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Phenotypic and genotypic characterization of carbapenem-resistant Gram-negative organisms, Beni-Suef, Egypt

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Abstract

Background Carbapenem-resistant Gram-negative organisms (CRGNO) are a growing threat. We aimed in our study to determine the genotype of carbapenemases at Beni-Suef University Hospital by using newly introduced lateral flow assays in comparison with molecular techniques and test the effectiveness of ceftazidime/avibactam against them.

Methods Screening for carbapenemase production was done by mSuperCARBA (CHROMagar™ company). Genotypic characterization was done using 3 different kits of lateral flow assays: the NG-Test CARBA5 assay (NG Biotech, Guipry, France), RESIST-3 O.K.N. (Coris BioConcept, Belgium) and Carbapenem-resistant K.N.I.V.O Detection K-Set (Beijing Gold Mountain river Tech Development Co, China), whereas genotypic characterization was done for blaVIM blaIMP, blaKPC, blaOXA-48, and blaNDM by PCR.

Results The high prevalence of CRGNO in Beni-Suef University Hospital (29%) was dominated by *Klebsiella pneumonia* (83.3%) harboring OXA-48 (92%). Lateral flow immunoassays showed high sensitivity and specificity for each type of carbapenemases in comparison with PCR.

Conclusion The coexistence of multiple carbapenemases genes in the same isolate increased resistance to new therapeutic options, e.g., CZA/AVI. Proper implementation of isolation measures in health care facilities can render the spread of CRGNO.

Keywords Carbapenem resistance, Lateral flow assay, Carbapenemases, Ceftazidime/avibactam

1 Background

According to the latest publications in Egypt, carbapenem-resistant Gram-negative organisms (CRGNO) is a growing threat [1, 2]. Knowing the genotype of CRGNO helps proper choice of newly introduced treatment options such as ceftazidime/avibactam (CZA/AVI) [3]. So, we aimed in our study to determine the genotype of carbapenemases at Beni-Suef University Hospital by using newly introduced lateral flow assays in comparison with molecular techniques and test the effectiveness of CZA/AVI against them.

2 Methods

All bacterial isolates described in this study were originally recovered from the microbiology lab at Beni-Suef University Hospital between June 2020 and January 2021. Gram-negative bacteria were isolated on MacConkey agar (Oxoid Co. England), and bacterial identification and susceptibility were completed by the

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vitek2 compact system (Biomerue, France) according to CLSI 2021 [4]. Screening for carbapenamase production was done by mSuperCARBA (CHROMagar™ company) according to manufacturer recommendations. Sixty isolates were selected to perform lateral flow assay by 3 available kits: The NG-Test CARBA5 assay (NG Biotech, Guipry, France), RESIST-3 O.K.N. (Coris BioConcept, Belgium) and Carbapenem-resistant K.N.I.V.O Detection K-Set (Beijing Gold Mountain river Tech Development Co, China) all were done according to manufacture recommendations. DNA was extracted according to [5, 6] with some modifications as follows: 1.5 ml of the culture was centrifuged for 10 min at 5000 rpm, and then the supernatant was discarded; 400 µl spheroblast buffer (10% sucrose, 25 mM Tris pH 8.4, 25 mM EDTA pH 8.0, 2 mg/ml lysozyme and 0.4 mg/ml RNase A) was added to the pellets and vortexed. Then, the tubes were incubated for 10 min at 37 °C. We added 1000 ml of 5% SDS (lysis buffer 1) and 5 M NaCl (lysis buffer 2) to the tubes and mixed well, and then, the tubes were incubated at 65 °C for 5 min. We added to the mixture, 200 µl neutralizing buffer which was consisted of 60 ml 5 M potassium acetate, 11.5 ml glacial acetic acid, and 28.5 ml dH2O. The mixture was put in ice for 5 min and then centrifuged for 15 min at 13,000 rpm at 4 °C. Approximately 500 µl of the supernatant was transferred to a new tube, and an equal amount of chloroform:isoamyl alcohol (24:1) was added, and then the new tube was incubated at room temperature for 5 min. Approximately 300 µl of the aqueous layer was transferred to a new tube, and an equal amount of isopropanol was added, and then the new tube was incubated at room temperature for 5 min. The mixture was centrifuged for 15 min at 13,000 rpm and the supernatant was removed and the pellets were washed by 70% ethanol and then centrifuged again for 5 min at 13,000 rpm. Finally, the pellets were air-dried from ethanol and dissolved in 50 µl water and stored at refrigerator 4 °C for the next step. Primers targeting blaVIM, blaIMP, blaKPC, blaOXA-48, and blaNDM were used to determine the presence of carbapenemases encoding genes as in Table 1 [7], whereas PCR amplification was performed in a total volume of 15 µl containing 0.75 µl of each primer, 7.5 µl PCR Master Mix (2X), 1 µl of genomic DNA and then the volume was completed to 15 µl with nuclease-free water. Thermal cycler was used under the following conditions for each gene as shown in Table 2. Amplification was performed using a programmable thermal controller PCR machine (Biometra, Germany); all genotypic work was carried out at the faculty of pharmacy, Beni-Suef University, Egypt. Then the presence of specific amplified DNA bands was detected by visualization with UV light at different wave lengths as shown in Figs. 1, 2, 3, 4 and 5.

