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Original article

In vitro activity of ceftazidime-avibactam and meropenem-vaborbactam against carbapenem-resistant Enterobacterales in Egyptian Hospitals: A challenge for clinical practice

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ABSTRACT

Background: Ceftazidime-avibactam (CZA) and meropenem-vaborbactam (MVB) are novel therapeutic options for infections caused by carbapenem-resistant Enterobacterales (CRE). Our study aimed to evaluate the *in vitro* activity of CZA and MVB, against a diverse collection of CRE and to assess the accuracy of the disc diffusion method compared to E-test. **Methods.** 70 CRE isolated from hospitalized patients in Egypt were included in our study. The *in vitro* susceptibility profiles of these isolates to CZA and MVB were determined using disc diffusion and E-test methodologies. The studied isolates were genetically identified as part of another study using multiplex-PCR. **Results.** Susceptibility rates to CZA and MVB were low, at 31.4% and 14.3% respectively with the best activity of CZA reported among isolates harboring OXA-48 gene alone followed by isolates producing a combination of OXA-48 and NDM-1 (50%, 42.9%, respectively) while the least activity recorded was against NDM-1 only producers (11.5%). The highest susceptibility rate for MVB was recorded among isolates harboring NDM-1 gene alone followed by the dual carbapenemase producers (19.2%, 11.9%, respectively) while the least activity was among OXA-48 only producers. The categorical agreement (CA) between disc diffusion and E-test of CZA and MVB was acceptable with low very major error (VME). However, a high major error (ME) was recorded. **Conclusions.** CZA and MVB have shown limited effectiveness against studied CRE isolates. Disc diffusion tests for CZA and MVB may not be reliable substitutes for E-tests. A comprehensive understanding of test performance within specific clinical settings is essential prior to definitive interpretation.

Introduction

Over the past 20 years, carbapenem-resistant Enterobacterales (CRE) have become a growing burden on healthcare systems due to rising costs and mortality [1]. The primary cause of carbapenem resistance in these bacteria is the production of carbapenemase enzymes [2]. Common carbapenemase enzymes contributing to CRE include those belonging to class A, such as KPC, class B metallo-beta-lactamases (MBLs) like NDM, VIM, and IMP, and class D enzymes, particularly OXA-48 [1]. The prevalence of CRE and the specific carbapenemase genes they carry exhibit significant geographic variability.

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Consequently, the distribution of carbapenemases differs substantially across countries and regions globally [3]. There have been reports of *bla$_{KPC}$* in China, America, and Europe. CRE harboring bla$_{NDM}$ are more frequently seen in Asia, Africa, China, and the United Kingdom [3], while bla$_{OXA-48}$ is thought to be indigenous to North Africa and the Middle East [4].

The threat posed by CRE is significant due to the limited and often suboptimal therapeutic options, which raise concerns about both efficacy and toxicity [5]. To address this issue, ceftazidime avibactam (CZA), a novel beta- lactam/beta-lactamase inhibitor (BL/BLI), was approved in 2015 for treating CRE [6]. Avibactam is effective against KPC enzymes and can also inhibit certain class D carbapenemases, such as OXA-48-like enzymes [7]. Another novel BL/BLI, meropenem-vaborbactam (MVB), was later approved in 2017 for treating adult patients with complicated urinary tract infections [8]. Vaborbactam, a cyclic boronic acid derivative, exhibits strong activity against Ambler class A and class C β-lactamases. It has shown significant *in vitro* potency and clinical efficacy, particularly against Ambler class A enzymes, particularly blaKPC [9]. Nonetheless, previous studies demonstrated that MVB may have retained *in-vitro* potency against non–KPC-producers [10].

