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In Vitro Efficacy of Ceftazidime-Avibactam among Carbapenem Resistant Enterobacterales and Pseudomonas aeruginosa Clinical Isolates in Specialized Pediatric Hospital

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ABSTRACT

Background: Carbapenem-resistant organisms (CRO) have been disseminated worldwide. Ceftazidime-avibactam (CZA/AVI) has been suggested as an alternative option.

Objectives: This study aims to assess the prevalence of CRO among clinical isolates and to investigate the in vitro antimicrobial activity of CZA/AVI.

Design: Our observational experimental in vitro study was conducted over one year.

Settings: Pediatric specialized teaching hospital.

Material and Methods: Identification was done by MALDI-TOF-MS. CZA/AVI susceptibility testing was done by disk diffusion. The presence of carbapenemases was detected by modified carbapenem inactivation method and multiplex PCR tests.

Main Outcome Measures: Prevalence of CRO was 72.6%; (80.0%) of our isolates were Klebsiella pneumoniae. The most frequently detected carbapenamase genes were blaNDM (70.0%), followed by blaOXA-48 (68.24%) and blaKPC (16.47%).

Sample size: 170 clinical isolates of Enterobacterales and Pseudomonas aeruginosa.

Result: CZA/AVI in vitro efficacy was 30.0%.

Conclusion: Due to the high prevalence of metallo B lactamases in our hospital CZA/AVI may not be a good therapeutic option for CRO infections, emphasizing the importance of improving infection prevention and control.

Limitations: larger sample size is needed and studying the in vivo response in correlation with the invitro results will be of great benefit.

Keywords: *Ceftazidime-avibactam, Carbapenem resistant, Multiplex PCR*

INTRODUCTION

Carbapenem resistant organisms (CRO) are described as infections that can produce a carbapenamase or are resistant to one type of carbapenem1. The spread of CRO has been observed worldwide 2,3. In Egypt, El-Kholy et al.4 have reported 28% resistance levels compared to 0–1% in the Gulf Cooperation Council and, 1.22.5 percent in the Levant and 2% in the remaining African nations4. That is why the world health organization gave the priority to research and developments of new antibiotic5. Ceftazidime-avibactam (CZA/AVI) is newly introduced to the Egyptian market. The reversable inhibition of β-lactamases is a unique character that other known β-lactamase inhibitors lack therefore, the activity of AVI is restored once acted 6-8. Infections caused by organisms that are resistant to several drugs are becoming more common in children, and developing treatments became significantly more difficult9. The aim of this is study was to assess the prevalence of CRO among clinical isolates and to investigate the in vitro antimicrobial activity of CZA/AVI.

METHODS

This cross-sectional study was performed at the clinical microbiology unit of Cairo University Specialized Pediatric hospital (CUSPH). A total of 170 CRO clinical isolates were collected between August 2019 and September 2020, without being duplicated every isolate was obtained from regularly sent-off cultures and cultivated aerobically on standard blood, chocolate, MacConkey, and CLED agar media (Oxoid, Basingstoke, United Kingdom) at 37ºc for (24-48) hours. Enterobacterales and Pseudomonas aeruginosa

Gram staining and matrix assisted laser desorption ionization-mass spectrophotometry MALDI-TOF MS were used for further identification.

Susceptibility to carbapenems was determined by

1- Breakpoints for carbapenems as determined by the clinical and laboratory standards institute are used in standard Kirby Bauer disc diffusion to identify CRO isolates10.

2- Modified carbapenem inactivation method (mCIM) 11.

Ceftazidime-avibactam susceptibility was established by using the standard Kirby Bauer disc diffusion method using 30/20 microgram disk content) LOT 448800, mast diagnostics, UK, 2019)10, 11.

For multiplex PCR testing, bacterial isolates were stored at -80°C while suspended in 20% glycerol trypticase soy broth according to Poirel et al. 2011, Multiplex PCR was used to detect the following carbapenamase genes utilising three distinct multiplex reactions for OXA-48, NDM, and KPC12. Quality assurance procedures were carried out throughout all of the tests, including those involving the culture media, biochemical processes, and antimicrobial discs.

