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Plazomicin activity against polymyxin-resistant Enterobacteriaceae, including MCR-1-producing isolates

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Objectives: Plazomicin, a novel aminoglycoside with *in vitro* activity against MDR Gram-negative organisms, is under development to treat patients with serious enterobacterial infections. We evaluated the activity of plazomicin and comparators against colistin-resistant enterobacterial isolates.

Methods: Susceptibility to plazomicin and comparators was tested by broth microdilution for a collection of 95 colistin-resistant enterobacterial isolates collected from 29 hospitals in eight countries. Forty-two isolates (*Klebsiella pneumoniae* and *Klebsiella oxytoca*) possessed chromosomally encoded resistance mechanisms to colistin, 21 isolates (*Escherichia coli* and *Salmonella enterica*) expressed the *mcr-1* gene, 8 isolates (*Serratia*, *Proteus*, *Morganella* and *Hafnia*) were intrinsically resistant to colistin and 24 isolates (*K. pneumoniae*, *E. coli* and *Enterobacter* spp.) had undefined, non-*mcr-1* mechanisms. Susceptibility profiles were defined according to CLSI for aminoglycosides and to EUCAST for colistin and tigecycline.

Results: Plazomicin inhibited 89.5% and 93.7% of the colistin-resistant enterobacterial isolates at $\leq$2 and $\leq$4 mg/L, respectively. MICs of plazomicin were $\leq$2 mg/L for all of the *mcr-1* positive isolates and $\leq$4 mg/L for all the intrinsic colistin-resistant Enterobacteriaceae. Non-susceptibility to currently marketed aminoglycosides was common: amikacin, 16.8%; gentamicin, 47.4%; and tobramycin, 63.2%. Plazomicin was the most potent aminoglycoside tested with an MIC_{90} of 4 mg/L, compared with 32, >64 and 64 mg/L for amikacin, gentamicin and tobramycin, respectively.

Conclusions: Plazomicin displayed potent activity against colistin-resistant clinical enterobacterial isolates, including those expressing the *mcr-1* gene. Plazomicin was more active than other aminoglycosides against this collection of isolates. The further development of plazomicin for the treatment of infections due to MDR Enterobacteriaceae is warranted.

Introduction

Acquired resistance to polymyxins is increasingly reported in Enterobacteriaceae, and particularly in *Klebsiella pneumoniae*. This is of great concern, considering that polymyxins are among the rare last-resort antibiotics for treating infections due to carbapenem-resistant Enterobacteriaceae.[1] The predominant mechanism of colistin resistance described to date among clinical enterobacterial isolates involves changes in the phosphate groups of lipid A by addition of 4-amino-4-deoxy-1-arabinose and/or phosphoethanolamine, resulting in reduced anionic charge of LPS.[2] Genetic alterations associated with resistance include mutations in the two-component regulatory systems PhoPQ and PmrAB, as well as inactivation of the *mgrB* gene.[1,3,4] More recently, a plasmid-encoded resistance mechanism (involving the *mcr-1* or *mcr-2* genes) has been described worldwide among enterobacterial isolates from animals, food and humans.[5,6] This plasmid-encoded mechanism may be associated with ESBL and carbapenemase genes, heightening concerns regarding the global spread of pandrug-resistant Enterobacteriaceae.[7–9]

Plazomicin (plazomicin sulphate, ACHN-490) is a novel semisynthetic aminoglycoside derived from sisomicin. Plazomicin is insensitive to classical aminoglycoside-modifying enzymes such as acetyl-, phosphoryl- and nucleotidyltransferases. Plazomicin is active against clinical isolates possessing a broad range of resistance mechanisms, including ESBLs, carbapenemases and

Table 1. Colistin-resistant isolates with intrinsic resistance, chromosomally acquired resistance or plasmid-mediated or unknown resistance mechanisms

Species	Number	Colistin resistance	Mechanism
Strains naturally resistant to colistin			
Morganella morganii	2	intrinsic resistance	NA
Proteus mirabilis	2	intrinsic resistance	NA
Proteus vulgaris	1	intrinsic resistance	NA
Serratia marcescens	2	intrinsic resistance	NA
Hafnia alvei	1	intrinsic resistance	NA
Strains resistant to colistin with an identified mechanism of resistance			
K. pneumoniae	41	acquired, chromosomal	3 strains: *pmrA* mutations 3 strains: *pmrB* mutations *phoP*, 25 nt deletion *phoQ* point mutation 3 strains with *mgrB* point mutations 7 strains with a truncated *mgrB* gene 15 with IS insertion into the *mgrB* gene 8 strains with a partial or total deletion of the *mgrB* gene
K. oxytoca	1	acquired, chromosomal	IS insertion in *mgrB*
E. coli	15	acquired, plasmid	*mcr-1* gene
E. coli	2	acquired, plasmid	*mcr-1* gene
S. enterica	4	acquired, plasmid	*mcr-1* gene
Strains resistant to colistin without an identified mechanism of resistance			
K. pneumoniae	9	acquired	unknown
E. coli	2	acquired	unknown
E. cloacae	12	acquired	unknown
Enterobacter asburiae	1	acquired	unknown

