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ORIGINAL ARTICLE

Evaluation of Low-cost, Manual Techniques for Rapid Detection of Ceftazidime-avibactam Susceptibility against Carbapenem-Resistant Enterobacterales

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ABSTRACT

Background: *Ceftazidime-avibactam (CAZ–AVI) is a new effective therapeutic combination that exhibits exceptional efficacy against clinically significant serine β-lactam-resistant bacteria. Emergence of CAZ–AVI resistance has been observed in carbapenem-resistant Enterobacterales (CRE). Therefore, rapid and accurate detection of CAZ–AVI susceptibility is time-saving for clinical treatment measures.* ***Objective****: To assess the efficacy and usage in clinical setting of two low-cost, rapid simple manual methods for screening of CAZ-AVI activity against CRE, to restrict spread of diseases and drug resistance through the implementation of rapid infection control programs.* ***Methodology:*** *The susceptibility of CAZ-AVI among CRE isolates was assessed using Rapid CAZ/AVI NP and Rapid ResaCeftazidime-avibactam-Enterobacterales NP tests along with broth microdilution method (BMD). Vitek- 2 compact was utilized to identify CRE isolates and CAZ/AVI sensitivity using GN and AST cards and Gene-Xpert system was used to determine the type of carbapenemase encoding genes.* ***Results****: This study utilized a total of 93 CRE isolates. Rapid CAZ/AVI NP test enabled the detection of CRE susceptibility to CAZ/AVI within three hours, with an overall percent agreement (OPA) of 97.9%, 2.8% major errors (MEs) and 0% very major errors (VMEs). In contrast, Rapid ResaCeftazidime-avibactam Enterobacterales NP test can be interpreted after 4 hours with an OPA of 98.9%, 1.4% MEs and 0% VMEs.* ***Conclusion****: These screening manual tests were rapid, simple, straightforward and easily applicable in routine microbiology laboratories. These tests are anticipated to be valuable tools for rapid clinical screening of CAZ-AVI susceptibility after further optimization of the test conditions in the near future.*

INTRODUCTION

The increasing rate of pathogenic multidrug-resistant (MDR) Gram-negative bacteria, particularly Enterobacterales, which acquire genes encoding multiple antibiotic resistance mechanisms has led to development of carbapenem resistance which become recently a significant worldwide public health interest[1,2].

The World Health Organization (WHO) has informed high morbidity and mortality of carbapenem-resistant Enterobacterales (CRE) infections as a category of MDR organisms highlighting urgent necessity for efficient and immediate antimicrobial remedies [3].

Ceftazidime-avibactam (CAZ–AVI) is a novel combination of 3rd generation cephalosporin, ceftazidime and non- β-lactam β-lactamase inhibitor avibactam. It was approved by the US Food and Drug Administration (FDA) for urinary tract infections with complication, intra-abdominal infections, nosocomial infections and ventilator-associated pneumonia [4]. It exhibits remarkable efficacy against clinically significant serine β-lactam resistant bacteria that produce class A enzymes (such as ESBLs and KPCs), class C enzymes (such as AmpC β-lactamases) and some class D enzymes (such as OXA-48). However, it does not show activity against class B metallo-β-lactamases (MBLs) such as NDM, VIM and IMP [5].

Despite effectiveness of CAZ-AVI, its resistance has emerged in Enterobacterales as a result of certain mutations in class A carbapenemases. These mutations, as well as modifications to target of antibiotic, cell permeability changes, efflux pumps overexpression, increased expression of KPC variants and overproduction and modifications in chromosome or plasmid encoding AmpC β-lactamases in

Enterobacterales are the most common mechanisms[6,7,8,9]. The European Center for Disease Prevention and Control (ECDC) has identified CAZ–AVI resistance as a significant threat requiring constant monitoring[10].Therefore,it is imperative to investigate the resistance mechanisms and develop precise and rapid methods for determining CAZ-AVI susceptibility [11].