While these novel agents demonstrate promising results, concerns persist regarding their spectrum of activity and the emergence of resistance [11]. In Egypt, the prevalence of CRE is escalating and the adoption of new antimicrobials like CZA has been quick, bolstered by clinical reports showing better efficacy compared to traditional treatment regimens [12]. Nonetheless, to optimize the utilization of these agents, a comprehensive assessment of susceptibility patterns within the local epidemiological context is imperative 10. Moreover, precise susceptibility testing is also essential for identifying resistance patterns and effectively utilizing these novel antimicrobials [13]. The limited availability of automated susceptibility systems in Egypt, coupled with the challenges associated with the reference broth microdilution (BMD) method for routine laboratories, underscores the need for alternative approaches. While E-test gradient strips offer reliable susceptibility testing for CZA and MVB against Enterobacterales, their cost is a concern. The disc diffusion test is a suitable option for most laboratories, given its affordability, convenience, and practicality [7], especially in resource-limited settings like Egypt. Accordingly, CZA has been extensively employed in our healthcare facilities primarily based on microbiology laboratories testing reports. However, these laboratory reports depend only on disc diffusion susceptibility testing results without taking into consideration the genetic layout of carbapenemases currently prevalent in our hospitals. Given that occurrences of disc errors could be influenced by the minimum inhibitory concentration (MIC) values and the genetic makeup of the tested isolates. In this study, our aim was to assess the *in-vitro* activity of CZA and MVB against carbapenem resistant Enterobacterales isolated from two Egyptian tertiary care hospitals and to evaluate the performance of the CZA and MVB disc diffusion tests against these isolates using the FDA-Cleared Liofilchem MTS™ MIC test strips as a gold standard.

Methods:

This cross-sectional study, approved by the Cairo University Research Ethics Committee (N172-2023) and adhering to the Helsinki Declaration, analyzed 70 non-duplicate CRE isolates collected from hospitalized patients at two tertiary care hospitals in Cairo and Alexandria between September 2021 and June 2023. All isolates exhibited resistance to either meropenem or imipenem.

***In vitro* activity of Ceftazidime-avibactam and Meropenem-Vaborbactam against CRE isolates:**

The *in vitro* susceptibility of the study strains to CZA and MVB was determined using both disc diffusion and E-test.

Antibiotic susceptibility testing:

For disc diffusion susceptibility method, CZA (30/20 µg) and MVB (20/10 µg) antibiotic discs (Mast, UK) were used. The test was performed precisely according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [14]. For gradient strips (E-test), FDA-cleared Liofilchem MTS™ MIC test strips (Liofilchem, Italy) with concentration ranges of 0.016/4-256/4 µg/ml for CZA and 0.016/8-256/8 µg/ml for MVB were used. The test was performed in strict accordance with the manufacturer's instructions. For each bacterial isolate, bacterial suspensions were standardized to a 0.5 McFarland turbidity and a standardized inoculum was used to inoculate the Mueller-Hinton agar (MHA) agar plates used for both the disc diffusion method and E-test

simultaneously. Both methods were interpretated according to the CLSI breakpoints [14], (table 1). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as experimental quality control isolates. The tests were considered as valid only when the results for all quality control isolates were within the acceptable range established by the CLSI [14].

Agreement Analysis

Previous studies have demonstrated that the gradient diffusion MIC strip method exhibited an excellent linear correlation, with the reference method BMD validating the use of this approach as an alternative to the standard method [15–17]. Accordingly, the disc diffusion test was evaluated using the E-test as a gold standard method.

Using the E-test as a reference standard, calculated categorical agreement (CA), major error (ME), very major error (VME), and minor error (mE) rates were calculated according to CLSI M52 guidelines [18]. CA represents the concordance of susceptibility categorization between the E-test and disc diffusion. Error rates, including VME (false susceptibility), ME (false resistance), and mE (discrepancy between intermediate and susceptible/resistant categories), were determined and compared to CLSI-defined acceptable limits of 1.5% for VME, 3% for ME, and 10% for mE.

Genetic identification of carbapenemase producing genes:

Our studied isolates were subjected to genetic identification of genes encoding for carbapenemases using multiplex PCR as a part of another recently published study [19].

Statistical analysis:

Data was coded and entered using SPSS version 28. Descriptive statistics, including frequencies and percentages, were calculated for the dataset. To compare categorical data, the Chi-square test was employed. However, when expected cell counts were less than five, an exact test was utilized instead. Statistical significance was set at a p-value of 0.05 or less.