Table 1 Primers targeting blaVIM, blaIMP, blaKPC, blaOXA-48, and blaNDM

Gene	Primer	Reverse primer	References
KPC	CATTCAAGGGCTTTCTTGCTGC	ACGACGGCATAGTCATTTGC	[6]
OXA-48	GCTTGATCGCCCTCGATT	GATTTGCTCCGTGGCCGAAA	[6]
NDM	GGTTTGGCGATCTGGTTTTC	CGGAATGGCTCATCACGATC	[6]
VIM	GGTGTTTGGTCGCATATCGCAA	ATTCAGCCAGATCGGCATCGG	[7]
IMP	TCGTTTGAAGAAGTTAACG	ATGTAAGTTTCAAGAGTGATGC	[7]

Table 2 Thermal cycler conditions and detection for each gene

	OXA-48	KPC	NDM	VIM	IMP
Initiation step	95 °C for 5 min	95 °C for 5 min	95 °C for 5 min	95 °C for 5 min	95 °C for 5 min
Denaturation	94 °C for 45 s	94 °C for 45 s	94 °C for 45 s	94 °C for 45 s	94 °C for 45 s
Annealing	54 °C for 45 s	54 °C for 45 s	54 °C for 45 s	58 °C for 45 s	46.5 °C for 45 s
Extension	72 °C for one min	72 °C for one min	72 °C for one min	72 °C for one min	72 °C for one min
The steps of denaturation, annealing and extension were repeated for 35 cycles					
Final extension	72 °C for 10 min	72 °C for 10 min	72 °C for 10 min	72 °C for 10 min	*t* 72 °C for 10 min
Molecular size Base par (bp)	238 bp	498 bp	521 bp	502 bp	568 bp
References	[6]	[6]	[6]	[7]	[7]