Due to its cost-effectiveness and simplicity, disc diffusion method (DDM) is the preferred approach in Egypt for determining CAZ-AVI susceptibility. In most clinical laboratories in our country, commercially available broth microdilution plates (BMD), gradient diffusion tests such as E-Test Strip and automated system such as Vitek-2 are not favored for many reasons including time-consuming, necessity for overnight results, false resistance results, high cost of BMD,Vitek-2 and E-test [12,13,14].Consequently,it is imperative to develop alternative methods to assess the susceptibility of CAZ–AVI. Therefore, our study was done to assess the efficacy of two low-cost, simple, straightforward manual methods for rapidly screening CAZ–AVI activity against CRE. The aim of this study was to assess feasibility of applying these techniques to decrease cost for expensive equipment and tests, optimize clinical treatment time and minimize the spread of disease and drug resistance.

METHODOLOGY

Bacterial strains identification:

This cross-sectional study included a total of 93 meropenem-resistant Enterobacterales isolates that were analyzed for CAZ/AVI susceptibility using Vitek-2 compact. These isolates included *K. pneumoniae* (n=44), *E.coli* (n=29) and *Enterobacter cloacae* (n=20).The isolates were obtained from patients in Zagazig University Hospitals, Egypt, during the study period. Ethical approval was obtained from the Medical Research Ethical Committee at Zagazig University (IRB#: 455/30-July-2024). They were obtained from different clinical samples including urine (28), sputum (27), pus (20), blood (11) and ear discharge (8). These strains were identified by standard bacteriological methods and confirmed by Vitek-2 using GN cards (bioMérieux, France). A total of 21 CAZ –AVI resistant strains were detected by BMD including *E. coli* (n=7), *K. pneumoniae* (n=10) and *E. cloacae* (n=4). Also, 72 CAZ–AVI susceptible strains were detected by BMD, that include *E. coli* (n=22), *K. pneumoniae* (n=34) and *E. cloacae* (n=16).

Detection of carbapenems resistance and production of ESBL:

The isolates were selected based on their AST results by Vitek-2, obtained using AST-XN12 cards. The isolates were either resistant to carbapenems alone or in combination with ESBL production.

Identification of carbapenemases genes:

Gene-Xpert system, which utilizes Xpert CARBA-R assay (Cepheid, Sunnyvale, CA, USA) was employed to detect regions encoding carbapenemase enzyme, including (blaNDM, blaVIM, blaKPC, blaIMP and blaOXA-48). The results were obtained within 1h.

Susceptibility testing of CAZ –AVI was done by:

1- Vitek-2 compact: It monitors growth of Gram-negative bacteria in each well of the card for a specified period 18 h.

All CAZ/AVI susceptible and resistant strains were recorded. *E. coli* with ATCC 2955 was utilized as Quality control (QC) strain with expected range 0.12-0.5 ug/ml.

2- Broth Microdilution method (BMD):

Cation-adjusted Mueller-Hinton broth (CAMHB) micro-dilution method was used to determine Minimal inhibitory concentrations (MICs) of CAZ–AVI for bacterial isolates according to the CLSI 2020 protocol [15]. In this method 96-well micro-titer plates, 2-fold dilutions between 0.125 and 64 µg/ml for CAZ and a fixed 4ug/mL for AVI were prepared, respectively. Finally, we added a bacterial suspension of $5x10^5$ CFU/mL from freshly prepared isolates to each well then, they were incubated with CAZ–AVI at 37°C for 18 h. The studied Enterobacterales were set to be susceptible to CAZ–AVI at MIC ≤ 8/4 µg/mL and resistant at MIC ≥16/4 µg /mL as in CLSI 2020 and EUCAST 2022 protocols [15,16]. We compared BMD method with rapid CAZ–AVI tests. *E.coli* with ATCC 2955 was utilized as QC strain with expected range 0.06-0.5ug/ml.

