



In Vitro Activity of Plazomicin among Carbapenem-resistant Enterobacteriaceae

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Abstract

BACKGROUND: Carbapenem-resistant Enterobacteriaceae (CRE) have been disseminated worldwide and became a global threat. Due to limited therapeutic drugs plazomicin - a new semisynthetic aminoglycoside - have been suggested as an alternative option due to its stability against aminoglycosides modifying enzymes.

AIM: This study aims to assess the in vitro activity of plazomicin against CRE isolates and to detect different types of carbapenemases among these isolates.

METHODS: In this study, 102 CRE isolates were collected from different clinical samples at Cairo University hospitals and the presence of carbapenemases was detected by modified carbapenem inhibition method and multiplex polymerase chain reaction tests. Plazomicin susceptibility testing was done using E-test.

RESULTS: The most frequently detected carbapenemase genes were *bla*_{NDM} in 75 (73.5%) isolates, followed by *bla*_{OXA-48} in 57 (55.9%) and *bla*_{KPC} in 16 (15.5%) isolates. Plazomicin was active against 32 (31.4%) isolates. Among the isolates carrying *bla*_{NDM} gene only and those carrying *bla*_{OXA-48} gene only, 21% and 41% were sensitive to plazomicin, respectively. Plazomicin showed the highest sensitivity against CRE isolates compared to the other tested antibiotics.

CONCLUSION: Plazomicin might be a good option for treatment of infections caused by CRE. In health-care settings where *bla*_{NDM} gene is prevalent, plazomicin may not be a good therapeutic option for CRE infections.

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Introduction

Carbapenem-resistant Enterobacteriaceae (CRE) are resistant to almost all β -lactams leaving only few and unfortunately older, antimicrobial classes with adequate activity [1]. These limited therapeutic options are highlighting the need for new antibiotics to treat serious infections caused by these resistant pathogens. Since the early 1980s, carbapenems are considered the last line of defense against multidrug-resistant (MDR) Gram-negative organisms [2]. CRE are defined as pathogens that are resistant to at least one carbapenem or are proved to produce a carbapenemase [3]. Over the past two decades, dissemination of CRE has been observed worldwide [4], [5]. *Klebsiella pneumoniae* (*K. pneumoniae*) isolates showed the highest rate of carbapenem resistance among Enterobacteriaceae [6], [7].

In Egypt, Kotb *et al.*, 2020, have reported that 1105 (47.9%) of the 2306 Enterobacteriaceae isolates included in the surveillance from 2011 to 2017 were CRE [8]. The highest percentage of CRE was among *Klebsiella* spp. 53.7% followed by *Enterobacter* spp. 43.5%, while a smaller percentage of *Escherichia coli* (*E. coli*) isolates 27.1% were CRE.

Few antibiotics are still active against CRE, since they usually carry resistance genes to β -lactams, aminoglycosides, and fluoroquinolones. Older antimicrobials such as fosfomycin and polymyxins that were rarely used in the past due to efficacy and toxicity concerns may be considered [9]. Plazomicin is a semisynthetic aminoglycoside that inhibits bacterial protein synthesis. It has an important *in vitro* activity against MDR Enterobacteriaceae including those producing aminoglycosides modifying enzymes (AMEs), extended spectrum beta-lactamases, and carbapenemases. Enhanced activity of plazomicin against Enterobacteriaceae is due to its stability to commonly encountered AMEs that inhibit the activity of other aminoglycosides [10], [11].

In June 2018, plazomicin was approved by the US Food and Drug Administration (FDA) for treatment of complicated urinary tract infections, including acute pyelonephritis [12] and is waiting for FDA approval for acute bloodstream infections caused by MDR Enterobacteriaceae infections, including CRE [13].

Aim of the study

In the light of the above, this study aimed at assessing the *in vitro* activity of plazomicin against CRE

isolates and to detect different types of carbapenemases among these isolates.

Methods

This cross-sectional study was performed in Cairo University Hospitals, Clinical Pathology Department. A total of 102 CRE isolates from different clinical specimens were collected without duplication from October 2019 to October 2020. All isolates were collected from cultures sent to the laboratory as a part of the routine medical service provided to the patients and were cultured aerobically on routine blood, chocolate, MacConkey, and CLED agar media at 37°C for (24–48) h and *Enterobacteriaceae* were further identified by Gram staining and conventional biochemical reactions that include triple sugar iron, lysine iron agar, motility indole ornithine, simon citrate, and urease agar.