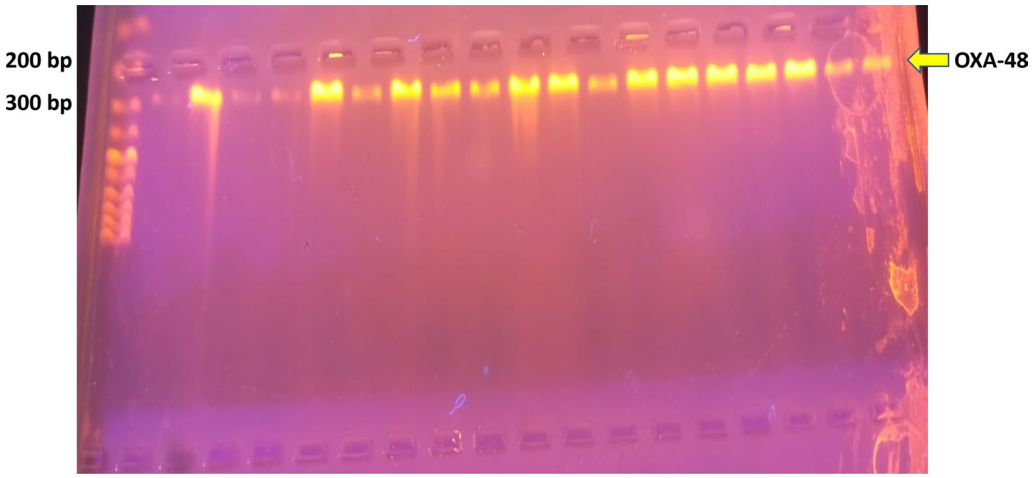

Fig. 1 Detection of OXA- 48

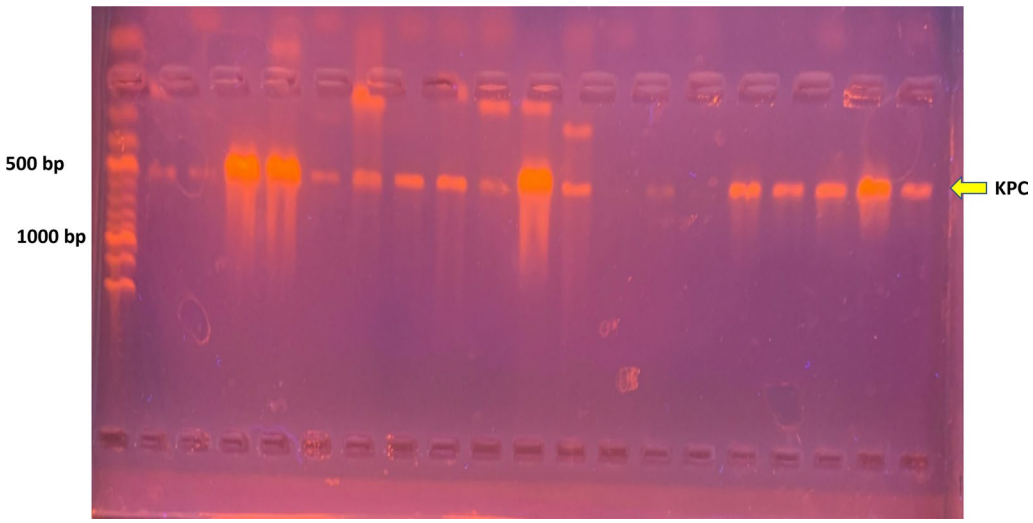

Fig. 2 Detection of KPC

3 Results

The total number of samples collected over 8 months was 1000 samples, 300 of them gave no growth, 370 of them revealed growth of Gram-positive bacteria, and 330 of them were Gram-negative bacteria, out of which 96 isolates were carbapenem-resistant 96/330 (29%). Complete data analysis was done for our studied isolates; among the 96 cases, the most common clinical samples were from blood cultures (46/96; 47.9%) followed by sputum culture (30/96; 31.3%); data analysis showed that Klebsiella spp was the most prevalent organism in carbapenem resistance (78.1%). The results of lateral flow immunoassays in comparison with the results of PCR in each gene, the sensitivity and specificity of NG-Test Carba 5 were in range from 96.5 to 100% and 96.7 to 100%, respectively. Also, the sensitivity of RESIST-3 O.K.N-SeT was in range from 98 to 100% and the specificity was 100%. After exclusion of the isolates of *Acinetobacter* spp., the overall sensitivity to (CAZ-AVI) was 15%. Table 3 shows the distribution of the CRGNO over various sample types, carbapenemases genes and CZA/AVI susceptibility while Table 4 shows the evaluation of rapid phenotypic methods for detection of CRGNO.

4 Discussion

In a wide Egyptian study covering a period from 2011 to 2017, Kotb et al. (2020) reported a high prevalence of carbapenems resistance (54.1%) among *Enterobacterales*. In