3- Rapid ResaCeftazidime-avibactam Enterobacterales NP Test:

Negative and positive controls (confirmed by Vitek-2 and BMD) were one CAZ/AVI susceptible (KPC-producing *K. pneumoniae*) and one resistant (NDM-producing *E. coli*) bacterial isolates. Following methodology of Feng et al.[17]. *Preparation:* Cation Adjusted Mueller-Hinton solution (CAMHS) (Sigma-Aldrich, Egypt) was divided into two parts: Solution (A) contains only CAMHS, while Solution (B) contains CAMHS at a specific concentration of CAZ of 14µg/ml and AVI of 4µg/ml. Sterile 96-well round base polystyrene microplate was divided into two partitions. Partition(A) contains lines (A, C, E, G), while partition(B) contains lines (B, D, F, H). To prepare bacterial suspensions,1 McFarland was diluted from fresh culture for each of negative and positive controls and tests at a ratio of 1:20 using CAMHS.*Indicator:* 10% (v/v) concentration of resazurin-PrestoBlue solution (Biotium, USA). *Procedure steps:* 180 µL of solution (A) and (B) were added to corresponding partitions (A) and (B), respectively. Subsequently,20 µl of blank (CAMHS) was added. Diluted bacterial suspensions of negative and positive controls and tests were added to corresponding wells. Finally, we added

22 µl of the indicator to all wells. Samples were incubated at 37°C in ambient temperature with agitation for 4 h. Following that, medium's color change was visually assessed. Interpretation: Blank; The two wells remain blue (no medium contamination), susceptible or negative; one well turns purple or pink while the other well contains CAZ/AVI remains blue, positive or resistant; both wells turn purple or pink.

4- RapidCAZ/AVI NP Test:

One CAZ/AVI susceptible (KPC-producing *K. pneumoniae*) and one resistant (NDM-producing *E. coli*) isolates identified by Gene-Xpert, Vitek- 2 and BMD were used as negative and positive controls This was done following Nordmann et al. described method [18]. *Preparation*: We prepared 250 mL solution with final concentrations of 2.5% of Mueller-Hinton CA powder, 0.005% of phenol red indicator (Sigma –Aldrich, Egypt), 0.1 mol/L of zinc sulfate and1% of D (+)-glucose, then was divided into two parts: Solution(A) refers to the prepared solution alone, while Solution (B) represents solution (A) containing CAZ/AVI with a specific concentration of CAZ 128µg/ml and AVI 53µg/ml. Sterile 96-well round base polystyrene microplate was divided into two partitions: partition (A) includes lines (A, C, E, G) and partition (B) includes lines (B, D, F, H). *Bacterial suspensions:* 0.5 McFarland bacterial suspensions were prepared for each of the negative and positive controls and tests from fresh bacterial colonies on Muller Hinton agar using 0.85% saline solution. These suspensions should be used within 60 minutes as EUCAST guidelines recommendations for susceptibility testing. *Procedure steps:* Prior to use, all reagents should be pre-warmed at 37°C to avoid growth and color change delay. Afterward, each partition(A) and (B) should be filled with 150 µL of solution (A) and (B) respectively. Following this, 50 µL of a 0.85% saline solution should be added to the blank and bacterial suspensions prepared from negative and positive controls and tests in the corresponding wells. Finally, bacterial suspension was mixed by shaking the tray and then incubated in ambient air without sealing and shaking at 37°C for 3 hours. Subsequently, color change of media was visually inspected every 15 minutes. *Interpretation:* Blank; the 2 wells remain red (no medium contamination), susceptible or negative; One well turned yellow, indicating the tested isolate's viability and confirming the glucose metabolism while the other well containing CAZ/AVI remains red, positive or resistant; both wells turned yellow. This study required approximately the following amounts of reagents (2.1 mg of CAZ, 0.83 mg of AVI, and 65 ml of CAMHB) and only 4 microplates to complete Rapid ResaCAZ/AVI- Enterobacterales NP and Rapid CAZ/AVI NP tests.

Data Analysis:

We used BMD as a gold standard for CAZ/ AVI susceptibility. In addition, we determined discrepancies for each method to assess its performance. As previously described in Garrett et. al.[19], positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA) were calculated as follows: PPA= [true positive/ (all positive by gold standard)] ×100%, NPA= [true negative/ (all negative by gold standard)] ×100% and OPA=[(all true (positive + negative)/ (all tested isolates by gold standard)] ×100%. Major errors (ME) and very major errors (VME) were calculated according to CLSI protocol (CLSI, 2015) [20]. When BMD test showed susceptibility and studied Rapid test showed resistance, results were classified as ME while, when BMD test showed resistance and Rapid test showed susceptibility, results were classified as VME. CA, EA, OPA > 90%, ME < 3%, and VME < 1.5% were deemed acceptable criteria.

