



Comparative evaluation of plazomicin MICs obtained using agar dilution versus broth microdilution methods and impact of inoculum size against ESBL-producing *Escherichia coli*, carbapenemase-producing *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus* clinical isolates

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ABSTRACT

MICs of plazomicin were determined by agar dilution and broth microdilution in 187 ESBL-producing *Escherichia coli* ($n = 73$), carbapenemase-producing *Klebsiella pneumoniae* ($n = 55$) methicillin-resistant *Staphylococcus aureus* ($n = 59$) clinical isolates. Inoculum effect was determined by broth microdilution assay using two different inocula; $1-5 \times 10^5$ (standard inoculum) and $1-5 \times 10^8$ CFU/mL. For all isolates tested >98% categorical agreement and $\geq 95\%$ of essential agreement ($\pm 1 \log_2$) was found. At high inocula, MICs of plazomicin increased \geq eight-fold for 25% of *E. coli*, 24% of *K. pneumoniae* and 7% of *S. aureus* isolates tested. The results indicate that agar dilution and broth microdilution methods were equally suitable for determining plazomicin MICs. Inoculum effect was observed for plazomicin in *Escherichia coli* and *Klebsiella pneumoniae* isolates. Further studies that establish the genetic background of the isolates are required to better understand the reasons behind the inoculum effect.

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1. Introduction

Plazomicin (ACHN-490, Achaogen) is a next-generation aminoglycoside derived from sisomicin that contains structural modifications that allow it to retain antibacterial activity in the presence of aminoglycoside-modifying enzymes (Aggen et al. 2010). It has enhanced activity against many multidrug resistant Gram-negative bacteria and methicillin-resistant *S. aureus* isolates. It has potent activity versus carbapenem-resistant isolates, including those with multidrug resistant phenotype (ESBL, KPC, and metallo- β -lactamases that do not co-express a 16S rRNA methyltransferases resistance mechanism) (Rodríguez-Avial et al. 2015; López Díaz et al., 2017a; López-Díaz et al., 2017b).

Plazomicin was approved by the US Food and Drug Administration (FDA) in June 2018 for adults with complicated urinary tract infections, including pyelonephritis, caused by certain Enterobacteriaceae in patients who have limited or no alternative treatment options (ZEMDRITM, 2018) (product package insert). Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210303Orig1s000lbl.pdf. Accessed 26 July 2018.

As with any new antimicrobial agent, investigating the impact of the testing methodology is critical to detect changes in its in vitro antimicrobial activity profile. The minimal inhibitory concentration of an antibiotic may be also affected by the initial inoculum used in the antimicrobial susceptibility testing. This is a laboratory phenomenon known as inoculum effect (IE) (Brook 1989).

The IE generally occurs with beta-lactam antibiotics in relation to beta-lactamase-producing bacteria, but it also has been observed for other classes of antimicrobials, including aminoglycosides (Kelly and Matsen 1976). In this study we compared agar dilution (AD) method versus broth microdilution (BMD) for plazomicin MIC determination. We also determined on how variations of the standard inoculum testing parameters affected the antimicrobial activity of plazomicin.

2. Materials and methods

2.1. Bacterial isolates

A total of 187 selected organisms previously characterized were tested as follows: *Escherichia coli* ESBL-producing isolates ($n = 73$ strains), *Klebsiella pneumoniae* carbapenemase-producing isolates ($n = 55$ strains) and methicillin-resistant *Staphylococcus aureus* ($n = 59$ strains) [2–4].

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2.2. Antimicrobial susceptibility testing

Organisms were tested for susceptibility to plazomicin by AD and BMD in Mueller-Hinton agar and cation-adjusted Mueller-Hinton broth according CLSI specifications (CLSI 2018). Plazomicin was supplied by Achaogen, Inc. (South San Francisco, CA) and the concentration range tested was 0.06–8 µg/mL. *S. aureus* ATCC 25923 and *E. coli* ATCC 29212 were used as quality control strains in MIC determination. Plazomicin breakpoints were those established by the FDA: ≤2 µg/mL as susceptible, 4 µg/mL as intermediate, and ≥8 µg/mL as resistant. Available at <https://www.fda.gov/drugs/development-resources/plazomicin-injection>

For the broth microdilution method, two different inocula were employed whose densities differed 1000-fold: $1-5 \times 10^5$ (standard inoculum) and $1-5 \times 10^8$ CFU/mL (high inoculum) Tests were carried out in duplicate, and inoculum-size controls were provided by quantitative subculture. *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 were used as quality control strains. An inoculum effect was defined as an eight-fold or greater increase in MIC value upon testing with the highest inoculum (Thomson and Moland 2001).