NA, not applicable.

fluoroquinolone target site mutations. This novel antibiotic has the potential to address an unmet medical need for patients with serious MDR Enterobacteriaceae infections, including those caused by carbapenem- and colistin-resistant isolates. Few studies have been conducted so far to evaluate the activity of plazomicin against MDR Gram-negative organisms, but a recent study showed that this molecule retained excellent activity against carbapenemase and ESBL producers, including those combining aminoglycoside resistance mechanisms.[10]

The first step in aminoglycoside uptake by Gram-negative bacteria involves electrostatic binding of the positively charged antibiotic to negatively charged sites on the outer membrane (OM), including LPS.[11] Because common polymyxin resistance mechanisms in Enterobacteriaceae lead to a reduction in the negative charge of the OM, there is a theoretical possibility that colistin resistance may impact the activity of aminoglycosides, including plazomicin. This was of concern in the context of the emergence of the transmissible MCR-1/-2 resistance determinants, as stated above. In this study, we evaluated the activity of plazomicin and comparators against colistin-resistant clinical enterobacterial isolates with resistance conferred by a wide variety of genetic mechanisms.

Materials and methods

Bacterial isolates

A total of 95 colistin-resistant clinical Enterobacteriaceae were evaluated in this study. The strains were collected from 29 hospitals in eight countries (Angola, Colombia, France, Portugal, South Africa, Spain, Switzerland and Turkey). Forty-two isolates (*K. pneumoniae* and *Klebsiella oxytoca*) had identified chromosomal colistin resistance mechanisms (e.g. *mgrB*, *phoPQ* or *pmrAB* mutations), 21 isolates (*Escherichia coli* and *Salmonella enterica*) expressed *mcr-1*, 8 isolates (*Serratia*, *Proteus*, *Morganella* and *Hafnia*) were intrinsically resistant to colistin[12] and 24 isolates (*K. pneumoniae*, *E. coli* and *Enterobacter* spp.) had undefined, non-*mcr-1*-related colistin resistance mechanisms (Table 1). In addition, *E. coli* TOP10 WT reference strain and its counterpart *E. coli* transconjugant TOP10 producing MCR-1 were tested, corresponding to two isogenic strains expressing or not expressing a colistin resistance mechanism.[7]

In vitro susceptibility testing methods

MICs were determined following CLSI broth microdilution guidelines,[13,14] with the exception of colistin and tigecycline, for which EUCAST breakpoint criteria were applied.[15] Frozen pre-loaded antibiotic-growth-medium microtitre plates were purchased from Thermofisher (OH, USA) by Achaogen, Inc. (South San Francisco, CA, USA) for MIC testing.

All isolates were tested for their susceptibility to the following antibiotics: colistin, amikacin, gentamicin, plazomicin, tobramycin, piperacillin plus tazobactam at a fixed concentration (4 mg/L), ceftazidime, ceftriaxone, doripenem, imipenem, meropenem, aztreonam, levofloxacin, tigecycline and trimethoprim plus sulfamethoxazole. The range of concentrations tested was: 0.06–128 mg/L for plazomicin and colistin; 0.015–32 mg/L for doripenem, imipenem, ceftazidime, ceftriaxone, aztreonam and tigecycline; 0.03–64 mg/L for the aminoglycosides amikacin, gentamicin and tobramycin; 0.004–512 mg/L for meropenem; 0.004–8 mg/L for levofloxacin; 0.06–64 mg/L for piperacillin plus 4 mg/L tazobactam; and

Table 2. *In vitro* activities of plazomicin, colistin, amikacin, gentamicin, tobramycin, imipenem, doripenem, meropenem, tigecycline, levofloxacin, ceftazidime, ceftriaxone, aztreonam, piperacillin/tazobactam and trimethoprim/sulfamethoxazole against colistin-resistant Enterobacteriaceae