Results:

This observational study involved 70 CRE strains obtained from various clinical samples at two tertiary care hospitals in Egypt. Most of the included isolates were *Klebsiella pneumoniae* (n=52, 74.3%) followed by *Enterobacter* species and *E. coli* (n=9, 12.9% for each). The study evaluated the *in vitro* effectiveness of CZA/MVB against these 70 isolates using both disc diffusion and E-test methods (table 2).

***In vitro* activity of Ceftazidime-avibactam and Meropenem-Vaborbactam against CRE isolates**:

Most isolates were resistant to CZA [77.1% (n=54) and 68.6% (n=48)] and MVB [91.4% (n=64) and 80% (n=56)] by disc diffusion and E-test, respectively. Only 1.43% (n=1) and 14.3% (n=10) of the isolates were sensitive (S) to MVB by both disc diffusion and E-test, respectively. On the other hand, 7.1% (n=5) and 5.7% (n=4) were shown to be intermediate (I) by disc diffusion and E-test, respectively (figure1).

Agreement Analysis between disc diffusion method and E-test as a reference method:

The CA between the CZA disc diffusion method and E-test was 88.6% with ME of 10% and VME of 1.43%. Meanwhile, the CA between the MVB disc diffusion method and E-test was 82.9% with ME of 7.1%, mE of 10%.and no VME.

The inhibitory profiles of CZA and MVB in relation to the detected carbapenemases genes:

Among the 70 CRE isolates, 60% of isolates carried both NDM-1 and OXA-48 genes, while 37.1% were detected as NDM-1 and 2.9% carried the OXA-48 gene only [19].

The activity of CZA (as detected by E-test) was statistically different among isolates harboring different carbapenemases genes (p-value of 0.022), with the best activity reported among isolates harboring OXA-48 gene (one of the 2 isolates is sensitive), followed by combined OXA-48 and NDM genes (42.9% sensitive), while the least activity was reported among isolates harboring NDM gene (11.5% sensitive) (table 3).

Additionally, the activity of MVB (as detected by E-test) was different among isolates harboring different carbapenemases genes, however, the difference was not statistically significant. The best activity of the drug was reported among isolates harboring NDM genes (19.2% sensitive), followed by isolates harboring combined OXA-48 and NDM genes (11.9 % sensitive) while the least activity was reported among isolates harboring OXA-48 (none of the two isolates was sensitive) (table 3).

Table1. Interpretative criteria for ceftazidime-avibactam and Meropenem-vaborbactam

Agent	Disc diffusion zone diameter breakpoints (mm)			MIC breakpoints (µg/ml)		
	S	**I**	**R**	**S**	**I**	**R**
Ceftazidime-avibactam	≥ 21		≤ 20	≤ 8/4		≤ 16/4
Meropenem-vaborbactam	≥ 18	15-17	≤ 14	≤ 4/8	8/8	≤ 16/8

Table 2. Relation between disc diffusion and E-test results for CZA and MVB

Relation between CZA disc diffusion and E-test results				
		CZA E-test		
		R	**S**	**P value**
		Number (%)	**Number (%)**	
CZA disc	**R**	47 (97.9%)	7 (31.8%)	< 0.001
	S	1(2.1%)	15 (68.2%)	
	Total	48 (100%)	22 (100%)	