RESULTS

In this study, 93 strains of Enterobacterales were examined to assess performance of two Rapid tests (Rapid CAZ/AVI NP test and Rapid ResaCAZ-AVI-Enterobacterales NP test). The BMD method results indicated that 21 isolates were resistant (MICs ranging from 16/4 to >64/4 µg/ml) and 72 isolates were susceptible (MICs ranging from 0.125 to 8 µg/ml). Among the Enterobacterales, *K. pneumoniae* (n=44) and *E.coli* (n=29) represented the highest proportion followed by *E.cloacae* (n=20). The rapid methods yielded an equivalent number of CAZ-AVI-resistant strains (n=21) for Enterobacterales isolates as BMD (Table 1).

Genotype Determination:

Gene-Xpert system was used to detect regions encoding carbapenemase enzymes (Table 1). A total of 79 strains were revealed to have one or more of five types of resistance genes: *blaNDM, blaVIM, blaKPC, blaIMP* and *blaOXA-48*. *blaKPC* represented the highest proportions 57% (45/79), followed by *blaOXA-48* at 35.4% (28/79), NDM at 15.2% (12/79) and ESBL at 26.6% (21/79), respectively. Among them 15 isolates had two carbapenemase genes with and without ESBLs. Five isolates were CAZ/AVI resistant having one metallo-β-lactamase gene, while the remaining ten were sensitive having serine-β-lactamases only. The percentage of VIM and IMP were 7.6% (6/79) and 3.8%(3/79), respectively. Table 1 showed that among 21 CAZ/AVI resistant isolates, main cause of resistance was metallo-β-lactamases production which was detected in 95.2% (20/21), including *blaNDM* (n=12), blaVIM (n=6) and *blaIMP* (n=3). Furthermore, four serine-β-lactamases were detected, including KPC and Oxa-48. Nevertheless, two of each were associated with metallo-β-lactamases (Table 1). High-level resistance

was conferred by all of these resistant mechanisms with a range of (32-> 64/4 µg/ml). In addition, the remaining undiagnosed *E.coli* resistant isolate showed MICs of 32/4 µg/ml(1/21). 72 susceptible isolates were mainly serine-β-lactamases from classes A, C and D-β-lactamases, including OXA-48, KPC or both except 13 isolates with unidentified mechanisms (Table 1&2).

Performance of Vitek-2 Compact versus BMD test:

After assessing the isolates for CAZ/AVI susceptibility using Vitek-2, we also assessed its performance using the BMD method. We detected all 21 resistant isolates, while only one susceptible isolate yielded a positive (false-positive) result (71/72). Overall, the test showed that categorical agreement (CA) was 98.9% (92/93), essential agreement (EA) was 96% (90/94), ME was 1.1% (1/93) and 0.0% VMEs.

Performance of Rapid ResaCAZ-AVI-E NP test:

This test successfully recovered all 21 CAZ/AVI resistant isolates. Among 72 isolates that were susceptible to CAZ/AVI, 71 were identified as susceptible similar to BMD (Tables 1 & 2). One strain of *K. pneumoniae* with MIC of 8 µg/ml produced a false-positive result by both rapid tests. The overall performance of the test was OPA of 98.9%, PPA of 100% and NPA of 98.6% compared to the BMD. No VMEs and only 1.4% (1/72) ME (false positive) were observed (Table 3). The optimal final result was achieved after 4 hours of monitoring the results every 30 minutes.