		MIC (mg/L)			MIC interpretive criteria (mg/L)		
Colistin resistance mechanism/antibiotic	*n*	range	MIC_{50}[a]	MIC_{90}[a]	susceptible	intermediate	resistant
Intrinsic resistance	8						
plazomicin*		1–4			8	0	0
colistin**		16 to >128			0	—	8
amikacin		2–16			8	0	0
gentamicin		0.25–32			7	0	1
tobramycin		0.5–16			7	0	1
imipenem		0.25–16			2	0	6
doripenem		0.06–0.5			8	0	0
meropenem		0.03–0.12			8	0	0
tigecycline***		0.5–8			4	0	4
levofloxacin		0.03–2			8	0	0
ceftazidime		0.03 to >32			7	0	1
ceftriaxone		≤0.015 to >32			4	0	4
aztreonam		≤0.015 to 32			7	0	1
piperacillin/tazobactam		≤0.06/4 to 0.5/4			7	0	1
trimethoprim/sulfamethoxazole		≤0.25/4.75			6	—	2
Acquired, chromosomal	42						
plazomicin		0.25 to >128	0.25	1	38	0	4[b]
colistin		8–64	32	64	0	—	42
amikacin		1 to >64	16	64	30	7	5
gentamicin		0.25 to >64	32	>64	14	1	27
tobramycin		0.25 to >64	16	>64	5	7	30
imipenem		1 to >32	32	>32	1	2	39[c]
doripenem		0.06 to >32	8	>32	12	5	25[c]
meropenem		0.03 to >512	8	256	16	1	25[c]
tigecycline		0.25–4	2	4	19	17	6
levofloxacin		0.06 to >8	>8	>8	6	2	34
ceftazidime		0.5 to >32	>32	>32	6	0	36
ceftriaxone		0.03 to >32	>32	>32	3	3	36
aztreonam		0.12 to >32	>32	>32	7	0	35
piperacillin/tazobactam		4/4 to >64/4	>64/4	>64/4	4	0	38
trimethoprim/sulfamethoxazole		≤0.25/4.75 to >64/1216	>64/1216	>64/1216	10	—	32
Acquired, plasmid *mcr-1*	21						
plazomicin		0.5–2	1	2	21	0	0
colistin		4–16	8	8	0	—	21
amikacin		1–32	2	8	20	1	0
gentamicin		0.5–64	1	32	17	0	4
tobramycin		0.5–32	1	32	15	1	5
imipenem		1–8	4	4	4	5	12
doripenem		0.03–0.5	0.06	0.12	21	0	0
meropenem		0.03–0.5	0.03	0.06	21	0	0
tigecycline		0.25–2	0.5	1	19	2	0
levofloxacin		0.03 to >8	8	>8	10	0	11
ceftazidime		0.12 to >32	0.5	>32	14	0	7
ceftriaxone		0.03 to >32	0.12	>32	12	0	9
aztreonam		0.06 to >32	0.25	>32	13	0	8
piperacillin/tazobactam		1/4 to >64/4	4/4	>64/4	15	3	3
trimethoprim/sulfamethoxazole		≤0.25/4.75 to >64/1216	>64/1216	>64/1216	7	—	14
Unknown mechanism	24						
plazomicin		0.12 to >128	0.5	4	22	0	2[d]

Continued

Table 2. *Continued*

Colistin resistance mechanism/antibiotic	*n*	MIC (mg/L) range	MIC_{50}[a]	MIC_{90}[a]	MIC interpretive criteria (mg/L) susceptible	intermediate	resistant
colistin		4 to >128	64	>128	0	—	24
amikacin		0.5–64	4	32	21	1	2
gentamicin		0.15 to >64	4	64	12	1	11
tobramycin		0.25 to >64	8	32	8	4	12
imipenem		1 to >32	8	>32	2	3	19[e]
doripenem		0.03 to >32	2	16	10	3	11[e]
meropenem		0.008–64	1	16	12	4	8[e]
tigecycline		0.12–4	2	4	10	9	5
levofloxacin		0.03 to >8	8	>8	9	2	13
ceftazidime		0.12 to >32	>32	>32	5	0	19
ceftriaxone		0.03 to >32	>32	>32	3	2	19
aztreonam		0.06 to >32	>32	>32	5	0	19
piperacillin/tazobactam		0.5/4 to >64/4	>64/4	>64/4	6	1	17
trimethoprim/sulfamethoxazole		≤0.25/4.75 to >64/1216	16/304	>64/1216	11	—	13