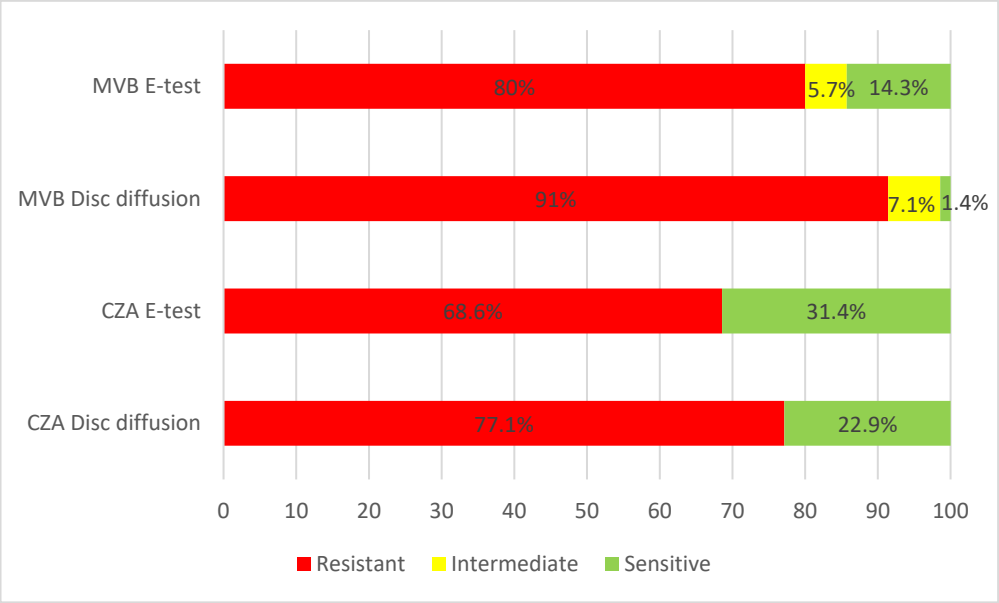
Relation between **MVB disc diffusion and E-test** results					
		MVB E-test			
		R	I	**S**	**P value**
		Number (%)	**Number (%)**	**Number (%)**	
MVB disc	**R**	56 (100.0%)	3 (75%)	5 (50%)	< 0.001
	I	0 (0%)	1 (25%)	4 (40%)	
	S	0 (0.0%)	0 (0%)	1 (10%)	
	Total	56 (100.0%)	4 (100%)	10 (100.0%)	

Table 3. Relation between results of CZA and MVB by E-test & carbapenemases genes.

I. CZA activity in relation to carbapenemases genes distribution								
		PCR						
		OXA-48		**NDM**		**NDM& OXA**		**P value**
		Number	**%**	**Number**	**%**	**Number**	**%**	
CZA E-test	**R**	1	50.0%	23	88.5%	24	57.1%	0.022
	S	1	50.0%	3	11.5%	18	42.9%	
	Total	2	100%	26	100%	42	100%	

II. **MVB activity in relation to carbapenemases genes distribution**								
		PCR						
		OXA		**NDM**		**NDM& OXA**		**P value**
		Number	**%**	**Number**	**%**	**Number**	**%**	
MVB E-test	**R**	1	50.0%	20	76.9%	35	83.3%	0.081
	I	1	50.0%	1	3.8%	2	4.8%	
	S	0	0.0%	5	19.2%	5	11.9%	
	Total	2	100.0%	26	100.0%	42	100.0%	

Figure 1. *In vitro* activity of CZA and MVB against CRE isolates using E-test and disc diffusion method