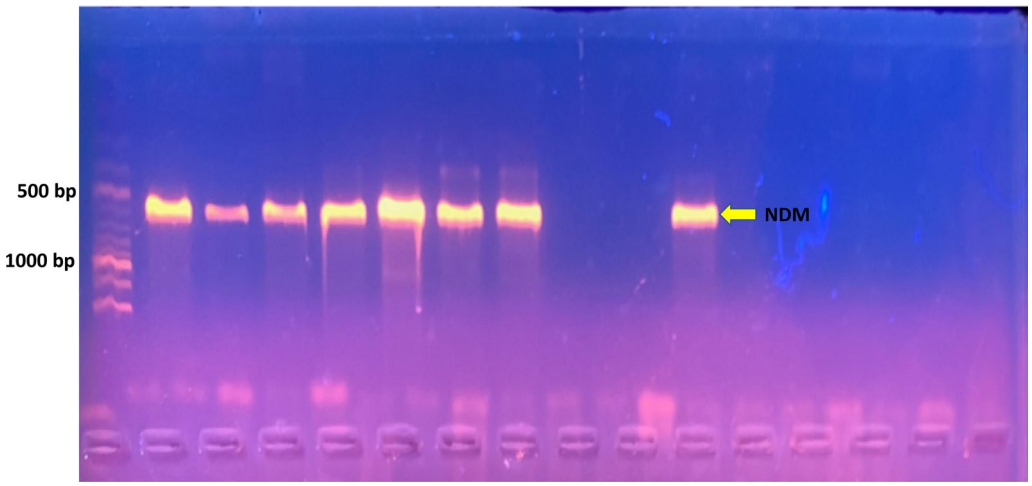

Fig. 3 Detection of NDM

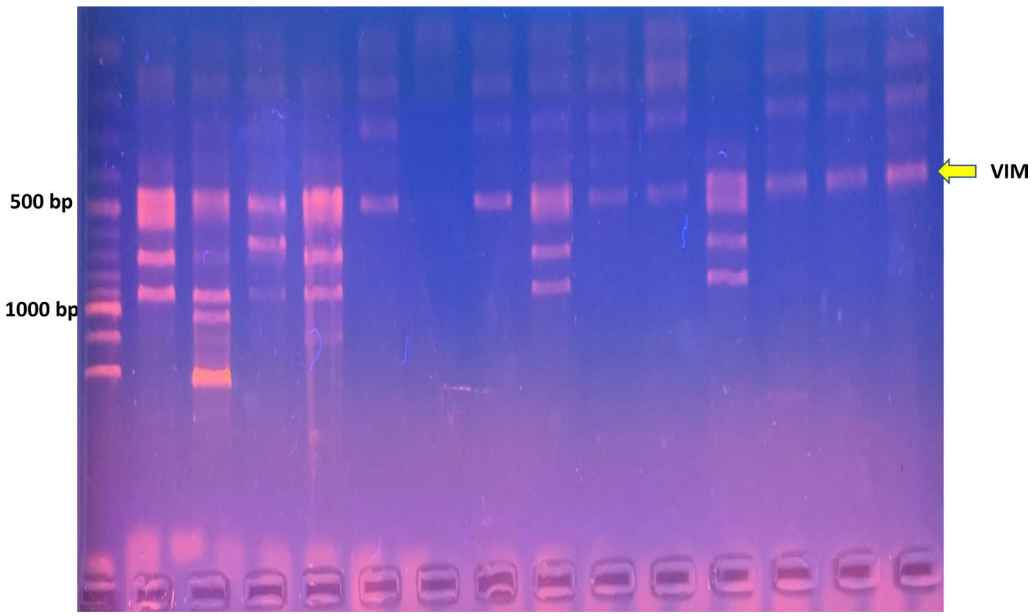

Fig. 4 Detection of VIM

our study, the prevalence of CRGNO in Beni-Suef University Hospital was (29%) which is like that was reported in a study done by Haji et al. (2021) in the Iraqi Kurdistan region (30.9%) [1, 8]. Resistance to carbapenem antibiotics is different all over the world a previously published review showed that resistance to carbapenems was reported in 10–66% of Gram-negative isolates in Saudi Arabia, which was lower than that in Iran (86%). On the contrary, in Europe, 4% and 85% of gram-negative isolates were carbapenem-resistant in the north and south of Europe, respectively [9]. This may be due to differences in the degree of performance of the infection control measures in different countries and hospitals.

Klebsiella pneumonia was the most prevalent isolate-producing carbapenemases in our study and in many other Egyptian studies whether as a colonizer or true pathogen, especially in neonatal intensive care units [10–13].