Performance of Rapid CAZ/AVI NP test:

This test accurately detected all 21 CAZ/AVI resistant isolates. 70 out of 72 CAZ/AVI susceptible isolates by BMD were identified as susceptible (Tables 1, 2). Furthermore, this test yielded a positive (false-positive) result for 2 out of 13 undiagnosed susceptible isolates (*K. pneumoniae* and *E. coli*, one of each) with a MIC of 8 µg/ml. In comparison to BMD, the test demonstrated an OPA of 97.9%, PPA of 100% and NPA of 97.2%. No VMEs and only 2.8% ME (false positive) were observed (Table 3a). The result was continuously monitored at 15-minute intervals for a duration of 4 h and the optimal final outcome was achieved at 30 min, 2 h, and 3 h.

Performance of each Enterobacterales:

E. cloacae exhibited the highest performance in both tests with OPA of 100%, PPA of 100%, NPA of 100%, 0% MEs and 0% VMEs. The performance for *K. pneumoniae* was consistent in both tests with OPA of 97.7%, PPA of 100%, NPA of 97.1%, 2.9% MEs and 0% VMEs. Furthermore, both tests classified the same strain as false positive. The efficiency of Rapid ResaCAZ-AVI-E NP test for *E. coli* was comparable to that of *E. cloacae*. However, Rapid CAZ/AVI NP test yielded OPA of 96.6%, PPA of 100%, NPA of 95.5%, MEs of 4.5% and 0% VMEs (Table 3b).

Table 1: The distribution of different carbapenem resistance mechanisms among susceptible and resistant -CAZ/AVI isolates

Carbapenem Resistance Mechanisms					Organisms			BMD-CAZ–AVI susceptible phenotype
Gene type	N	Gene type	N	Total	K.P	E. coli	EBC	
NDM	**6**	**NDM + ESBL**	**4**	**10**	**7**	**3**	**-**	**R**
KPC + VIM	**1**	**KPC+ IMP**	**1**	**2**	**1**	**1**	**-**	**R**
VIM	**3**	**VIM + ESBL**	**1**	**4**	**1**	**1**	**2**	**R**
IMP	**1**	**-**	**-**	**1**	**-**	**-**	**1**	**R**
IMP+ OXA-48	**1**	**-**	**-**	**1**	**1**	**-**	**-**	**R**
NDM + VIM	**1**	**-**	**-**	**1**	**-**	**-**	**1**	**R**
NDM + Oxa-48 + ESBL	**1**	**-**	**-**	**1**	**-**	**1**	**-**	**R**
Not detected	**1**	**-**	**-**	**1**	**-**	**1**	**-**	**R**
KPC	23	KPC+ ESBL	10	33	12	10	11	S
OXA-48	12	OXA-48+ ESBL	4	16	8	5	3	S
OXA-48+ KPC	9	OXA-48+ KPC+ ESBL	1	10	6	3	1	S
Not detected	13	-	-	13	8	4	1	S

KPC, Klebsiella pneumoniae carbapenemase; NDM, New Delhi metallo-beta-lactamase; VIM, Verona integron-borne metallo-beta-lactamase; IMP, imipenemase; OXA, oxacillinases, ESBL; extended-spectrum β-lactamase, K.P; Klebsiella pneumoniae, E. coli; Escherichia coli, EBC; Enterobacter cloacae.

Table 2: Frequency of studied Enterobacterales isolates with ceftazidime-avibactam MIC (mg/mL) by broth microdilution method

	No. of isolates with ceftazidime-avibactam MIC (µg/ml)										BMD results	
Species	0.125	0.25	0.5	1	2	4	8	16	32	≥64	No. of resistant isolates	No. of susceptible isolates
E. coli	1	2	4	2	6	6	1	1	2	5	7	22
K. pneumoniae	2	2	7	8	10	4	1	1	3	6	10	34
E. cloacae	1	4	2	3	4	2	0	1	0	3	4	16
Total											21	72

Table 3: Performance of the Rapid CAZ/AVI NP test and Rapid ResaCeftazidime-avibactam Enterobacterales NP test compared with BMD method for Enterobacterales.