Discussion

Limited therapeutic choices are available for treating infections caused by CRE, and such infections are linked to high clinical failure and mortality rates, particularly in vulnerable patients. Hence, there is a critical need to promptly initiate effective antimicrobial therapy [20,21]. This study investigated the *in vitro* efficacy of the latest approved BL/BLIs, CZA and MVB, against 70 CRE isolates retrieved from hospitalized patients in Egypt. Overall, susceptibility rates to both CZA and MVB were low, at 31.4% and 14.3%, respectively. These low rates are explained by the predominance of NDM and OXA-48 gene carriers, which were later identified among our studied isolates. Avibactam, a component of CZA, effectively targets KPC and OXA-48 enzymes but lacks activity against MBLs like NDM-1. Similarly, vaborbactam, a component of MVB, potently inhibits KPC enzymes but exhibits limited activity against OXA-48 producers and no activity against MBL producers [9]. Consistent results were reported by previous studies conducted in the same geographical region, where *Ahmed et al* reported similar CZA susceptibility rates (30%) among CRE strains isolated from pediatric hospital in Cairo [22]. Comparable susceptibility rates (23.5%) were reported in Zagazig among CRE strains isolated from ICU patients [23]. Meanwhile, lower susceptibility rates were detected among CRE strains isolated from adult and neonatal ICU from the same Egyptian city (13.3% and 8%, respectively). [12,24]. Regarding MVB, few Egyptian studies investigated the susceptibility of CRE to MVB and they reported higher sensitivity rates (48%, 58%) than our study [25,26]. In contrast to our findings, studies from other geographical regions reported higher susceptibility rates. For example, *Nordmann et al* reported moderate susceptibility rate to CZA and MVB among CRE isolates recently recovered in Switzerland (63%, 77%, respectively) [27]. Comparable rates were reported by *Huang et al.* in China with susceptibility rates of (60%, 83%) for CZA and MVB, respectively [15]. Meanwhile, in the United States, higher susceptibility rates were reported by *Sader et al.*, (82.6%, 81.7% for CZA and MVB, respectively) [28]. These discrepancies can be attributed to variations in the characteristics and genetic makeup of the tested isolates. While NDM and OXA-48 are predominant carbapenemase genes in Egypt [12,22]. KPC enzymes were historically more prevalent in China and Europe, however, a notable shift in the carbapenemase landscape has been observed, with increasing prevalence of MBLs and OXA-48-like enzymes in recent studies [29,30]. In contrast, KPC remains the dominant carbapenemase type in the United States [3].

Given the distinct inhibitory profiles of CZA and MVB against different carbapenemases, understanding the susceptibility patterns of these agents against specific carbapenemase producers can optimize antibiotic selection [27]. Our study found that 50% of isolates producing only OXA-48

were susceptible to CZA, while none were susceptible to MVB. These findings align with the known inhibitory spectrum of avibactam (active against OXA-48) and vaborbactam (limited activity against OXA-48). Our results corroborate those of *Gandor et al.,* who reported a 44% CZA susceptibility rate among OXA-48 producers [23]. On the other hand, our results partially matches previous study conducted by *Nordmann et al.,* where they reported in their investigation that their studied OXA-48 only producers' strains were susceptible to both CZA and MVB [27]. *Shrief et al.,* also reported MVB susceptibility rate of 50% among OXA-48 CRE strains [25]. The observed susceptibility to MVB in these strains was primarily attributed to the underlying susceptibility to meropenem, independent of vaborbactam's inhibitory effect. Previous research in a neutropenic murine infection model demonstrated limited efficacy of MVB against OXA-48-producing Enterobacterales, despite a significant proportion of isolates falling within the susceptible range according to EUCAST and CLSI guidelines [31]. Consequently, careful interpretation of *in vitro* susceptibility data for OXA-48-producing Enterobacterales is essential to predict clinical outcomes effectively [32].

Our study evaluated the effectiveness of CZA and MVB against isolates identified as OXA-48 and NDM co-producers. CZA demonstrated slightly higher susceptibility rates (42.9%) compared to MVB (11.9%). Limited research has investigated the *in vitro* activity of new BL/BLIs against these complex isolates. In their study, *Nordmann et al.* reported that all their tested isolates producing a combination of OXA-48-like and NDM enzymes were resistant to CZA and only 20% were susceptible to MVB [27]. While, *Shrief et al.* reported moderate MVB susceptibility among OXA-48 and NDM co-producers (50%) [25]. Interestingly, among NDM-1 only producers, 11.5% of our tested isolates were susceptible to CZA while 19.2% were susceptible to MVB. In accordance, *Sader et al.* [28] reported low susceptibility rates to CZA and MVB (2.6%, 15.8% respectively) among their NDM producers. *Nordmann et al.* reported similar susceptibility rate to MVB (20%), however they stated that no NDM producing isolates were susceptible to CZA [27]. On the contrary *Huang et al.* reported higher susceptibility rates among their NDM isolates (33.3%, 70% for CZA and MVB, respectively) [15]. The discrepancy between the genotypic and phenotypic susceptibilities of NDM-only producers or dual carbapenemase-producing organisms might be attributed to either low carbapenemase production or reduced carbapenemase affinity for the tested antibiotic combinations. These conflicting findings pose a challenge for treatment decisions, as there is limited research available to guide therapeutic approaches in such cases [33].