Susceptibility to carbapenems was determined by the standard Kirby–Bauer disk diffusion method to detect CRE isolates according to clinical and laboratory standards institute breakpoints for carbapenems [14]. Bacteriological strains were suspended in 20% glycerol trypticase soy broth and stored at –80°C for further laboratory testing. Subculture of the stored isolates were done on blood agar and incubated aerobically at 37°C for 24 h, then subjected to:

Phenotypic detection of carbapenemase enzymes activity

Phenotypic detection of carbapenemase enzymes activity was detected by modified carbapenem inactivation method (mCIM) and EDTA-mCIM (eCIM) [15].

Detection of carbapenemase genes by conventional multiplex polymerase chain reaction (PCR)

DNA extraction, multiplex conventional PCR, and gel electrophoresis according to Poirel *et al.* [16]. Multiplex conventional PCR was performed to detect the following carbapenemase genes using three different multiplex reactions:

- Reaction 1: OXA-48, NDM, KPC, and BIC
- Reaction 2: AIM, GIM, SIM, and DIM
- Reaction 3: IMP, VIM, and SPM.

Plazomicin susceptibility testing

Plazomicin susceptibility was determined using minimal inhibitory concentration (MIC) method on Muller–Hinton agar using plazomicin E test (PLZ;

0.016–256 µg/mL) (Iiofilchem diagnostics, Italy) and the MIC value was determined [17].

Quality control measures were performed all through the different tests including the culture media, biochemical reactions, and antimicrobial discs.

- *E. coli* ATCC 25922 was used for plazomicin susceptibility testing control.
- *K. pneumoniae* NCTC 13443 was used for PCR technique as a positive control of NDM.

Statistical analysis

Data were statistically described in terms of range, mean ± standard deviation (±SD), and percentages. A probability value $p < 0.05$ was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2010 and Statistical Package for the Social Science version 23 for Microsoft Windows. This study was approved by the ethical committee of faculty of medicine Cairo University.

Results

During the study period, a total of 10,964 clinical samples were sent to the Clinical Pathology Department of Cairo University Hospitals. Gram-negative pathogens were identified in 3301 (30.1%) samples. Among the 3301 Gram-negative isolates, 2229 (67.5%) were *Enterobacteriaceae* isolates; out of which 131 (5.8%) isolates were CRE, and a total of 102 CRE isolates were randomly collected.

Out of 102 CRE isolates, 97 (95%) were *klebsiella* species, while 5 (5%) isolates were other types including *E. coli*, *Proteus*, and *Citrobacter* species.

Results of mCIM and eCIM tests are illustrated in Tables 1 and 2, respectively.

Table 1: Results of mCIM test according to CLSI [15]

Result	Number	Percent
Positive ^a	65	63.7
Negative ^b	24	23.5
Indeterminate ^c	13	12.7
Total	102	100

^a: Positive: Interpret eCIM, ^b: Negative: Do not interpret eCIM, ^c: Indeterminate: The presence or absence of Carbapenemase cannot be confirmed thus do not interpret eCIM. mCIM: Modified carbapenem inactivation method, CLSI: Clinical and Laboratory Standards Institute.

Table 2: Results of eCIM test according to CLSI [15]

Result	Number	Percent
MBL positive	50	49
MBL negative	15	14.7
Not applicable	37	36.3
Total	102	100

eCIM: EDTA-modified carbapenem inactivation method, CLSI: Clinical and Laboratory Standards Institute, MBL: Metallo-β-lactamases.

Results of multiplex PCR are illustrated in Table 3.

Plazomicin susceptibility testing have detected that 32 (31.4%) isolates were sensitive as shown in Table 4.

Table 3: Carbapenemase genes detected by PCR

Carbapenemase class	Carbapenemase gene	Number of positive isolates	Percent
Class A	<i>bla</i> _{KPC}	16	15.7
Class B	<i>bla</i> _{NDM}	75	73.5
	<i>bla</i> _{SIM}	5	4.9
	<i>bla</i> _{VIM}	4	3.9
	<i>bla</i> _{BIC}	3	2.9
	<i>bla</i> _{SIM}	3	2.9
	<i>bla</i> _{AIM}	1	1
	<i>bla</i> _{DIM}	1	1
	<i>bla</i> _{SPM}	0	0
	<i>bla</i> _{IMP}	0	0
	Class D	<i>bla</i> _{OXA-48}	57

PCR: Polymerase chain reaction.

Plazomicin susceptibility among isolates carrying *bla*_{NDM} or *bla*_{OXA-48} gene only is shown in Table 5.

Table 4: Susceptibility rate of plazomicin and MIC values among CRE isolates

Susceptibility pattern	MIC (µg/ml)	Number	Total number	Percent
Sensitive	0.25	1	32	31.4
	0.38	12		
	0.5	10		
	0.75	2		
	1	2		
	1.5	5		
Intermediate	6	1	1	1
Resistant	>256	69	69	67.6
Total		102	102	100

MIC: Minimal inhibitory concentration, CRE: Carbapenem resistant *Enterobacteriaceae*.