The control for CZA susceptibility testing was E. coli ATCC 25922.

K. pneumoniae NCTC 13443 served as a positive control for NDM in the PCR process.

Statistical analysis

The terms range, mean, standard deviation (SD), and percentages were employed to describe the data statistically. A probability value (P value) less than 0.05 was used to indicate statistical significance. All statistical computations were performed using Microsoft Excel 2010 and SPSS (Statistical Package for the Social Science) version 23 for Windows.

RESULTS

During the study period a total of 1150 clinical samples were identified as Enterobacterales isolates and Pseudomonas aeruginosa out of which 835 isolates were carbapenem resistant, the prevalence of carbapenem resistance was 72 .6%. All data is shown in Table 1 while Figure 1 illustrates the susceptibility rates of ceftazidime-avibactam and other antibiotics among CRO. Figure 2 shows the PCR gel electrophoresis for the carbapenemases genes.

TABLE 1: Distribution of the organisms, device utilization associated with CRO infections, the results of mCIM , carbapenemases genes and CZA/AVI susceptibility

		Department		Sample type								MCIM			Gene						CZA		
				Respiratory 69	Blood 42		Urine 8		CSF 7														
		ICU	ward	Vent 40 57.97%	No vent	CVL30 71.43%	No CVL	Catheter	No catheter	Shunt	NO	Wound 19	Positive	indeterminate	Negative	OXA-48	P value	NDM	P value	KPC	P value	Sensitive	Resistant	
Klebsiella pneumoniae	136			33	2341.1%	24 61.5%	15 38.5%	3 42.9%	4 57.1%	3 100%	0	31	125 91.91%	7 5.15 %	4 2.94%	106 77.9%	<0.001*	92 67.6%	0.06	26 19.1%	0.04	45 (88.24%)	91 (76.47%)	
Pseudomonas aeruginosa	26			7 53.8%	6	3 100%	0	0	1 100%	0	0	9	24 92.3%	0 0%	2 7.7%	5 19.2%	<0.001*	22 84.6%	0.07	1 3.8%	0.04	3 (5.88%)	23 (19.33%)	
E. Coli	4 2.35%									1		3	4 100%	0%	0%	2 50%		3 75%		0%		1 (1.96%)	3 (2.52%)	
Enterobacter hormaechei	2					1	1						2 100%	0%	0%	2 100%		0%		0%		2 (3.92%)	0%	
Klebsiella oxytoca	1					1							1 100%	0%	0%	0%		1 100%		0%		0%	,1 (0.84%)	
Proteus mirabilis	1					1							0 0%	1 100 %	0%	1 100%		1 100%		1100%		0%	1 (0.84%)	
Total	170	137	33										156 91.76%	8 4.71%	6 3.53%	116 68.24%		119 70%		28 16.47%		51 30%	119 70%	

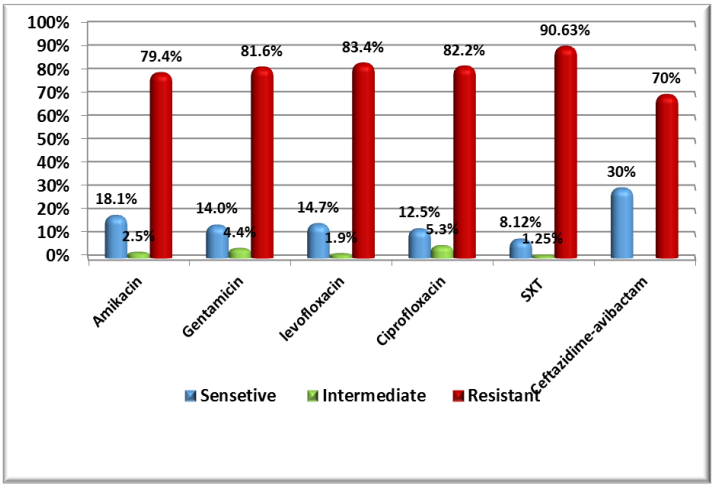

FIGURE 1: Susceptibility rates of ceftazidime-avibactam and other antibiotics among CRO. SXT: sulfamethoxazole-trimethoprim.

