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Plazomicin Activity against 346 Extended-Spectrum-β-Lactamase/AmpC-Producing *Escherichia coli* Urinary Isolates in Relation to Aminoglycoside-Modifying Enzymes

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ABSTRACT The activity of plazomicin and clinically relevant aminoglycosides was tested against 346 extended-spectrum-β-lactamase/AmpC-producing *Escherichia coli* urinary isolates, and the results were correlated with the presence of aminoglycoside-modifying enzymes (AMEs). Data showed that plazomicin was very active against all ESBL/AmpC-producing *E. coli* urinary isolates. Its activity was not related to the AME genes studied.

KEYWORDS AMEs, ESBL, *Escherichia coli*, plazomicin, aminoglycosides

Escherichia coli strains producing extended-spectrum β-lactamases (ESBLs) have emerged as major global pathogens, primarily associated with urinary tract infections (1). These strains possess plasmids that carry genes conferring resistance to multiple antibiotic classes (2). As a result, therapeutic options against these β-lactam-resistant *E. coli* infections are extremely limited.

Aminoglycoside resistance in Gram-negatives is mainly conferred by production of aminoglycoside-modifying enzymes (AMEs) (3). Genes encoding AMEs are located on mobile genetic elements along with other resistance determinants, resulting in multidrug-resistant (MDR) isolates (3). Plazomicin is a next-generation aminoglycoside modified to evade AMEs. The compound is currently under clinical development for the treatment of complicated urinary tract infections (cUTIs) and acute pyelonephritis as a single agent (4, 5).

In this study, we evaluated the activity of plazomicin and clinically relevant aminoglycosides against 346 ESBL/AmpC-producing *E. coli* urinary isolates. The presence of four AME genes was also investigated, and the relationship between the AME genes detected and the resistance phenotype found was determined.

The isolates were obtained prospectively during 2013 at the Hospital Clínico San Carlos (Madrid, Spain). Only one isolate per patient was included. PCR characterization (6, 7) showed 302 ESBL producers and 44 AmpC producers.

MICs of gentamicin, tobramycin, amikacin, and plazomicin were determined by the agar dilution method. MICs of plazomicin were also determined by the broth microdilution method (8). Antimicrobial agents were obtained from their respective manufacturers. Plazomicin was supplied from Achaogen (South San Francisco, CA). The results were interpreted according the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (9).

All isolates resistant to at least one of the aminoglycosides studied were tested by PCR for the presence of AME genes. Sets of primers for the following genes were

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TABLE 1 *In vitro* susceptibility to different aminoglycosides of 346 *Escherichia coli* isolates in relation to the β-lactamase type produced[a]

Aminoglycoside	ESBL (n = 302 isolates)				AmpC (n = 44 isolates)			
	MIC_{50} (mg/liter)	MIC_{90} (mg/liter)	Range	No. (%) of resistant isolates	MIC_{50} (mg/liter)	MIC_{90} (mg/liter)	Range	No. (%) of resistant isolates
Gentamicin	1	64	≤0.125 to >64	86 (28.6)	1	64	0.5 to >64	11 (25)
Tobramycin	1	32	≤0.125 to >64	123 (40.9)	1	8	0.5 to 32	12 (27.3)
Amikacin	4	8	0.5 to >64	5 (1.7)	2	4	2 to 8	0
Plazomicin	1	1	0.25 to 4	NA	1	1	0.5 to 2	NA

[a]Resistance data were determined on the basis of EUCAST susceptibility breakpoints. NA, not applicable.

included in the PCR assay: *aac(3)-IIa* (10); *aac(6′)-Ib* (11); *ant(2″)-Ia* (12); and *aph(3′)-Ia* (11).

Comparisons of MICs for each antibiotic between groups were performed by Mann-Whitney U test. Correlations between pairs of variables were calculated by Spearman's rank test. The significance level was considered a P value of ≤0.05. Statistical analysis was performed using IBM SPSS 20 (SPSS Inc., Chicago, IL).

Overall, the highest resistance rate was observed for tobramycin (38.3%), followed by gentamicin (27.7%). Amikacin showed good activity, with a 1.5% resistance rate and a MIC_{90} of 8 mg/liter; similar results were previously reported in ESBL-producing *E. coli* (13, 14).

Plazomicin showed the best activity, with a MIC range of 0.25 to 4 mg/liter and MIC_{50} and MIC_{90} values of 1 mg/liter against all ESBL- and AmpC-producing *E. coli* isolates (data obtained by agar method). No significant differences were found between agar dilution MICs and microdilution MICs (MIC_{50}, 0.5 mg/liter; MIC_{90}, 1 mg/liter; MIC range, 0.125 to 4 mg/liter). Our findings are consistent with previous studies that showed plazomicin to be highly active against MDR clinical isolates of *Enterobacteriaceae*, including ESBL-producing *E. coli* isolates (15, 16).

The levels of susceptibility to aminoglycosides in relation to the β-lactamase type produced by the isolates are summarized in Table 1. All the AmpC isolates were amikacin susceptible, with MICs of ≤8 mg/liter.