Susceptibility pattern of the 102 CRE isolates to different antibiotics is illustrated in Figure 1.

Table 5: Susceptibility of plazomicin among isolates carrying bla_{NDM} or bla_{OXA-48} gene only

Isolates gene group	Sensitive (%)	Resistant (%)	Total (%)	p-value
Isolates carrying <i>bla</i> _{NDM} gene only	4 (21)	15 (79)	19 (100)	0.19
Isolates carrying <i>bla</i> _{OXA-48} gene only	7 (41)	10 (59)	17 (100)	

Discussion

In this study, the most common isolated pathogen was *Klebsiella* spp. in 95% of the isolates. Similarly, other studies reported that *Klebsiella* spp. was the most common isolated pathogen [18], [19]. In our study, we found that mCIM was positive in 63.7% of CRE isolates. Recently, similar results were reported by other studies [20], [21].

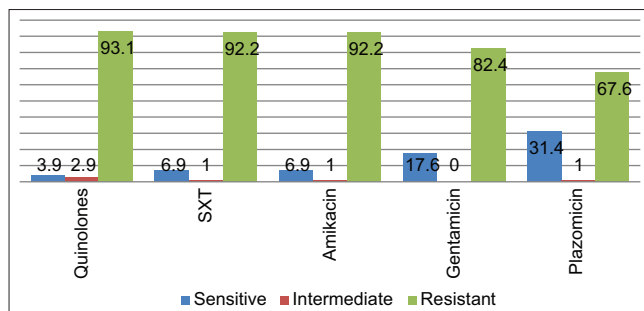


Figure 1: Susceptibility rates of plazomicin and other antibiotics among carbapenem resistant *Enterobacteriaceae* isolates. SXT: Sulfamethoxazole-trimethoprim

Regarding carbapenemase genes, we found that the most frequently detected gene was *bla*_{NDM},

followed by *bla*_{OXA-48} and *bla*_{KPC}. Comparable results were reported by a previous study [18]. While, other studies reported that *bla*_{OXA-48} gene was the most prevalent followed by *bla*_{OXA-48} [20], [22]. Although the latter studies had the same geographical distribution as this study, they showed different gene prevalence. This might be explained by the difference in sample size between our study and their as our sample size was almost double the sample size they tested.

In the current study, plazomicin was active against 31.4% of the isolates. While among the isolates carrying *bla*_{NDM} gene only and those carrying *bla*_{OXA-48} gene only, 21% and 41% were sensitive to plazomicin, respectively. Earlier studies reported similar sensitivity rates of plazomicin among *bla*_{NDM} carrying isolates. While, higher sensitivity rates were detected among isolates carrying *bla*_{OXA-48} genes [10], [23].

On the other hand, Plazomicin showed the higher sensitivity rates in other studies [24], [25], [26]. The discrepancy between our results and these studies may be related to the different characteristics of the tested isolates, as most of our isolates were carrying *bla*_{NDM} genes 73.5%. As previously discussed, *bla*_{NDM} genes are commonly co-expressed with 16S-RMTases; the main mechanism of resistance to plazomicin [27].

We found also that plazomicin showed the highest susceptibility rate (31.4%) among CRE isolates in comparison to the other routinely tested antibiotics (quinolones [3.9%], sulfamethoxazole-trimethoprim [6.9%], amikacin [6.9%], and gentamicin [17.6%]). Similar findings were reported in various studies [23], [25], [26], [28].

Our study results revealed that the infections caused by CRE represent a serious public health problem even in the presence of new antibiotics like plazomicin.

Limitations of the study

Detection of plazomicin resistance mechanism in plazomicin-resistant isolates was not performed. Larger sample size is recommended for more accurate evaluation of plazomicin activity.

Conclusion

Further efforts should be exerted toward the control of spread of *Klebsiella* spp. infections being the most commonly isolated. In addition, more studies should be directed to CRE isolates carrying *bla*_{NDM}, *bla*_{OXA-48}, and *bla*_{KPC}, respectively. Although plazomicin showed the highest sensitivity against CRE isolates compared to the other tested antibiotics thus it might be a good option for treatment of infections caused

by CRE, in health-care settings where *bla*_{NDM} gene is prevalent, it may not be a good therapeutic option for CRE infections.

Recommendations

Our study results recommend further studies with larger sample size to evaluate plazomicin activity against different species of MDR bacteria including CRE. In addition, studies that assess synergy between plazomicin and other antibiotics are recommended.

Furthermore, clinical trial studies are recommended to evaluate the efficacy of plazomicin in the treatment of infections caused by CRE.

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