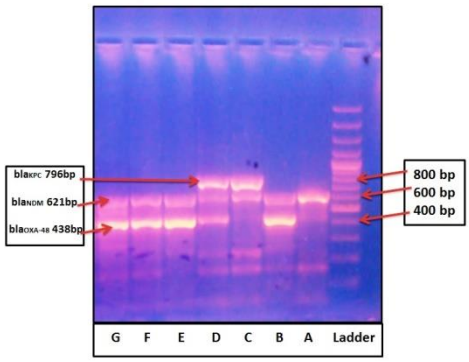

FIGURE 2: Gel electrophoresis of PCR reaction . Isolate (A) shows blaNDM genes. Isolate (B) shows blaOXA-48 and blaNDM genes. Isolate (C) shows blaNDM and blaKPC. Isolate (D) shows blaOXA-48 blaKP, and blaNDM genes.

DISCUSSION

Our study reported high prevalence of carbapenem resistant Enterobacteriaceae and Pseudomonas aeruginosa among clinical isolates (72.6%) similarly to Kotob et al., 47.9% of the Enterobacterales isolates monitored from 2011 to 2017 were carbapenem resistant 13.

Regarding the risk factors associated with carbapenem resistance in our study 80.59% of the isolates were related to ICUs patients. 71.43% of isolates were from patients having central venous line, 57.97% of respiratory samples were from patients on mechanical ventilator. Previous studies found that CRE infections are frequently linked to admission to the intensive care unit (ICU), an extended hospital stay, and the use of medical equipment, including central venous catheters and mechanical ventilation 14-16.

In this study, Klebsiella pneumoniae was the most prevalent isolated pathogen in 80.0% of the isolates. According to other studies reported that Klebsiella pneumoniae was the most prevalent isolated organism17, 18.

We found that mCIM was positive in 91.76% of CRO in our study, Similarly, Gelmez et al., 2020 have evaluated mCIM and other phenotypic methods for detection of carbapenemases in Enterobacteriaceae and found that mCIM was positive 90.5% among isolates carrying blaOXA-48 and blaNDM genes19. On the other hand, Raheel et al. 14 have reported that mCIM was positive in67.9% of CRO. This may be attributed to lower levels of carbapenemases expression also may explained by reduced hydrolytic activity of OXA-48 carbapenemases20.

Regarding carbapenamase genes, we found that in 70% of the isolates, the gene blaNDM was the one that was most found , followed by blaOXA-48 in 68.24% and blaKPC in 16.47% of the isolates. An earlier study17 reported comparable outcomes. While previous studies revealed that the blaOXA-48 gene and blaNDM were the two most common variants 14, 20. Also, in a previous study was done by Wassef et al. 21 in CUSPH reported that blaNDM was detected in 24% of studied isolates. It was shown that, frequency of MBL producing Enterobacterales is increasing in our hospital.

In this study, 30% of the isolates were sensitive to ceftazidim-avibactam. The most prevalent carbapenamase gene among isolates sensitive to CZA/AVI was blaOXA-48 and most of them were Klebsiella pneumoniae. Similarly, a low sensitivity rate was reported by Amer et al 2019 in a study done at EL Zagazige, Eygpt 22. On the other hand, in previous studies, ceftazidime-avibactam demonstrated higher sensitivity rates23,24. Given that the majority of our tested isolates were 70% blaNDM gene carriers, the gap between our results and those of prior studies may be due to differences in the tested isolates' features. As was previously mentioned, the primary mechanism of resistance to CZA/AVI is blaNDM genes8.

We also found that ceftazidime-avibactam demonstrated showed the highest sensitivity rate (30%) among CRO compared to the other commonly used antibiotics (levofloxacin (14.7%), SXT (8.12%), amikacin (18.1%) and gentamicin (14.0%). Different susceptibility results were reported in several studies25, 26.

