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Killing activity of meropenem in combination with amikacin against VIM- or KPC-producing *Enterobacteriaceae* that are susceptible, intermediate, or resistant to amikacin

Brandon Kulengowski, Justin A. Clark, David S. Burgess *

College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA

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ABSTRACT

Amikacin is administered with a carbapenem to treat serious infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE). The varying degrees of activity of the individual agents correspond to differences in activity of the 2 in combination. Amikacin and meropenem are not bactericidal against amikacin-resistant CRE.

Carbapenem-resistant *Enterobacteriaceae* (CRE) exhibit resistance to most available antimicrobial agents and cause significant mortality, and there is no current treatment standard. Carbapenem-containing combination regimens have been the front-running choice clinically, along with recently approved contenders such as ceftazidime-avibactam and meropenem-vaborbactam which show activity against KPC-producing CRE (Perez et al., 2016). The present study aimed to evaluate the killing activity of amikacin (AMK) in combination with meropenem (MEM) against KPC- or VIM-producing CRE exhibiting moderate resistance to MEM (MICs 16–32 mg/L) and variable activity ranging from susceptible to resistant with AMK (MICs 8–64 mg/L).

The selection of isolates and time-kill methodology has been previously described (Kulengowski et al., 2018). In brief, CRE isolates were identified as part of routine patient care, and the carbapenemase gene was confirmed by the Verigene® system. MICs of AMK and MEM were determined by broth microdilution according to CLSI guidelines, and 4 isolates were chosen for further study. A VIM-producing AMK susceptible *Enterobacter cloacae* (MIC 8 mg/L; isolate 169), a KPC-producing AMK susceptible *Klebsiella pneumoniae* (MIC 16 mg/L; isolate 32), a KPC-producing AMK intermediate *Klebsiella pneumoniae* (MIC 32 mg/L; isolate 22), and a KPC-producing AMK resistant *Klebsiella pneumoniae* (MIC 64 mg/L; isolate 37) were selected. The first 3 isolates exhibited MEM MICs of 16 mg/L, and the AMK-resistant isolate had an MEM MIC of 32 mg/L. Time-kill assays were performed in at least duplicate using clinically achievable concentrations with typical human dosing regimens. AMK alone (8 and 16 mg/L), MEM alone (4 and 16 mg/L), and all possible combinations were evaluated (Kovacevic et al., 2016; Usman et al., 2016). Standard definitions of bactericidal activity, bacteriostatic activity, regrowth, synergy, indifference, and antagonism were utilized (Kulengowski et al., 2018).

AMK and MEM, when used alone, resulted in regrowth except for the highest MEM concentration (16 mg/L) against the VIM-producing isolate, even though the MEM MICs for most isolates were 16 mg/L (Fig. 1). All combinations maintained bactericidal activity against the 2 amikacin-susceptible CRE (isolates 169 and 32), and synergy was demonstrated in 5 of 8 combinations (Figs. 1 and 2). Synergy was not determinable in the remaining 3 combinations because the most active single agent was within 2-log CFU/mL of the lower limit of quantification of the laser colony counter. Against the amikacin-intermediate isolate, only the highest MEM-AMK combination maintained synergy and bactericidal activity (Figs. 1 and 2). However, no combination maintained bactericidal activity against the amikacin-resistant strain, and none were synergistic (Figs. 1 and 2). Antagonism was never observed with any isolate.

These data suggest that amikacin and meropenem are only reliably synergistic and bactericidal when bacterial strains are susceptible to amikacin (MICs ≤16 mg/L) and are exhibiting meropenem MICs of at most 16 mg/L. Previous data with polymyxins in combination with a carbapenem have suggested that in vitro killing activity (bactericidal activity and synergy) is dependent on the carbapenem MIC (Kulengowski et al., 2017). We have not observed a similar relationship for amikacin in combination with meropenem against other

* Corresponding author. Tel.: +1-859-218-0948; fax: +1-859-323-0069.
E-mail address: david.burgess@uky.edu (D.S. Burgess).

