Activity of Plazomicin (ACHN-490) against MDR clinical isolates of *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter* spp. from Athens, Greece

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The *in vitro* activity of plazomicin was evaluated against 300 multidrug resistant (MDR) (carbapenemase and/or ESBL-producing) isolates from four hospitals in Athens, an area where carbapenemase-producing organisms are endemic. Most of the isolates were also resistant to the legacy aminoglycosides with the MIC_{50}/MIC_{90} to tobramycin, amikacin and gentamicin being 32/>32, 32/>32 and 4/>8 μg/ml, respectively. ACHN-490 retained activity (MICs ⩽ 4 μg/ml) against all isolates of *Klebsiella pneumoniae, Escherichia coli*, and *Enterobacter* spp. tested with MIC_{50} and MIC_{90} of 1 and 2 μg/ml, respectively, irrespective of their MDR phenotype and it represents a promising alternative for the treatment of the most problematic Gram-negative pathogens.

Keywords: Aminoglycosides, Multidrug resistant, VIM, KPC

Introduction

Bacterial resistance is an increasing threat to the successful treatment of both community- and hospital-acquired infections (http://www.earss.rivm.nl) and antimicrobials potent against multidrug resistant (MDR) pathogens are urgently needed.

Plazomicin (formerly ACHN-490) (Achaogen, South San Francisco, CA, USA) is a next-generation aminoglycoside, currently in early clinical development (FDA, http://clinicaltrials.gov/), with enhanced activity against many MDR Gram-negative bacteria and *Staphylococcus aureus* including methicillin resistant S. aureus isolates (MRSA) (MIC_{90}, 2 μg/ml).[1] Plazomicin is not affected by any of known aminoglycoside-modifying enzymes, except AAC(2′)-Ia, -Ib and -Ic (only found in *Providencia spp*), it retains the favourable bactericidal properties of the aminoglycoside class and has demonstrated potent in vivo efficacy in two animal infection models.[2] Methylation of 16S ribosomal RNA (rRNA) confers MICs of >8 μg/ml for plazomicin, as well as high-level resistance to all parenterally administered aminoglycosides that are currently in clinical use.[3,4] The compound is currently under development for the treatment of complicated urinary tract infections and acute pyelonephritis as a single agent.

After intravenous administration of plazomicin to humans at a dose of 15 mg/kg, the maximum concentraration was 113 μg/ml, the area under the curve (0–24) was 239 hours μg/ml, the half-life was 3.0 hours and the steady-state volume of distribution was 0.24 l/kg.[5] Human phase I and II studies to date have not reported nephrotoxicity or ototoxicity, and lack of ototoxicity has been reported in the guinea pig model.[5]

In the present work, we analyzed the *in vitro* activity of plazomicin against a collection of 300 MDR clinical isolates of *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter* spp. recently collected at four tertiary-care Hospitals in Athens, Greece, the University General Hospital Attikon, the Laikon and Evaggelismos General Hospitals, and the private Hospital Hygeia.

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Material and Methods

Clinical isolates collected from January 2008 to November 2010, were studied and only one isolate per patient was accepted. Identification and MIC determinations were performed using an automated system (BD Phoenix automated microbiology system; BD Diagnostic Systems, Sparks, MD, USA). MICs of plazomicin (0.25–32 μg/ml), tobramycin (0.25–32 μg/ml), and fosfomycin (16–512 μg/ml) were determined by the agar dilution method following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (www.eucast.org), whereas those of doripenem and tigecycline were determined using E-test (AB Biodisk, Solna, Sweden), in accordance with the manufacturer's instructions. Plazomicin was supplied by Achaogen, Inc. Tobramycin and fosfomycin were purchased from Sigma-Aldrich (St Louis, MO, USA). Agar medium in which fosfomycin MICs were tested was supplemented with 25 μg/ml of glucose-6-phosphate (Sigma-Aldrich). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains. Results were interpreted in accordance with the EUCAST guidelines (www.eucast.org).

All isolates were screened for MBL and class A carbapenemase production with EDTA–meropenem and meropenem–boronic acid disc synergy tests,[6] respectively. The presence of KPC and VIM genes was confirmed by PCR with specific primers.[6] ESBL production was tested with the CLSI ESBL confirmatory test[7] and with a modified test using clavulanate in

Table 1 ***In vitro*** **activity of plazomicin and comparators against 300 MDR enterobacterial isolates**