MIC_{50}, MIC that inhibits 50% of the isolates; MIC_{90}, MIC that inhibits 90% of the isolates.
CLSI criteria were applied with the exception of *plazomicin, for which no breakpoint is available at the moment (4 mg/L was arbitrarily chosen to categorize the isolates), and **colistin and ***tigecycline, for which EUCAST breakpoint criteria were used.
[a]No MIC_{50} and MIC_{90} are given for isolates exhibiting intrinsic resistance to colistin due to a limited number of tested isolates.
[b]Four isolates expressed 16S rRNA methylases and three out of four expressed NDM.
[c]Twenty-seven isolates produced a carbapenemase, of which six were NDM producers.
[d]One isolate co-produced a 16S rRNA methylase and an NDM-type carbapenemase.
[e]Fifteen isolates produced a carbapenemase.

0.25–64 mg/L for trimethoprim plus 4.75–1216 mg/L sulfamethoxazole. *E. coli* ATCC 25922 was used for quality control according to CLSI guidelines.

Molecular testing methods

Enterobacterial isolates with plazomicin MICs ≥8 mg/L were screened by PCR for the presence of 16S methyltransferase genes *armA*, *npmA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, *rmtF*, *rmtG* and *rmtH*, and for the different carbapenemase genes,[16,17] as resistance to plazomicin has been associated with the presence of 16S methylases that modify the intracellular target of all aminoglycosides.[18,19]

Results and discussion

Plazomicin inhibited 89.5% (85/95) and 93.7% (89/95) of the colistin-resistant Enterobacteriaceae isolates at ≤2 and ≤4 mg/L, respectively (Table 2). For strains with a well-defined chromosomally encoded resistance mechanism to colistin, the MIC_{90} value (i.e. the MIC that inhibits 90% of the isolates) was 2 mg/L for plazomicin. All of the *mcr-1*-positive isolates were inhibited at ≤2 mg/L, while the naturally colistin-resistant bacteria were inhibited at ≤4 mg/L. No difference was observed between *E. coli* TOP10 and *E. coli* TOP10 producing the plasmid-encoded MCR-1, both exhibiting an MIC of colistin of 0.25 mg/L. The MIC_{90} value was 2 mg/L for isolates with unknown colistin resistance mechanisms.

Overall, five *K. pneumoniae* isolates had a plazomicin MIC of ≥128 mg/L; none of these isolates carried *mcr-1*. A 16S methyltransferase gene was detected in all of these isolates (*armA* in three isolates, *rmtC* and *rmtF* in one isolate each; data not shown) and the bla_{NDM} carbapenemase gene was also detected in four of these isolates (data not shown). One *Enterobacter cloacae* isolate had an elevated plazomicin MIC (8 mg/L), but a methyltransferase gene was not detected in this isolate.

Plazomicin was more active than currently marketed aminoglycosides against this collection of colistin-resistant isolates. The plazomicin MIC_{90} value was 4 mg/L, compared with 32, >64 and 64 mg/L for amikacin, gentamicin and tobramycin, respectively. Non-susceptibility to amikacin, gentamicin and tobramycin was common [16.8% (16/95), 47.4% (45/95) and 63.2% (60/95) of the isolates, respectively] (Table 2).

This study shows that plazomicin remains effective against colistin-resistant Enterobacteriaceae, regardless of the mechanism of polymyxin resistance (intrinsic, acquired, chromosome- or plasmid-encoded). Importantly, the emergence of plasmids encoding the MCR-1/MCR-2 polymyxin resistance traits has not altered the susceptibility of Enterobacteriaceae to this antibiotic.

By circumventing the main mechanisms of resistance to aminoglycosides, i.e. the aminoglycoside-modifying enzymes, plazomicin may be seen as an interesting molecule for treating infections due to MDR Enterobacteriaceae. Consistent with known limitations of this drug, and the aminoglycoside class in general, isolates with high plazomicin MICs (>128 mg/L) carried 16S rRNA methylase-encoding genes, often in association with NDM-encoding genes in this isolate collection. There were a total of four isolates co-producing an NDM-type carbapenemase and a 16S rRNA methylase, and two isolates producing an NDM carbapenemase only.

Taking into account the irreversible spread of multiresistance in Enterobacteriaceae, it is expected that plazomicin may find its place in the armamentarium against those bacteria. All aminoglycosides, including plazomicin, possess rapid bactericidal activity and favourable chemical and pharmacokinetic properties, making this class of antibiotics a therapy of choice for treating many bacterial infections.

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Transparency declarations

L. E. C. and K. M. K. are employees of and shareholders in Achaogen, Inc. All other authors: none to declare.

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