Regarding the genotype profile, OXA-48 stile the most prevalent carbapenemase gene in our study (92%) in agreement with Muhammad et al. (2021) who reported 72.5% in Al-Zahraa and Helwan University hospitals,

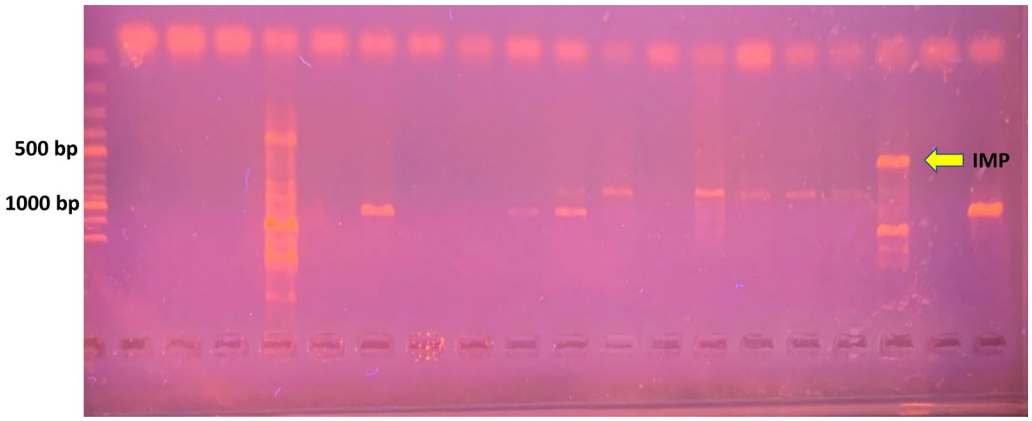

Fig. 5 Detection of IMP

also Osama et al. (2021) who worked on clinical isolates obtained from pediatric cancer patients in Egypt yet, Wassef et al. (2020) worked on colonizers [14–16].

The second prevalent carbapenemase gene was NDM (85%); Wassef et al. (2016) detected NDM in only 24% of isolates in 2016 [17]. Yet, in 2020 Tawfick et al. detected NDM with a high percentage of 68.88% in diverse biological specimens collected from patients admitted to Cancer Institutes in Cairo [18]. The percentage of KPC gene is increasing in Egypt; in our study, it was detected in 73% of the isolates. This may be due to increased prevalence of multidrug-resistant *Klebsiella spp* and increased usage of carbapenem drugs in treatment of these cases.

Lateral flow immunoassays provide shorter turnaround time than the conventional bacterial culture and susceptibility testing methods. This new assay will be useful in hospital infection control contact isolation policy, as well as identification and treatment of resistant strains due to simple operation and cost-effectiveness.

The used lateral flow assays showed high sensitivity and specificity of each type of carbapenemases in comparison with PCR as shown in Table 2. Other studies that showed lower sensitivities as Baer et al. (2021) reported lower sensitivity (89.5%) and low negative predictive value (66.7%) due to dilution with saline in the simulated blood cultures. The lower sensitivities were noted when the NG-Test Carba 5 was used for detecting NDM, IMP, or OXA-48-like carbapenemases and NG-Test Carba 5 failed to identify some of the carbapenemases in organisms with combinations of KPC + OXA48, KPC + NDM + OXA48, and KPC + NDM + IMP [19].

In our study, the sensitivity of RESIST-3 O.K.N-SeT in detecting the most prevalent carbapenemase genes (OXA-48, KPC, NDM) in the 60 isolates was in the range from 98 to 100% and specificity was 100% this is like Wink et al.(2019) who used clinical CRGNB obtained from a previous surveillance study in southern Brazil [20]. On the other hand, Bogaerts et al. (2020) reported a sensitivity and specificity of RESIST-4 O.K.N.V-SeT 100% in comparison with two multiplex immunochromatographic assays besides, RESIST-4 and NG-CARBA5, providing direct results from clinical isolates or positive blood culture broth in a maximum of 15 min. Yet, RESIST-3 O.K.N. which was used in our study has a limitation in terms of detecting other carbapenemases such as IMP and VIM producers [21].

Recently, Josa et al. (2022) used two lateral flow immunoassay NG-CARBA5 and RESIST-4O.K.N.V-SeT for detecting carbapenemases *in Enterobacterales and P.aeruginosa* and compared them with phenotypic synergy tests. According to the results of PCR, they reported that the two types of lateral flow immunoassay correlated 100% with molecular characterization by PCR [22].

In our study, we used Carbapenem-resistant K.N.I.V.O Detection K-Set (Beijing Gold Mountain river Tech Development Co, China) to be evaluated for the first time with a sensitivity in the range from 96.5 to 100% and the specificity was in the range from 93.5 to 100%, respectively.