As novel antimicrobial agents targeting carbapenem-resistant Gram-negative bacteria are introduced into clinical practice [13], accurate susceptibility testing would be essential for effective antimicrobial therapy. While the reference BMD remains the gold standard method, its complexity and resource-intensive nature limit its use in routine clinical laboratories, [13]. Previous studies have demonstrated the reliability of the gradient diffusion MIC strip method for determining susceptibility to CZA and MVB, In addition to gradient strip methods, the disc diffusion method offers a simpler and more cost-effective approach for susceptibility testing, particularly in resource-limited settings. This study evaluated the disc diffusion method for determining the susceptibility of CRE to CZA and MVB, using the MIC strip method as a reference standard. The overall agreement between the two methods was acceptable for both antibiotics (88.6% and 82.9% for CZA and MVB respectively) with low VME of (1.43%, 0%, respectively). Nonetheless, the disc diffusion method demonstrated significant limitations in correctly identifying susceptible isolates, with ME of (10%, 7.1%, for CZA and MVB, respectively) and mE error for MVB (10%).

Regarding CZA, our results matches previous studies [13,34] which reported acceptable CA values (76%, 87%) and no VME (0%) between disc diffusion and BMD as a reference method, however high number of false resistant isolates were observed; ME (16%, 24.3%). On the other hand, multiple studies reported much better performance of disc diffusion method with CA (98%-100%) with BMD method, ME (0-1%) and VME (1%-3%) [7,16]. For MVB, limited data exists, only one previous study evaluated MVB disc diffusion using BMD as a reference method, they reported slightly better performance than our study with 90% CA between the two methods and 3.3% ME, 6.7% mE and no VME [17]. The higher rate of false-resistant results in our study may be attributed to the prevalence of isolates with elevated MIC values,

which can impact the accuracy of disc diffusion [35]. To minimize errors, determining the MIC when zone diameters are close to the breakpoint is recommended [36]. Accordingly, when the zone diameters of CZA/MVB against Enterobacterales is at or near the breakpoint, MIC should be determined to avoid false-susceptible or false-resistant results [14]. Continuous monitoring of the disc diffusion method's performance is essential, as the emergence of novel resistance mechanisms to CZA and MVB could lead to an increased prevalence of clinical isolates with MIC values approaching or exceeding the breakpoint, even among genetically susceptible strains [36]. Clinicians and microbiologists should be aware of the limitations of current susceptibility testing methods for these novel antibiotics and consider additional testing, such as reference methods or genotypic analysis, when discrepancies arise [37].

Conclusion

In conclusion, our study demonstrated that the latest approved BL/BLIs such as CZA and MVB are generally ineffective against CRE currently prevalent in our hospitals. While CZA showed slightly better performance than MVB, overall susceptibility rates were low. Accordingly, CZA should be reserved for targeted therapy based on accurate susceptibility results and MIC values, rather than used empirically. Continuous monitoring of CRE strains and their characteristics is crucial to assess the ongoing utility of these new antibiotics. The study also highlighted the limitations of disc diffusion testing, emphasizing the need for more accurate methods like the E-test, particularly for isolates with disc zone diameters at or near the breakpoint. Despite acceptable CA and low rates of VME, the disc diffusion method demonstrated a propensity to overestimate resistance, potentially leading to suboptimal and potentially harmful therapeutic choices.

Conflicts of Interest:

All authors declare no conflict.

Ethical Approval

The current work was approved by the the Research Ethics Committee, Faculty of Medicine, Cairo University (N172-2023).

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Author Contributions:

Conceptualization:

ElFeky D.S., Awad A.M. **Methodology and validation:** ElFeky D.S., Awad A.M. and Baddour M.M. **Writing original draft and figure preparation:** ElFeky D.S., Hamed R.M. and Mowafy H.L. **Writing review and editing:** ElFeky D.S., Awad A.M., Baddour M.M., Mowafy H.L., Hamed R.M. **Supervision:** ElFeky D.S., Awad A.M, Baddour M.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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