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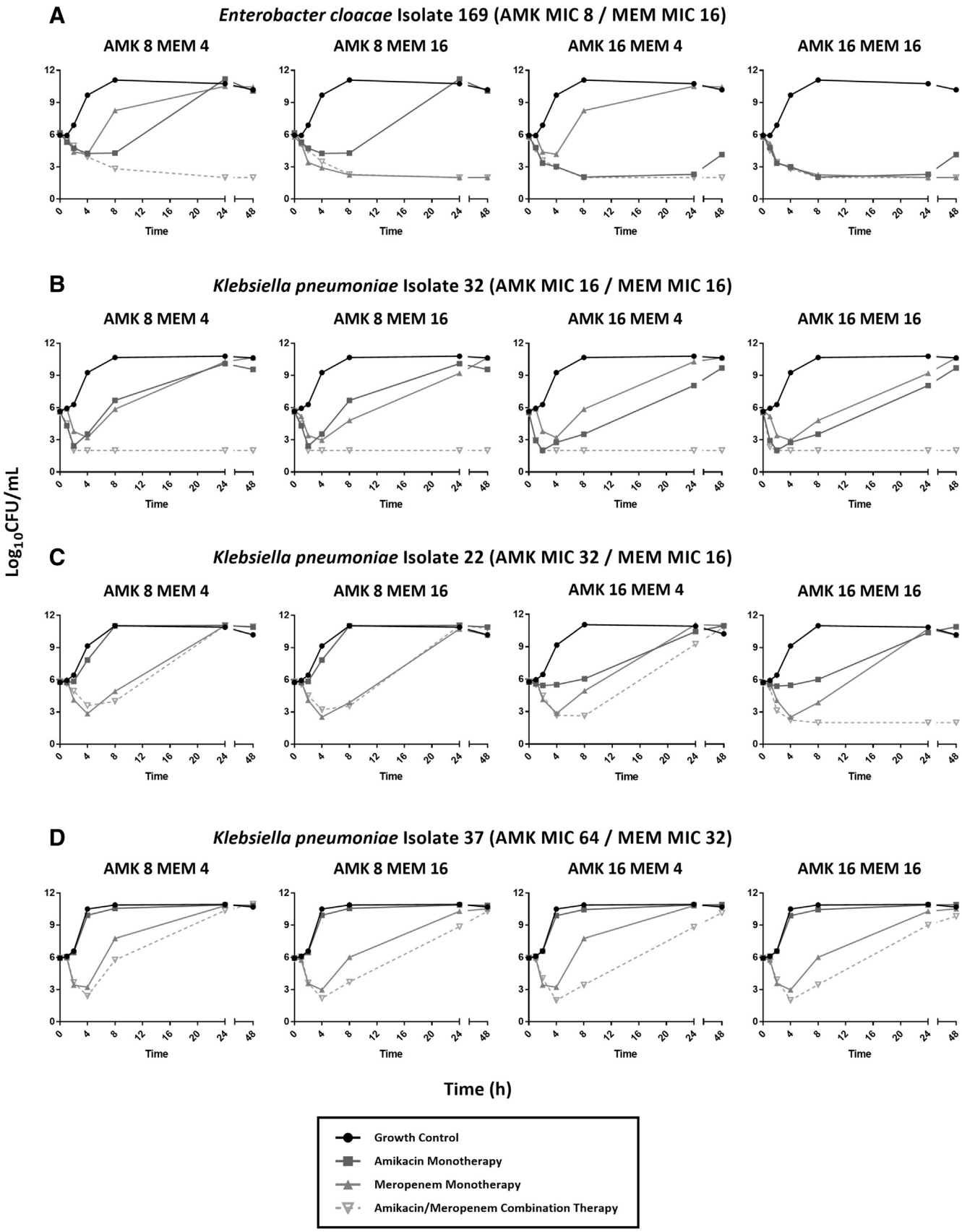

Fig. 1. Time kill curves of meropenem and amikacin alone and in combination against carbapenem-resistant *Enterobacteriaceae*. Filled circles represent growth controls. Filled squares represent amikacin alone. Filled triangles represent meropenem alone. Inverted hollow triangles represent amikacin and meropenem in combination. Data points are geometric means of replicate experiments ($n = 2$–3). The lower limit of quantification was 10^2 CFU/mL.