2.3. Data analysis

Concordance criteria included categorical agreement (CA) and essential agreement (EA). Categorical agreement (CA) is defined as the qualitative interpretation of MIC from the AD agreeing with the reference BMD method; the bacteria are categorized as either susceptible, intermediate or resistant. The results were considered to be in essential agreement when the AD result was within ± 1 dilution of the BMD value. The percent of CA and EA was calculated by using the number of AD results in agreement as the numerator and the total number of organisms tested as the denominator.

3. Results

A total of 187 isolates were tested by both methods. For MRSA the MIC₅₀/MIC₉₀ by BMD were 0.5/ 1 µg/mL (range 0.12–2 µg/mL) and 0.5 /1 µg/mL by AD (range 0.12–2 µg/mL). For ESBL- producing *E. coli* isolates the MIC₅₀/MIC₉₀ by BMD was 0.5/ 1 µg/mL (range 0.25–2 µg/mL) and 0.5/1 µg/mL (range 0.12–4 µg/mL) by AD. For carbapenemase-producing *Klebsiella pneumoniae* (CPKP) isolates the corresponding values were 0.25/0.5 µg/mL (range 0.25–2 µg/mL) and 0.25/1 µg/mL (range 0.12–2 µg/mL) by BMD and AD, respectively. Overall, MICs by AD were lower than by BMD in 43 (23%) cases, higher in 42 (22.4%) cases and identical in 102 (54.6%) cases. By species MICs by AD tended to be higher for *K. pneumoniae* (29%) and *E. coli* (25%) while in the case of *S. aureus*, 34% of the isolates had lower plazomicin MICs by AD than by BMD. (Fig. 1).

EA was 96.2% (51/55) for CPKP isolates 95.8% (70/73) for *E. coli* and 95% (56/59) for MRSA. CA was 100% for *Klebsiella pneumoniae* and *Staphylococcus aureus* isolates. Among *E. coli* isolates, one isolate showed a plazomicin MIC of 4 µg/mL when tested by AD and 2 µg/mL by BMD (CA rate 98.6%).

The results of the effect of the inoculum size are shown in Table 1. When an inoculum 1000-fold greater than standard was used, IE was detected in 25% of *E. coli*, 24% of *K. pneumoniae* and 7% of *S. aureus* isolates tested. Overall, both, the MIC₅₀ and MIC₉₀, of this new aminoglycoside increased 4-fold at the higher inoculum rates for the three groups of bacteria and this had an impact in the categorical interpretation; at standard inoculum no isolate showed a MIC >2 µg/mL, however, at the higher inoculum, the percentage of isolates showing a plazomicin MIC of >2 µg/mL was 45%, for *E. coli*, 10% for *K. pneumoniae* and 13% for *S. aureus* isolates. No inoculum effect was observed in the quality control strains included.