It is recommended to provide additional therapeutic choices, such as probiotics, as our study's findings showed that CRO infections still pose a severe public health threat even when new antibiotics like ceftazidime-avibactam are present 27.

CONCLUSION

This study showed a high 72.6% and a rising burden of CRO in CUSPH, enhancing the value of improving infection prevention and control. Our study revealed that Klebsiella pneumoniae was the organism that was most isolated organism in 80.0 %and the most often found carbapenamase genes among CRO isolates were blaNDM, blaOXA-48 and blaKPC in 70.0%, 68.24% and 16.47%, respectively.

Comparing to the other tested antibiotics, ceftazidime-avibactam showed the highest sensitivity (30%) against CRO, so CZA may be a potential therapeutic alternative in Egypt. However, due to the increasing MBL producing Enterobacterales isolates, CZA should be integrated into clinical use, considering the carbapenamase epidemiology or in vitro susceptibility results.

RECOMMENDATIONS

The findings of our study suggest more research with a bigger sample size to assess ceftazidime - avibactam activity against various blaOXA-48 and blaKPC of all alone. It's also advised to do studies evaluating the interactions between ceftazidime-avibactam, azteronam, and other antibiotics. Additionally, clinical trial studies are advised to assess ceftazidime-effectiveness avibactam's in treating infections brought on by CRO.

This study was approved by the ethical committee of faculty of medicine Cairo University.

List of Abbreviations

CRO: Carbapenem-resistant organisms
CZA/AVI: Ceftazidime- avibactam
mCIM: modified carbapenem inhibition method
MDR: multidrug-resistant
MHA: Muller Hinton agar
SXT: sulfamethoxazole-trimethoprim

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. Lee BY, Bartsch SM, Wong KF, et al. The potential trajectory of carbapenem-resistant Enterobacteriaceae, an emerging threat to health-care facilities, and the impact of the Centers for Disease Control and Prevention toolkit. Am J Epidemiol. 2016; 183(5):471-9.
2. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17(10):1791.
3. Schwaber MJ, Carmeli Y. Carbapenem-resistant Enterobacteriaceae: A potential threat. Jama. 2008; 300(24):2911-3.
4. El-Kholy A, El-Mahallawy HA, Elsharnouby N, et al. Landscape of multidrug-resistant gram-negative infections in Egypt: Survey and literature review. Infect Drug Resist. 2021:1905-20.
5. Kiratisin P, Kazmierczak K, Stone GG. In vitro activity of ceftazidime/avibactam and comparators against carbapenemase-producing Enterobacterales and Pseudomonas aeruginosa isolates collected globally between 2016 and 2018. J Glob Antimicrob Resist. 2021; 27:132-41.
6. Dietl B, Martínez LM, Calbo E, et al. Update on the role of ceftazidime-avibactam in the management of carbapenemase-producing Enterobacterales. Future Microbiol. 2020; 15(7):473-84.
7. Sader HS, Castanheira M, Shortridge D, et al. Antimicrobial activity of ceftazidime-avibactam tested against multidrug-resistant Enterobacteriaceae and Pseudomonas aeruginosa isolates from US medical centers, 2013 to 2016. Antimicrob. Agents Chemother. 2017;61(11):e01045-17.
8. Di Bella S, Giacobbe DR, Maraolo AE, et al. Resistance to ceftazidime/avibactam in infections