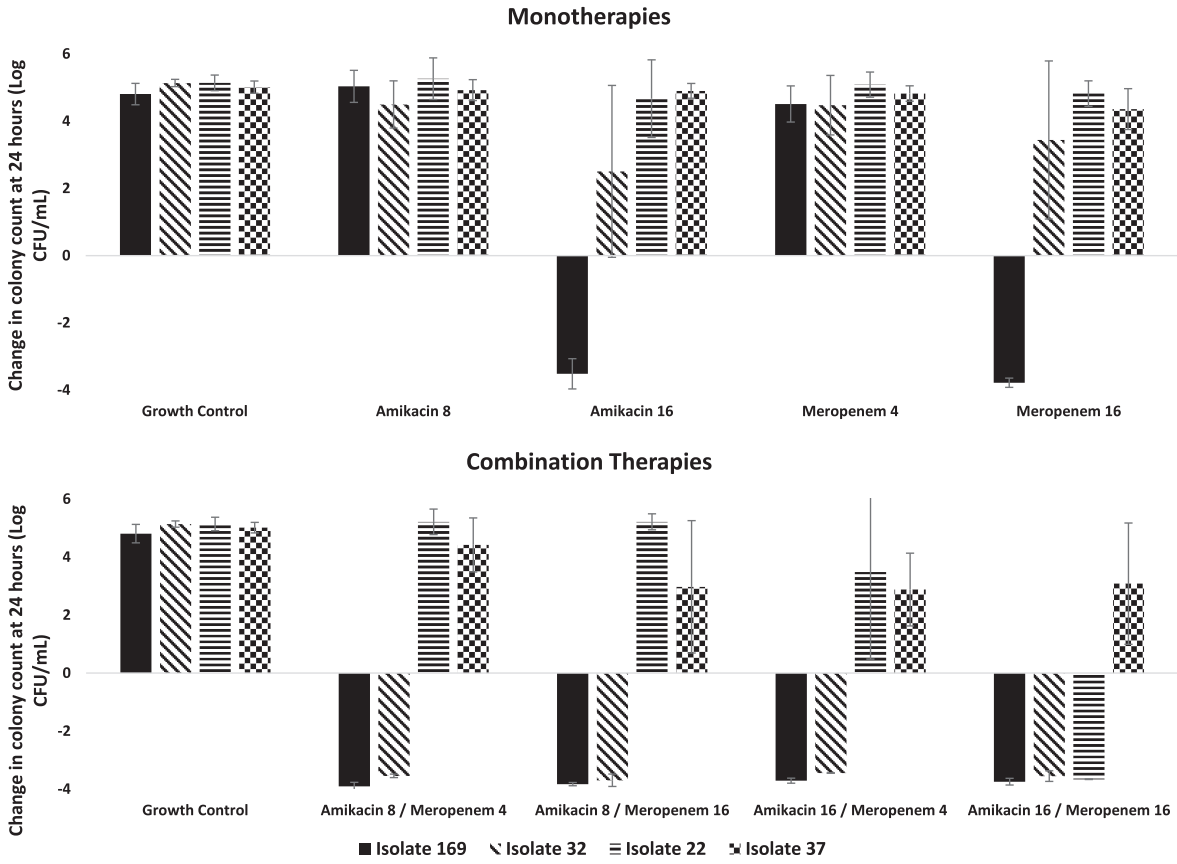

Fig. 2. Twenty-four–hour change in colony count for meropenem and amikacin alone and in combination against carbapenem-resistant *Enterobacteriaceae*. Data are differences of geometric means at time points 0 h and 24 h with standard deviations as error bars of replicate experiments ($n = 2–3$).