Table 3a: Rapid CAZ/AVI NP test compared with susceptible CAZ/AVI isolates by BMD

Species	PPA	NPA	OPA	MEs	VMEs	N	Type
Overall	100%	97.2%	97.9%	2.8%	0.0%	2/72	FP
E. coli	100%	95.5%	96.6%	4.5%	0.0%	1/22	FP
K.pneumoniae	100%	97.1%	97.7%	2.9%	0.0%	1/34	FP
E. cloacae	100%	100%	100%	0.0%	0.0%	0/16	-

Table 3b: Rapid ResaCAZ-AVI -E NP test compared with susceptible CAZ/AVI isolates by BMD

Species	PPA	NPA	OPA	MEs	VMEs	N	Type
Overall	100%	98.6%	98.9%	1.4%	0.0%	1/72	FP
E. coli	100%	100%	100%	0.0%	0.0%	0/22	-
K.pneumoniae	100%	97.1%	97.7%	2.9%	0.0%	1/34	FP
E. cloacae	100%	100%	100%	0.0%	0.0%	0/16	-

PPA; Positive percent agreement, NPA; negative percent agreement, OPA; overall percent agreement for discrepant results, MEs; major errors and VMEs; very major errors; FP; false positive

DISCUSSION

Multi-drug resistant Gram negative bacteria specially Enterobacterales producing carbapenemases, ESBLs and AmpC Beta lactamases remain a considerable threat to public health worldwide [21]. CAZ-AVI exhibits remarkable efficacy against serine β-lactam resistant bacteria but its resistance is expected to increase consistently as long as its usage in different clinical situations [5].

In this study, rate of resistance among CRE isolates was 22.3% (20%, 22.2%, and 24.1%) for *E. cloacae*, *K. pneumoniae*, and *E. coli* respectively, this result is similar to an Egyptian study which reported that 12% of CRE isolates were resistant to CAZ/AVI by E- test [22]. The rate of CRE isolates in both studies were lower than those represented in other Egyptian researches, in which CAZ/AVI resistance rate of 90% among CRE isolates [23] and 91.3% among CR *K. pneumoniae* isolates [24]. In contrast, the findings of the present study and other Egyptian studies were significantly higher than those of several worldwide surveillance programs that reported resistance rates ranging from 0.3% to 5.4% for Enterobacterales [25,26,27,28] and 18.6% for *E. cloacae* [28]. We hypothesize that it may be due to different carbapenem resistance mechanisms and that these mechanisms appear to be affected by geographical factors.

As regard genotypic detection of carbapenemases genes by gene Xpert in the current study, bla_{KPC} represented the highest proportions (57%), followed by bla_{OXA-48} (35.4%), *NDM* (15.2%), *VIM* (7.6%) and *IMP* (3.8%). *Elsawy et. al.* [29], analyzed carbapenemases encoding genes in *P. aeruginosa* by Gene-Xpert and reported that 23 isolates (13.7%) had bla_{NDM} gene and 53 isolates (31.5%) had bla_{OXA-48} gene a results which are quite similar to results of the current study. However, other carbapenemases genes weren't detected which is different from our results. In addition, *El Brardei et. al.* [30], by conventional PCR reported that bla_{OXA-48} was detected in 16 isolates (66.67%), followed by bla_{NDM-1} in 15 isolates (62.5%). However bla_{IMP} and bla_{KPC} were not found at all among the 24 isolates, however, bla_{VIM} was detected in only one isolate. In contrast *Hassan et. al.* [31], reported that the most frequently detected gene was *VIM* (83.3%) followed by *NDM* (69.8%), *OXA- 48* (62.5%), whereas the least numerous was *KPC* (19.8%). These results are inconsistent with the current study. We contributed cause of difference to different types of specimens,

bacterial isolates, antimicrobial protocols and infection control programs.

Analysis of CAZ-AVI resistant isolates in the current study revealed that 95.2% (20/21) were carbapenemase producers of the MBL type. Additionally, 28.6% and 19% of isolates were coproducers with ESBL or serine-β- lactamases, respectively.