and colonisations by KPC-producing Enterobacterales: A systematic review of observational clinical studies. J Glob Antimicrob Resist.. 2021; 25:268-81.
9. Gatti M, Cojutti PG, Campoli C, et al. A proof of concept of the role of TDM-based clinical pharmacological advices in optimizing antimicrobial therapy on real-time in different paediatric settings. Front Pharmacol. 2021;12.
10. CLSI. Performance standards for antimicrobial susceptibility testing. 29th ed. CLSI supplement M100. Wayne, PA: Clinical and laboratory standards institute; 2019.
11. CLSI. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and laboratory standards institute; 2020.
12. Poirel L, Walsh TR, Cuvillier V, et al. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 2011; 70(1):119-23.
13. Kotb S, Lyman M, Ismail G, et al. Epidemiology of carbapenem-resistant Enterobacteriaceae in Egyptian intensive care units using National Healthcare–associated Infections Surveillance Data, 2011–2017. Antimicrob. Resist. Infect. Control. 2020;9(1):1-9.
14. Raheel A, Azab H, Hessam W, et al. Detection of carbapenemase enzymes and genes among carbapenem-resistant Enterobacteriaceae isolates in Suez Canal University Hospitals in Ismailia, Egypt. Microb Infect Dis. 2020;1(1):24-33.
15. Ghaith DM, Mohamed ZK, Farahat MG, et al. Colonization of intestinal microbiota with carbapenemase-producing Enterobacteriaceae in paediatric intensive care units in Cairo, Egypt. Arab J Gastroenterol. 2019;20(1):19-22. doi: 10.1016/j.ajg.2019.01.002.
16. Makharita RR, El-Kholy I, Hetta HF, et al. Antibiogram and genetic characterization of carbapenem-resistant gram-negative pathogens incriminated in healthcare-associated infections. Infect Drug resist. 2020;13:3991.
17. Tawfick MM, Alshareef WA, Bendary HA, et al. The emergence of carbapenemase bla NDM genotype among carbapenem-resistant Enterobacteriaceae isolates from Egyptian cancer patients. Eur J Clin Microbiol Infect Dis. 2020:1-9.
18. Gelmez GA, Can B, Hasdemir U, et al. Evaluation of phenotypic tests for detection of carbapenemases: New modifications with new interpretation. J Infect Chemother. 2021;27(2):226-31.
19. Tamma PD, Simner PJ. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. J Clin Microbiol. 2018;56(11):e01140-18.
20. Ghaith DM, Zafer MM, Said HM, et al. Genetic diversity of carbapenem-resistant Klebsiella Pneumoniae causing neonatal sepsis in intensive care unit, Cairo, Egypt. Eur J Clin Microbiol Infect Dis 2020;39(3):583-91.
21. Wassef M, Abdelhaleim M, Ghaith D, et al. Emerging New Delhi metallo-β-lactamase-1-type-producing gram-negative bacteria isolated from Cairo University Pediatric Hospital, Cairo, Egypt. J. Glob. Antimicrob. Resist. 2016;7:84-7.
22. Aamir R, Ateya RM, Arafa M, et al. Ceftazidime/avibactam efficiency tested In vitro against carbapenem-resistant Klebsiella pneumoniae isolated from neonates with sepsis. Microb Infect Dis. 2021;2(3):529-40.
23. Ozger HS, Evren E, Yildiz SS, et al. Ceftazidime–Avibactam susceptibility among carbapenem-resistant Enterobacterales in a pilot study in Turkey. Acta Microbiologica et Immunologica Hungarica. 2021;68(4):256-61.
24. Yin D, Wu S, Yang Y, et al. Results from the China Antimicrobial Surveillance Network (CHINET) in 2017 of the in vitro activities of ceftazidime-avibactam and ceftolozane-tazobactam against clinical isolates of Enterobacteriaceae and Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 2019;63(4):e02431-18.
25. Sonnevend Á, Ghazawi A, Darwish D, et al. In vitro efficacy of ceftazidime-avibactam, aztreonam-avibactam and other rescue antibiotics against carbapenem-resistant Enterobacterales from the Arabian Peninsula. Int J Infect Dis. 2020;99:253-9.
26. Barber KE, Pogue JM, Warnock HD, et al. Ceftazidime/avibactam versus standard-of-care agents against carbapenem-resistant Enterobacteriaceae harbouring bla KPC in a one-compartment pharmacokinetic/pharmacodynamic model. J Antimicrobi Chemother. 2018;73(9):2405-10.
27. Abdelhalim MM, Saafan GS, El-Sayed HS, et al. In vitro antibacterial effect of probiotics against Carbapenamase-producing multidrug-resistant Klebsiella pneumoniae clinical isolates, Cairo, Egypt. J Egypt Public Health Assoc. 2022;97(1):1-7.