Microorganism	Antimicrobial agent	Breakpoints (S, R)	Range (μg/ml)	MIC_{50}	MIC_{90}	Susceptibility rate (%)
All	Plazomicin	NA	≤0.25 to 4	1	2	NA
	Amikacin	≤8, >16	≤8 to >32	32	>32	17.7
	Gentamicin	≤2, >4	≤2 to >8	4	>8	37.3
	Tobramycin	≤2, >4	0.5 to >32	32	>32	6.7
	Imipenem	≤2, >8	≤1 to >8	>8	>8	24.7
	Meropenem	≤2, >8	≤1 to >8	>8	>8	30.3
	Doripenem	≤1, >4	0.032 to >32	8	>32	18.3
	Piperacillin-Tazobactam	≤8, >16	≤4/4 to >64/4	>64/4	>64/4	3.7
	Ciprofloxacin	≤0.5, >1	≤0.5 to >2	>2	>2	10.0
	Fosfomycin w/G6P	≤32, >32	≤16 to >512	≤16	128	56.0
	Colistin	≤2, >2	≤1 to >2	≤1	>2	77.7
	Tigecycline	≤1, >2	0.125 to 16	2	4	32.0
Klebsiella pneumoniae	Plazomicin	NA	≤0.5 to 4	1	2	NA
	Amikacin	≤8, >16	≤8 to >32	32	>32	10.8
	Gentamicin	≤2, >4	≤2 to >8	4	>8	34.0
	Tobramycin	≤2, >4	0.5 to >32	32	>32	3.7
	Imipenem	≤≤2, >8	≤1 to >8	>8	>8	21.6
	Meropenem	≤2, >8	≤1 to >8	>8	>8	23.2
	Doripenem	≤1, >4	0.032 to >32	8	>32	12.9
	Piperacillin to Tazobactam	≤8, >16	≤4/4 to >64/4	>64/4	>64/4	0.4
	Ciprofloxacin	≤0.5, >1	≤0.5 to >2	>2	>2	4.6
	Fosfomycin w/G6P	≤32, >32	≤16 to >512	≤16	256	53.5
	Colistin	≤2, >2	≤1 to >2	≤1	>2	73.0
	Tigecycline	≤1, >2	0.125 to 16	2	4	25.7
Escherichia coli	Plazomicin	NA	≤0.25 to 2	1	2	NA
	Amikacin	≤≤8, >16	≤≤8 to >32	≤8	>32	54.5
	Gentamicin	≤2, >4	≤2 to >8	8	>8	30.3
	Tobramycin	≤2, >4	1 to >32	32	>32	21.2
	Imipenem	≤2, >8	≤1 to >8	≤1	>8	54.4
	Meropenem	≤2, >8	≤1 to >8	≤1	>8	78.8
	Doripenem	≤1, >4	0.032 to >32	0.5	16	60.6
	Piperacillin-Tazobactam	≤8, >16	≤4/4 to >64/4	>64/4	>64/4	30.3
	Ciprofloxacin	≤0.5, >1	≤0.5 to >2	>2	>2	30.3
	Fosfomycin w/G6P	≤32, >32	≤16–256	≤16	32	84.8
	Colistin	≤2, >2	≤1 to 2	≤1	≤1	100
	Tigecycline	≤1, >2	0.25 to 16	1	2	69.7
Enterobacter spp	Plazomicin	NA	≤0.5 to 2	1	1	NA
	Amikacin	≤8, >16	≤8 to >32	16	>32	34.6
	Gentamicin	≤≤2, >4	≤2 to >8	2	4	76.9
	Tobramycin	≤2, >4	1 to >32	32	32	15.3
	Imipenem	≤2, >8	≤1 to >8	>8	>8	15.4
	Meropenem	≤2, >8	≤1 to >8	>8	>8	34.6
	Doripenem	≤1, >4	0.032 to >32	32	>32	15.4
	Piperacillin-Tazobactam	≤8, >16	≤4/4 to >64/4	>64/4	>64/4	0.0
	Ciprofloxacin	≤0.5, >1	≤0.5 to >2	2	>2	34.6
	Fosfomycin w/G6P	≤32, >32	≤16 to 256	32	128	42.3
	Colistin	≤2, >2	≤1 to >2	≤1	≤1	92.3
	Tigecycline	≤1, >2	0.125 to 8	2	4	42.3

combination with boronic acid and EDTA in Enterobacteriaceae that produced KPC or VIM enzymes.[8]

Isolates with MIC to plazomicin of 4 μg/ml were examined by PCR for the presence of 16S rRNA methylase genes (i.e., *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, and *npmA*) and the most common aminoglycoside-modifying enzymes in Gram-negative pathogens, using primers and conditions previously reported.[9–11] In particular, the following genes were investigated: *aac(6′)-Ia, aac(6′)-Ib*, *aac(6′)-IIα*, *ant(2′)-Ia*, *aac (3)-Ia, aac(3)-IIa*, *aac(3)-IVα* and *aph(3′)-VIa*.

Results and Discussion

The studied isolates included 241 *K. pneumoniae*, 33 *E. coli* and 26 *Enterobacter* spp. derived from blood (65.7%), pus (5.3%), bronchial secretions (1.7%), urine (8.0%), and fecal carriage (19.3%).

Among the *K. pneumoniae* isolates, 138 (57.3%) were class A carbapenemase producers; 75 (31.1%) showed a positive EDTA-meropenem disc synergy test, which was suggestive of MBL production and 14 (5.8%) were designated both class A carbapenemase and MBL producers. PCR amplification confirmed the presence of bla_{KPC} and bla_{VIM} genes in all class A carbapenemase and MBL producers, respectively. ESBL production was confirmed in 113 (81.9%) KPC producers, in 43 (57.3%) VIM producers and in 4 (28.6%) KPC and VIM producers. The remaining 14 *K. pneumoniae* isolates were all ESBL producers. Among the *E. coli* isolates, 15 (45.5%) were designated ESBL producers, whereas 9 (27.3%) were identified as KPC and 9 (27.3%) as VIM producers. Four of the VIM-positive *E. coli* isolates were also ESBL producers. Twenty-one (80.8%) of the *Enterobacter* spp. isolates (19 *E. cloacae* and 7 *E. aerogenes*) harboured the bla_{VIM} gene, while four (15.4%) harboured the bla_{KPC} and one isolate possessed both bla_{VIM} and bla_{KPC}.