Of the 346 *E. coli* isolates, 144 (41.5%) were resistant to at least one of the aminoglycosides studied. Five different resistance phenotypes were observed among these 144 resistant isolates. Resistance to gentamicin/tobramycin was observed for 86 strains; resistance to tobramycin for 45 strains; resistance to gentamicin for 8 strains; resistance to amikacin/gentamicin/tobramycin for 3 strains; and resistance to amikacin/tobramycin for 2 strains.

As can be seen in Fig. 1, the median MICs of the respective aminoglycosides were significantly higher for the tobramycin- and gentamicin-susceptible and -resistant isolates. Amikacin comparisons showed results that were analogous to those obtained with tobramycin and plazomicin but not to those obtained with gentamicin. The level of correlation between the MICs for plazomicin and amikacin by Spearman's rank test was higher (r = 0.600; $P < 0.001$) than the level of correlation between the MICs for plazomicin and tobramycin (r = 0.271; $P < 0.001$) and gentamicin (r = 0.112; P = 0.038), respectively. This is the first description of a correlation between the MICs of plazomicin and those of amikacin, tobramycin, and gentamicin in *E. coli* isolates, although a correlation between the plazomicin MICs and those of gentamicin has been reported in carbapenemase-producing *Klebsiella pneumoniae* (17).

The prevalence of combinations of AME genes among 144 *E. coli* isolates resistant to aminoglycosides is shown in Table 2. At least one AME gene was detected in 94.5% of strains. The most common AME gene was *aac(6′)-Ib* (90 strains; 62.5%), followed by *aac(3)-IIa* (37 strains; 25.7%), *aph(3′)-Ia* (30 strains; 20.8%), and *ant(2″)-Ia* (19 strains; 13.2%). In total, 98 isolates (68%) contained only one of the evaluated AME genes, 36 (25%) contained two of them, and 2 (1.4%) harbored three. Using EUCAST breakpoints

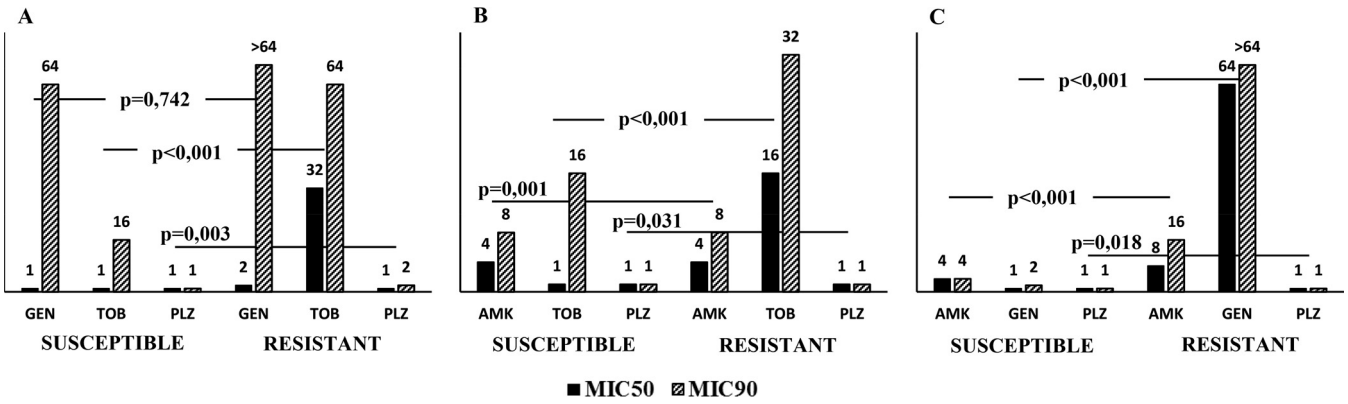

FIG 1 *In vitro* susceptibility to various aminoglycosides stratified by (A) amikacin (AMK) susceptibility, (B) gentamicin (GEN) susceptibility, and (C) tobramycin (TOB) susceptibility. *P* values denote differences in median MIC values of the respective aminoglycosides between the amikacin-, gentamicin-, and tobramycin-susceptible and -resistant isolates. The epidemiological cutoff was considered for amikacin resistance (MIC > 8 mg/liter). The MIC_{50} and MIC_{90} values are shown above the respective bars. PLZ, plazomicin.

(9), the AME presence was not always correlated with aminoglycoside resistance. Similar results were previously reported both in *E. coli* isolates and in *K. pneumoniae* isolates (17, 18).

PCR screening showed that *aac(6′)-Ib* was the most prevalent gene. Similar results have been reported by other authors (17, 18). All strains with *aac(6′)-Ib* were tobramycin resistant. Only 2 of the 58 isolates that harbored the *aac(6′)-Ib* gene alone expressed phenotypic resistance to amikacin, although it has been reported that *aac(6′)-Ib* confers resistance to both antibiotics (3). These observations were consistent with previous studies where, despite the possession of *aac(6′)-Ib*, low amikacin MICs have been reported for *E. coli* and *K. pneumoniae* strains (14, 17). In our study, moreover, there was a statistically significant relationship ($P < 0.01$) between the presence of an *aac(6′)-Ib* gene and an amikacin MIC of >8 mg/liter. These data showed that the presence of *aac(6′)-Ib* is required for expression of amikacin MICs above the epidemiological cutoff value (ECOFF).