Other studies have shown that all Enterobacterals isolates resistant to CAZ-AVI were carbapenemase producers and exhibited either ESBL or AmpC co-production in 100% and 88% of isolates, respectively. Furthermore, nearly 50% of resistant strains were found to be MBL producers [32]. Conversely, some studies have demonstrated that the majority of MBL-positive strains exhibit resistance to CAZ-AVI with resistance rates ranging from 90.8% to 98.6% [27,33,34].

Treatment of patients with severe CRE infections with CAZ-AVI has been correlated with an improved clinical cure rate, with a significant reduction in mortality [35]. This is the first study in Egypt to assess the efficacy of two rapid, simple, straightforward and cost-effective manual methods for immediate screening of CAZ-AVI susceptibility in comparison to BMD. BMD method is laborious and time-consuming, E-test strip method is high cost also Vitek-2 system however, it has excellent performance compared to BMD method as it achieved a CA of 98.9% (92/93), EA of 96% (90/94), ME of 1.1% (1/94) and 0.0% VME which is similar to Humphries et.al.[36], it is expensive and not available to most laboratories. In addition, these methods need additional overnight incubation period [14,37]. Therefore, all available CAZ-AVI susceptibility tests are not appropriate.

In our study, we compared rapid ResaCeftazidime-avibactam Enterobacterales NP test with BMD. In comparison to BMD, the test results agree with CLSI 2015 document. Nevertheless, our findings surpassed the findings of Feng et. al. [17] regarding OPA and MEs (96% and 7%, respectively) but yielded similar results for VMEs (0.0%). The assay duration (4 h) is considered the 2nd short-duration method among all other methods and could be used for this purpose.

Furthermore, we compared the rapid CAZ/AVI NP test with BMD. Our analysis of 93 Enterobacterales isolates revealed that the test results were similar to BMD. The results were monitored at 15-minute intervals for a duration of 4 hours. The optimal final result was achieved within the time range of 150 min to 180 min, which is widely regarded as the shortest duration among all tested methods. A previous study examined Enterobacterales species using the same technique and found that the test achieved an overall performance of 99%, PPA of 100% and NPA of 98.5% compared to the E-test as a reference method. Except for MEs, the reference method had only 1.5% MEs and 0% VMEs [18], which closely aligns with our results. The discrepancy in our findings can be considered false-positive results, which had MICs at the critical breakpoint (MIC = 8 µg/ml).

In this study, we assessed performance of both rapid methods for the studied species. By comparing the performance of our results with that of another study using Rapid CAZ-AVI-E NP test, we can conclude that the performance of the test regarding *E. coli* and *E. cloacae* was comparable with an excellent performance for *E. cloacae*. Nevertheless, the test's performance in our study related to *K. pneumoniae* was lower with 0.0% VMEs in both studies[18]. Additionally, comparing performance of rapid ResaCeftazidime-avibactam Enterobacterales NP test in our study with another study, we observed that the test for *E.coli* had outstanding performance in both studies, OPA of 100%,PPA of 100%, NPA of 100%, 0% MEs and 0% VMEs. The performance for *K. pneumoniae* is nearly comparable (NPA; 97.1% vs. 98%, OPA; 97.7% vs. 99%, MEs; 2.9% vs. 2%). However, the performance of our results regarding *E. cloacae* was excellent compared with the other study (OPA; 100% vs. 87%, NPA; 100% vs.75% and MEs; 0% vs. 25%) [17].

Finally, the advantages of these methods are rapid (results appear during duty hours), simple (one-step method) and cost-effective (no need for expensive instruments or specialized professional staff). These factors substantiated usage of these tests as an alternative to other methods. However, the primary drawbacks are that the MIC values cannot be reported as the results are subjective and dependent on color change and the results at the critical breakpoints are equivocal (may be true or false positive). In addition, the strains examined in this study were restricted necessitating additional research with more isolates to verify the efficacy of these rapid tests.

CONCLUSION

In conclusion, both examined manual screening tests are rapid, simple, easy, straightforward, cost-effective and readily applicable in standard microbiology laboratories. After further optimization of the test conditions in the near future, these methods are anticipated to be convenient for rapid clinical screening of CAZ-AVI susceptibility for Enterobacterales.

Conflict of interest:

The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript. Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it. This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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