed among CZA-resistant isolates. Among MBL producers 74.2% (23/31) demonstrated susceptibility to CZA+ATM with a higher susceptibility observed among isolates co-producing NDM and OXA-48 when compared to *NDM*-only producers (78.9% and 66.7%, respectively). Notably, all OXA-48-producing isolates were susceptible to CZA-ATM, with two out of three isolates being sensitive to CZA.

These results are consistent with earlier research showing the successful use of the CZA-ATM combination against GN bacteria that produce MBL. Taha *et al.* reported a 90% susceptibility rate among their isolates, all of which produced both MBLs and serine β-lactamases [21]. Similarly, Jayol *et al.* observed synergistic effects for all their isolates. Demonstrating its effectiveness against MBL-producing GN isolates, particularly those harboring multiple carbapenemase genes [27]. In contrast, Bedawy *et al.* reported lower susceptibility rates, with 60% susceptibility among MBL producers and 46.4% among non-MBL producers [28]. Nevertheless, CZA and ATM together continue to be a very successful treatment choice for infections brought on by Gram-negative bacteria that are resistant to carbapenem, especially those that produce MBLs either individually or in conjunction with other serine β-lactamases [21]. Given the increasing resistance trends, these findings underscore the importance of antimicrobial stewardship programs in optimizing the use of combination therapies like CZA-ATM to preserve

their efficacy and minimize further resistance development.

From a microbiological standpoint, the choice of effective therapy for infections caused by Gram-negative bacteria that produce MBL is essential and depends on the specific microbial species involved [3]. This study observed susceptibility to CZA+ATM in 85% of CRE isolates and 82.4% of CZA-resistant isolates. Similar results were reported by Mishra *et al.* (89% susceptibility in *Klebsiella* isolates) [10], and Rawson *et al.* (89% efficacy restoration in resistant *Enterobacterales*) [29]. Other researchers observed 100% susceptibility to the combination in MBL-positive *Enterobacterales* [18,30]. These findings are further supported by clinical data demonstrating lower failure rates, shorter hospital stays, and reduced mortality in patients treated with aztreonam/avibactam for severe resistant infections caused by NDM *Enterobacterales* [31]. Globally, less than 10% of MBL-producing *Enterobacterales* have been found to be resistant to ATM/AVI. Nevertheless, it has been noted that CMY β-lactamases and changes in PBP3 (induced by amino acid insertions) have decreased *E. coli's* susceptibility to ATM/AVI, emphasizing the need for careful and judicious use of available antibiotics [32]. While *in vitro* synergy was observed, clinical effectiveness may not always be guaranteed, as real-world outcomes are influenced by host factors, bacterial load, and pharmacokinetic/pharmacodynamic parameters emphasizing the need for clinical validation before recommending the CZA-ATM combination for routine use.

While synergy between CZA and ATM has been well-documented for *Enterobacterales*, its efficacy against non-fermenting Gram-negative bacteria like *P. aeruginosa* and *Acinetobacter spp.* is less consistent [33]. In our study, the susceptibility rate to the CZA-ATM combination was 75% among all *Pseudomonas* isolates, but it was 0% among CZA-resistant isolates. In line with our findings, Deckers *et al.* reported a susceptibility rate of 68.7% for the combination across 16 *P. aeruginosa* strains, with a higher rate of 58% among CZA-resistant isolates [34]. However, a systematic review found that only 6.2% of MBL-producing *Pseudomonas* isolates were susceptible to ATM/AVI [35]. Other studies have also found that adding avibactam to aztreonam did not enhance aztreonam's efficacy against clinical *P. aeruginosa* isolates [36]. Furthermore, most clinical evidence supporting the co-administration of ATM and AVI in *P. aeruginosa* is derived from case reports, with no available clinical trial data on this combination [35]. The limited effectiveness of the combination among *P. aeruginosa* isolates in our study can be explained by the complex array of acquired and innate resistance mechanisms in multidrug-resistant P. a*eruginosa*, including chromosomally encoded AmpC β-lactamases, altered outer membrane porins, multiple efflux pumps, and novel PBP3 insertions, restricts the ability of avibactam to restore the activity of aztreonam [2]. Furthermore, *P. aeruginosa* has different types and frequencies of serine β-lactamases and MBLs than *Enterobacterales*, which makes it more difficult to extrapolate the effectiveness of aztreonam-avibactam against *Enterobacterales* that produce serine β-lactamases and MBLs to *P. aeruginosa* [37].

Similarly, while *Acinetobacter* species are often considered inherently resistant to aztreonam, combinations with ceftazidime/avibactam have shown some promise in this study, the disc elusion method revealed susceptibility rates of 60% for all *Acinetobacter* isolates and 50% for CZA-resistant isolates. These findings are in line with previous studies, including one by Crouch *et al.*, which showed that the addition of CZA to ATM reduced the MIC in 12 tested isolates [33]. However, other studies have demonstrated minimal activity of ATM, either alone or with avibactam, against *Acinetobacter spp.*[38,39], highlighting the complexity of treating infections caused by this pathogen. The variation in response may be due to strain-specific factors or additional resistance mechanisms, such as efflux pumps and porin mutations, that are not addressed by this combination.

Conclusions:

This study highlights the potential of the CZA and ATM combination as an effective treatment option for MDR *Enterobacterales*, particularly those producing or co-producing MBLs. However, the response is less consistent with *P. aeruginosa* and *Acinetobacter spp.*, due to complex intrinsic resistance mechanisms. This study adds to the expanding corpus of evidence demonstrating the effectiveness of CZA and ATM combinations in the treatment of resistant infections. However, variability in susceptibility rates across different

bacterial species suggests that individualized treatment strategies based on *in vitro* susceptibility testing are crucial. Moreover, the emergence of resistance to these combinations emphasizes the need for vigilant antimicrobial stewardship and ongoing surveillance to ensure effective clinical management of infections caused by MDR GN bacteria. Despite the promising results, there are several limitations to this study. First, the study focused on *in vitro* testing without clinical outcome data, limiting our ability to correlate the susceptibility results with treatment success or failure in patients. Second, the sample size was relatively small, which may affect the generalizability of the findings. This study highlights the potential of CZA-ATM against MDR *Enterobacterales*, with variable efficacy against *P. aeruginosa* and *Acinetobacter spp.* Future studies should focus on clinical validation, optimizing combination therapy, and integrating *in vitro* susceptibility testing into routine diagnostic workflows.

Conflicts of Interest:

All authors declare no conflict.

Ethical Approval

The current work was approved by the Research Ethics Committee, Faculty of Medicine, Cairo University (Code: N-152-2024).

Author Contributions:

Conceptualization: Sahloul N. Y., Mowafy H.L., El-Kashef S. M. **Methodology and validation: Writing original draft and figure preparation:** Mowafy H.L. **Writing review and editing:** Sahloul N. Y., Mowafy H.L., El-Kashef S. M. **Supervision:**

Sahloul N. Y., Mowafy H.L., El-Kashef S. M.

All authors reviewed the manuscript.

Data availability statement:

All data used in the current study are available from the corresponding author on reasonable request.

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