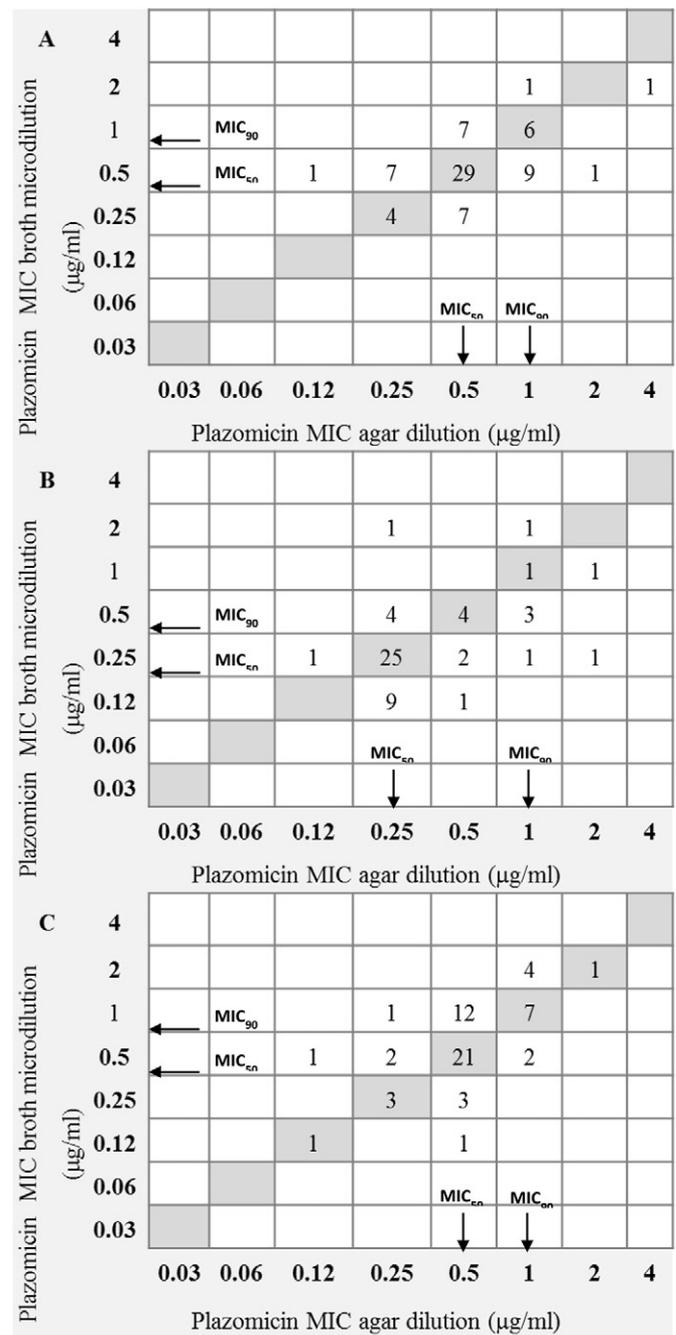


Fig. 1. Scattergram showing plazomicin MIC results obtained by agar dilution versus broth microdilution against *Escherichia coli* isolates (A), *Klebsiella pneumoniae* isolates (B) and *Staphylococcus aureus* isolates (C). Strains showing absolute agreement are gray shaded.

4. Discussion

Plazomicin has been recently approved by FDA for the treatment of complicated urinary tract infection (cUTI) including pyelonephritis. Investigating the impact of the testing methodology is critical to detect changes in the in vitro antimicrobial activity profile. In this study two standard methods were compared for testing plazomicin in vitro activity against 187 clinical bacterial isolates. Agar dilution and broth microdilution results were within ± 1 doubling dilution (essential agreement) for >95% of all study isolates and the category agreement rate based on proposed susceptible/intermediate/resistant breakpoints of ≤2/4/≥8 µg/mL was >98%. The data of the study show that there is a good correlation between plazomicin MICs determined by both dilution methods for all isolates tested.

Table 1
Plazomicin minimum inhibitory concentrations (MICs) according to inoculum size.

Isolate	MIC ($\mu\text{g/mL}$)						Number (%) of isolates with ≥ 8 -fold MIC increase
	Standard inoculum			High inoculum			
	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀	Range	
ESBL- <i>E. coli</i> (<i>n</i> = 73)	0.5	1	0.25–2	2	4	1–8	28 (25)
CPKP (<i>n</i> = 55)	0.25	0.5	0.12–2	1	2	0.5–8	13 (24)
MRSA (<i>n</i> = 59)	0.5	1	0.12–2	2	4	0.5–4	4 (7)

In our study, the impact of the bacterial load affected mainly the gram negative bacteria analyzed and to a lesser extent *Staphylococcus aureus*. Several studies have been shown that high bacterial inocula at the infection sites may decrease the activity of antibiotic (Docobo-Perez et al. 2013; Ferran et al. 2014; Rio-Marques et al. 2014; Tato et al. 2014). In a previous study, using an inoculum size 100-fold lower than the inoculum used here (5×10^6 CFU/mL), had minimal effects on the plazomicin MIC against *P. aeruginosa* and *A. baumannii* isolates. However, against 16 Enterobacteriaceae tested, plazomicin MICs remained within 2-fold for 87.5% of isolates (14/16) but in two cases (12.5%) an increase of 4-fold in the MIC was detected (Duncan et al. 2017).

This data show that there is an excellent agreement between plazomicin MICs determined by broth microdilution and agar dilution. Inoculum effect was observed for plazomicin in *Escherichia coli* and *Klebsiella pneumoniae* isolates. Differences in inoculum effect of the same antibiotic against different bacterial isolates have been previously observed (Lhermie et al. 2015). Further investigations are needed to better understand the reasons behind the inoculum effect. Since this effect was not observed uniformly, it would be interesting to know if the presence of the inoculum effect is related with the aminoglycoside resistance genes.

Conflict of interest

No conflict of interest.

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