amikacin-susceptible isolates (Kulengowski et al., 2018). Similar to the present study, Le et al. demonstrated synergy and bactericidal activity maintained in 2 of 3 KPC-3–producing amikacin intermediate isolates but not in the 1 amikacin-resistant isolate. Unlike our study, Le et al. did not analyze amikacin-susceptible isolates near the CLSI susceptibility breakpoint (16 mg/L). Interestingly, the rate of killing was faster in the present study with maximal killing at around 2–4 h except in the VIM-1–producing isolate where maximal killing was at around 8 h (Fig. 1). In the study by Le et al., maximal killing occurred at around 8–12 h, but meropenem MICs were reportedly higher (≥32 mg/L) (Le et al., 2011). We have previously observed slower killing rates as meropenem MIC increases with no change in rates of bactericidal activity or synergy in carbapenem-resistant *Enterobacter cloacae* (Kulengowski et al., 2018). Others have also reported in vitro synergy of amikacin and a carbapenem using an Etest® strip interaction assay against carbapenem-resistant *K. pneumoniae*. The improved activity of doripenem was found to be dependent on the susceptibility phenotypes of each drug alone since doripenem became significantly more active with the addition of amikacin only in amikacin-susceptible strains and not amikacin-resistant strains (Loho et al., 2018). Another study has quantified the MIC-lowering effect of amikacin on doripenem and concluded that the addition of amikacin to doripenem lowers doripenem MICs by 8–16-fold against KPC-producing *K. pneumoniae* (Clock et al., 2013).

In summary, amikacin in combination with meropenem demonstrates not only synergy but important bactericidal activity against amikacin-susceptible CRE, including VIM-producing CRE. Additional data are needed to ascertain the mechanism of reduced rates of killing against VIM-producing CRE and KPC-producing CRE with elevated carbapenem MICs (i.e., >32 mg/L). Additional data are also warranted in other MBL-producing CRE.

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Transparency

Declarations of interest: none.

References

Clock SA, Tabibi S, Alba L, Kubin CJ, Whittier S, Saiman L. In vitro activity of doripenem alone and in multi-agent combinations against extensively drug-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Diagn Microbiol Infect Dis 2013;76:343–6. https://doi.org/10.1016/j.diagmicrobio.2013.03.014.

Kovacevic T, Avram S, Milakovic D, Spiric N, Kovacevic P. Therapeutic monitoring of amikacin and gentamicin in critically and noncritically ill patients. J Basic Clin Pharm 2016;7:65–9. https://doi.org/10.4103/0976-0105.183260.

Kulengowski B, Campion JJ, Feola DJ, Burgess DS. Effect of the meropenem MIC on the killing activity of meropenem and polymyxin B in combination against KPC-producing *Klebsiella pneumoniae*. J Antibiot (Tokyo) 2017;70:974–8. https://doi.org/10.1038/ja.2017.73.

Kulengowski B, Rutter WC, Campion JJ, Lee GC, Feola DJ, Burgess DS. Effect of increasing meropenem MIC on the killing activity of meropenem in combination with amikacin or polymyxin B against MBL and KPC producing *Enterobacter cloacae*. Diagn Microbiol Infect Dis 2018;92:262–6. https://doi.org/10.1016/j.diagmicrobio.2018.06.013.

Le J, McKee B, Srisupha-Olarn W, Burgess DS. In vitro activity of carbapenems alone and in combination with amikacin against KPC-producing *Klebsiella pneumoniae*. J Clin Med Res 2011;3:106–10. https://doi.org/10.4021/jocmr551w.

Loho T, Sukartini N, Astrawinata DAW, Immanuel S, Aulia D, Priatni I. In vitro antibacterial interaction of doripenem and amikacin against multidrug-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* isolates. Can J Infect Dis Med Microbiol 2018;2018:1047–67. https://doi.org/10.1155/2018/1047670.

Perez F, El Chakhtoura NG, Papp-Wallace K, Wilson BM, Bonomo RA. Treatment options for infections caused by carbapenem-resistant *Enterobacteriaceae*: can we apply "precision medicine" to antimicrobial chemotherapy? Expert Opin Pharmacother 2016;17:761–81. https://doi.org/10.1517/14656566.2016.1145658.

Usman M, Frey OR, Hempel G. Population pharmacokinetics of meropenem in elderly patients: dosing simulations based on renal function. Eur J Clin Pharmacol 2016;73:333–42. https://doi.org/10.1007/s00228-016-2172-4.