The second-most-common gene in our study was *aac(3)-IIa*, although in other studies, this was the most prevalent gene, followed by *aac(6′)-Ib* (14, 19). The 21 isolates with this resistance gene alone were resistant to gentamicin; the presence of *aac(3)-IIa* in any combination of genes was associated with gentamicin resistance ($P < 0.01$). The resistance to gentamicin observed in our study was predominantly caused by the presence of the *aac(3)-IIa* gene; similar findings have previously been reported (18).

TABLE 2 Prevalence of aminoglycoside-modifying enzyme gene combinations in 144 *Escherichia coli* isolates resistant to aminoglycosides in relation to aminoglycoside MICs[a]

AME gene(s)	No. (%) of isolates	MIC_{50}/MIC_{90} (mg/liter)				Range				No. (%) of resistant isolates			
		AMK	GEN	TOB	PLZ	AMK	GEN	TOB	PLZ	AMK	GEN	TOB	PLZ
aac(6′)Ib	64 (44.4)	8/16	2/>64	16/32	1/1	2 to 64	0.5 to >64	8 to 64	0.25 to 4	2 (3.1)	24 (37.5)	64 (100)	NA
aac(3)IIa	21 (14.5)	4/4	>64/>64	8/8	1/1	2 to 8	64 to >64	4 to 32	0.5 to 2	0 (0)	21 (100)	19 (90.5)	NA
aph(3′)Ia	9 (6.3)	4/8	64/64	8/32	0.5/2	2 to 8	2 to 64	4 to 32	0.5 to 2	0 (0)	8 (88.9)	7 (77.8)	NA
ant(2″)Ia	4 (2.8)	2/4	64/64	8/32	0.5/1	2 to 4	64 to 64	8 to 32	0.5 to 1	0 (0)	4 (100)	4 (100)	NA
aa(6′)Ib + *aph(3′)Ia*	13 (9.02)	8/16	64/64	32/32	1/1	4 to 64	0.5 to >64	16 to 32	0.5 to 1	1 (7.7)	8 (61.5)	13 (100)	NA
aac(6′)Ib + *ant(2″)Ia*	7 (4.9)	8/32	32/64	32/64	1/4	2 to 32	8 to 64	8 to 64	0.5 to 4	1 (14.3)	7 (100)	7 (100)	NA
aac(6′)Ib + *aac(3)IIa*	5 (3.5)	8/>64	>64/>64	32/>64	0.5/1	2 to >64	64 to >64	16 to >64	0.5 to 1	1 (20)	5 (100)	5 (100)	NA
aac(3)IIa + *ant(2″)Ia*	5 (3.5)	4/4	64/64	8/8	1/1	2 to 4	64 to 64	4 to 8	0.5 to 1	0 (0)	5 (100)	4 (80)	NA
aac(3)IIa + *aph(3′)Ia*	4 (2.8)	2/4	64/>64	8/8	0.5/1	2 to 4	64 to >64	8 to 8	0.5 to 1	0 (0)	4 (100)	4 (100)	NA
ant(2″)Ia + *aph(3′)Ia*	2 (1.4)	2/4	64/>64	16/16	0.5/1	2 to 4	64 to >64	16 to 16	0.5 to 1	0 (0)	2 (100)	2 (100)	NA
aac(6′)Ib + *aac(3)Iia* + *aph(3′)Ia*	1 (0.7)	8/8	32/32	16/16	1/1	8 to 8	32 to 32	16 to 16	1 to 1	0 (0)	1 (100)	1 (100)	NA
aac(3)IIa + *ant(2″)Ia* + *aph(3′)Ia*	1 (0.7)	8/8	>64/>64	16/16	2/2	8 to 8	>64 to >64	16 to 16	2 to 2	0 (0)	1 (100)	1 (100)	NA
None	8 (5.5)	4/8	32/64	8/32	0.5/2	2 to 8	1 to 64	1 to 32	0.5 to 2	0 (0)	7 (87.5)	5 (62.5)	NA

[a]Resistance data were determined on the basis of EUCAST susceptibility breakpoints. AME, aminoglycoside-modifying enzyme; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; PLZ, plazomicin; NA, not applicable.

The presence of the *ant(2″)-Ia* gene was found to be related to gentamicin resistance, as previously described (14, 19). The presence of *aph(3′)-Ia* was not associated with resistance to any of the studied aminoglycosides.

In conclusion, in this study, the strains exhibited a remarkable diversity of AMEs; the AME genes involved in clinical aminoglycoside resistance were *aac(3)-IIa*, *aac(6′)-Ib*, and *ant(2″)-Ia*. High amikacin MICs, above the ECOFF established by EUCAST, have been shown to be related to the presence of *aac(6′)-Ib*. The activity of plazomicin was excellent regardless of the AME pattern; it may become a welcomed addition for the treatment of cUTIs, but the real position of this antibiotic will be revealed once pending phase III studies are completed.

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