We tried to find the relation between the response to CZA/AVI and the genetic characterization of the 58 CRGNB isolates after the exclusion of *Acinetobacter spp*.; we conducted that the good response to that antibiotic was only in the isolates that harbored OXA-48 and KPC genes but the bad response in the isolates harbored Metallo- beta-lactamase genes NDM and VIM. The coexistence of multiple carbapenemases genes in the same isolate increased the CZA/AVI resistance as shown also in Ahmed et al.'s (2023) study [23]. Although new antibiotics give more choices for treatment, newly introduced safer options such as probiotics need to be more evaluated [24].

Table 3 Distribution of the CRGNO over various sample types and carbapenemases genes, and CZA/AVI susceptibility

Organism	#	Sex		Department			Sample type				PCR					CZA/AVI	
		Male	Female	Adult ICU	NICU	Other wards	Blood	Respiratory	Urine	Others	NDM	OXA-48	KPC	VIM	IMP	S	R
Klebsiella pneumoniae	50(83.3%)	34(68%)	16(32%)	16(32%)	18 (36%)	16(32%)	23(46%)	17(34%)	7(14%)	3(6%)	45(90%)	50(100%)	43(86%)	24(48%)	0	7(14%)	43(86%)
Acinetobacter baumannii	2(3.3%)	2(100%)	0	1(50%)	1(50%)	0	1(50%)	0	1(50%)	0	0	0	0	1(50%)	2(100%)	Not tested	
Pseudomonas aeruginosa	3(5%)	2(66.7%)	1(33.3%)	1(33.3%)	1(33.3%)	1(33.3%)	0	0	3(100%	0	3(100%)	2(66.7%)	0	3(100%)	0	0	3(100%)
Enterobacter cloaca	3(5%)	2(66.7%)	1(33.3%)	0	2(66.7%)	1(33.3%)	1(33.3%)	1(33.3%)	0	1(33.3%)	3(100%)	3(100%)	1(33.3%)	13(3.3%)	0	0	3(100%)
E. coli	2(3.3%)	1(50%)	1(50%)	0	2(100%)	0	0	0	2(100%)	0	–	–	–	–	–	2(100%)	0
Totals 60	60(100%)	41(68.3%)	19(31.7%)	18(30%)	24(40%)	18(30%)	25(41.7%)	18(30%)	13(21.7%)	4(6.6%)	51(85%)	55(92%)	44(73%)	29(48%)	2(3%)	9(15.5%)	49(84.5)

ICU intensive care unit, *NICU* neonatal intensive care unit, *CZA/AVI* ceftazidime-avibactam

Table 4 Evaluation of rapid phenotypic methods for detection of CRGNO

	NG-Test CARBA 5 (%)				RESIST-3 O.K.N. K-SeT (%)				Carbapenem-resistant K.N.I.V.O detection K-Set (GOLD MOUNTAINRIVER) (%)			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
OXA-48 gene	100	100	100	100	100	100	100	100	98.2	100	100	83.3
NDM gene	100	100	100	100	98	100	100	90	100	100	100	100
KPC gene	97.7	100	100	94.6	100	100	100	100	97.7	100	100	94
VIM gene	96.5	96.7	96.5	96.7	Not tested				96.5	93.5	100	93.3
IMP gene	100	100	100	100	Not tested				100	100	100	100

PPV positive predictive value, *NPV* negative predictive value

There are some limitations in this study as our study is performed only in one center (Beni-Suef University Hospital) not multicenter also, the small sample size.

5 Conclusion

The increase in the percentage of CRGNB in NICU is mostly due to defective infection control measures mainly hand hygiene. Also, the efficacy of CZA/AVI in the treatment of the cases is not promising due to the high percentage of NDM gene in Beni-Suef University Hospital. We recommend increasing training courses on infection control, especially for proper hand hygiene measures, and trying to search for other therapeutic options, e.g., probiotics and bacteriophage.

Abbreviations

CRGNO Carbapenem-resistant Gram-negative organisms
CZA/AVI Ceftazidime/avibactam

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by SM, DG, MS and RR. The first draft of the manuscript was written by DG, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data are available.

Declarations

Ethics approval and consent to participate

This is an in vitro experimental study that no ethical approval is required.

Consent for publication

Not applicable because this manuscript does not contain any individual personal data.

Competing interests

Authors declare no competing interests.

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