The susceptibility results for all tested antimicrobials are shown in Table 1. Of the 300 isolates tested nine were pandrug-resistant, 157 were extensively drug-resistant and the remaining 134 were MDR according to definition given by ECDC.[12] As shown, isolates were highly resistant not only to carbapenems ($MIC_{50} \geq 8$; $MIC_{90} > 8$ μg/ml) and piperacillin-tazobactam ($MIC_{50} > 64/4$; $MIC_{90} > 64/4$ μg/ml) but also to ciprofloxacin ($MIC_{50} > 2$; $MIC_{90} > 2$ μg/ml). Approximately 78% of the strains were susceptible to colistin displaying MICs ≤ 2 μg/ml, while tigecycline's MIC_{50} and MIC_{90} were 2 and 4 μg/ml, respectively (with 32% of the isolates being susceptible and 81% displaying MICs ≤ 2 μg/ml). Finally, fosfomycin demonstrated 56% susceptibility with an MIC_{50} of ≤ 16 and an MIC_{90} of 128 μg/ml.

Isolates were highly resistant to tobramycin with only 6.7% of the strains being susceptible. Amikacin was active against 17.7% and gentamicin against 37.3% of the isolates. The vast majority ($n = 242$, 80.7%) of the isolates tested was non-susceptible to both amikacin and tobramycin, whereas 167 (55.7%) of them were resistant or intermediately susceptible also to gentamicin.

Plazomicin had an MIC range of ≤ 0.25 to 4 μg/ml, with an MIC_{50} of 1 and an MIC_{90} of 2 μg/ml that were substantially lower than those for comparator aminoglycosides. Only thirteen *K. pneumoniae* isolates (4.3%) exhibited MICs of 4 μg/ml. These

Table 2 Activities of plazomicin against 300 MDR enterobacterial isolates with different resistance phenotypes

			MIC (μg/ml)						
Species	Phenotype	No. of isolates	0.25	0.5	1	2	4	8	16
Klebsiella pneumoniae	KPC	25		7	12	5	1		
	ESBL, KPC	113		15	69	26	3		
	VIM	32		4	14	10	4		
	ESBL, VIM	43		8	22	9	4		
	KPC, VIM	10			7	3			
	ESBL, KPC, VIM	4			3	1			
	ESBL	14			9	4	1		
	Total	241		34	136	58	13		
Escherichia coli	KPC	9		3	4	2			
	VIM	5		2	3				
	ESBL, VIM	4	1		3				
	ESBL	15		3	8	4			
	Total	33	1	8	18	6			
Enterobacter aerogenes	KPC	1			1				
	ESBL, KPC	1		1					
	VIM	5			4	1			
	Total	7		1	5	1			
Enterobacter cloacae	KPC	2		1	1				
	VIM	15		8	6	1			
	KPC, VIM	1							
	ESBL, VIM	1			1				
	Total	19		10	8	1			

included one pandrug-resistant, 10 extensively drug-resistant and two MDR isolates. Those were resistant to tobramycin and amikacin (one was intermediate), whereas five were resistant or intermediate to gentamicin. None of those isolates was found to carry ribosomal methylases, while 7/13 possessed aminoglycoside modifying enzymes. In four of those isolates *aac(6′)-Ib* was the only gene detected, while *aac(3′)-Ia* and *aac(3′)-IIa* were revealed in one and two isolates respectively. No clear correlation between the MICs of plazomicin and the presence of aminoglycoside-modifying enzymes was observed.

Plazomicin MICs against a subset of enterobacterial isolates exhibiting specific phenotypic characteristics are listed in Table 2. Enterobacterial isolates with plazomicin MICs≥8 μg/ml are not common. Landman has reported two *E. coli* isolates of the same ribotype, from two separate hospitals, in which genes for ribosomal methylases were not detected and the efflux pump inhibitor phenyl-arginine-beta-naphthylamide had no appreciable effect on the plazomicin MICs.[13] Also 16 isolates with the New Delhi (NDM-1) MBL also producing 16S (rRNA) methylases exhibited high MICs (≥8 μg/ml) to plazomicin.[4]

The low prevalence of 16S rRNA methylases in Enterobacteriaceae that has already been reported,[14] as well as the absence of isolates producing the NDM-1 in Greece,[15] is most probably the reason why we found no isolates with MICs>4 μg/ml.

The next-generation aminoglycoside plazomicin retains activity against all isolates of *K. pneumoniae, E. coli*, and *Enterobacter* spp. tested, including those with ESBL, KPC, and VIM-MBL resistance mechanisms and may represent a promising alternative for the treatment of MDR pathogens.

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