Diagnostic Microbiology and Infectious Disease 93 (2019) 261–264

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Staggering the administration of polymyxin B and meropenem in time-kill against carbapenem-resistant *Enterobacteriaceae* exhibiting a wide range of meropenem MICs

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ARTICLE INFO

Article history:
Received 7 August 2018
Accepted 25 September 2018
Available online 4 October 2018

Keywords:
Carbapenem-resistant *Enterobacteriaceae*
Polymyxin B
Meropenem
Time-kill
Staggered administration

ABSTRACT

Little is known regarding the appropriate timing and sequencing of a carbapenem and polymyxin in combination against carbapenem-resistant *Enterobacteriaceae*. Meropenem and polymyxin B were administered simultaneously or 1 agent 2 h prior to the other, in vitro. The carbapenem should be administered prior to the polymyxin when used in combination.

Carbapenem-resistant *Enterobacteriaceae* (CRE) are resistant to nearly all antimicrobials and are associated with staggering mortality (50%) (Perez et al., 2016). CRE are often treated with combination therapy, but optimal therapy has not been established. Polymyxin B (PMB) and meropenem (MEM) are commonly utilized against CRE, and the meropenem MIC is known to influence their interaction (Kulengowski et al., 2017). The aim of this study was to determine whether the sequence of administration of MEM or PMB affects the efficacy of this combination against CRE with varying MEM MICs.

CRE isolates expressing KPC-2 (isolates 10 and 17) or KPC-3 (isolates 24 and 44) with PMB MICs 0.06–0.125 mg/L and MEM MICs 8–128 mg/L were selected for analysis. Whole genome sequencing was previously performed on these isolates (Kulengowski et al., n.d., 2017). We conducted separate-day duplicate time-kill studies of clinically achievable concentrations by typical human dosing of PMB (0.25 and 1 mg/L) and MEM (4 and 16 mg/L) alone and in combination against 2 *Enterobacter cloacae* (MEM MICs: 8 and 32 mg/L) and 2 *Klebsiella pneumoniae* isolates (MEM MICs: 32 and 128 mg/L). MEM 64 mg/L was also tested alone and in combination with PMB 0.25 and 1 mg/L for the isolate with a MEM MIC of 128 mg/L. Sampling occurred at 0, 1, 2, 4, 8, 24, and 48 h. For simultaneous administration, both antimicrobials were added at time 0. Otherwise, either PMB or MEM was added at time 0, and the remaining antimicrobial (MEM or PMB) was added at 2 h. This 2-h delay was selected based on maximal killing for polymyxin B monotherapy occurring around that time (Fig. 1), and our working theory was that administering polymyxin B first may improve the permeability of meropenem to its target site. Samples were diluted; plated using a spiral plater, which controlled for antimicrobial carryover; and enumerated by a laser colony counter (CLSI, 1999; Yourassowsky et al., 1988). Kill curves were constructed to characterize the activity of staggered vs. simultaneous administration. Standard definitions of bactericidal activity, bacteriostatic activity (colony count less than or equal to starting inoculum but not bactericidal), regrowth (any increase in colony count from previous time point), synergy, indifference, and antagonism were utilized (CLSI, 1999).

For PMB monotherapy against all isolates, bactericidal activity was observed by 2 h, but regrowth occurred by 8 h (Fig. 1). For MEM monotherapy against all isolates, bacteriostatic activity was observed with regrowth by 24 h (Fig. 1). Administering polymyxin B first exhibited less killing activity than simultaneous administration or administering meropenem first for all isolates, regardless of MEM MIC (Figs. 1, 2). Against the isolate with a MEM MIC of 8 mg/L, bactericidal activity was maintained throughout 48 h only when meropenem was administered first (Fig. 1, panel A). Furthermore, all combinations of meropenem administered first were synergistic. Synergy and bactericidal activity at 24 h for simultaneous administration was only observed for the 2 higher meropenem combination concentrations (MEM 16 mg/L) (Fig. 1, panel A). There was not a clear difference between administering meropenem first and simultaneous administration when the MEM MICs were 32 mg/L, but both exhibited more killing activity than administering polymyxin B first (Figs. 1, panels B and C; 2). For the isolate with a MEM MIC of 128 mg/L, only when

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https://doi.org/10.1016/j.diagmicrobio.2018.09.014
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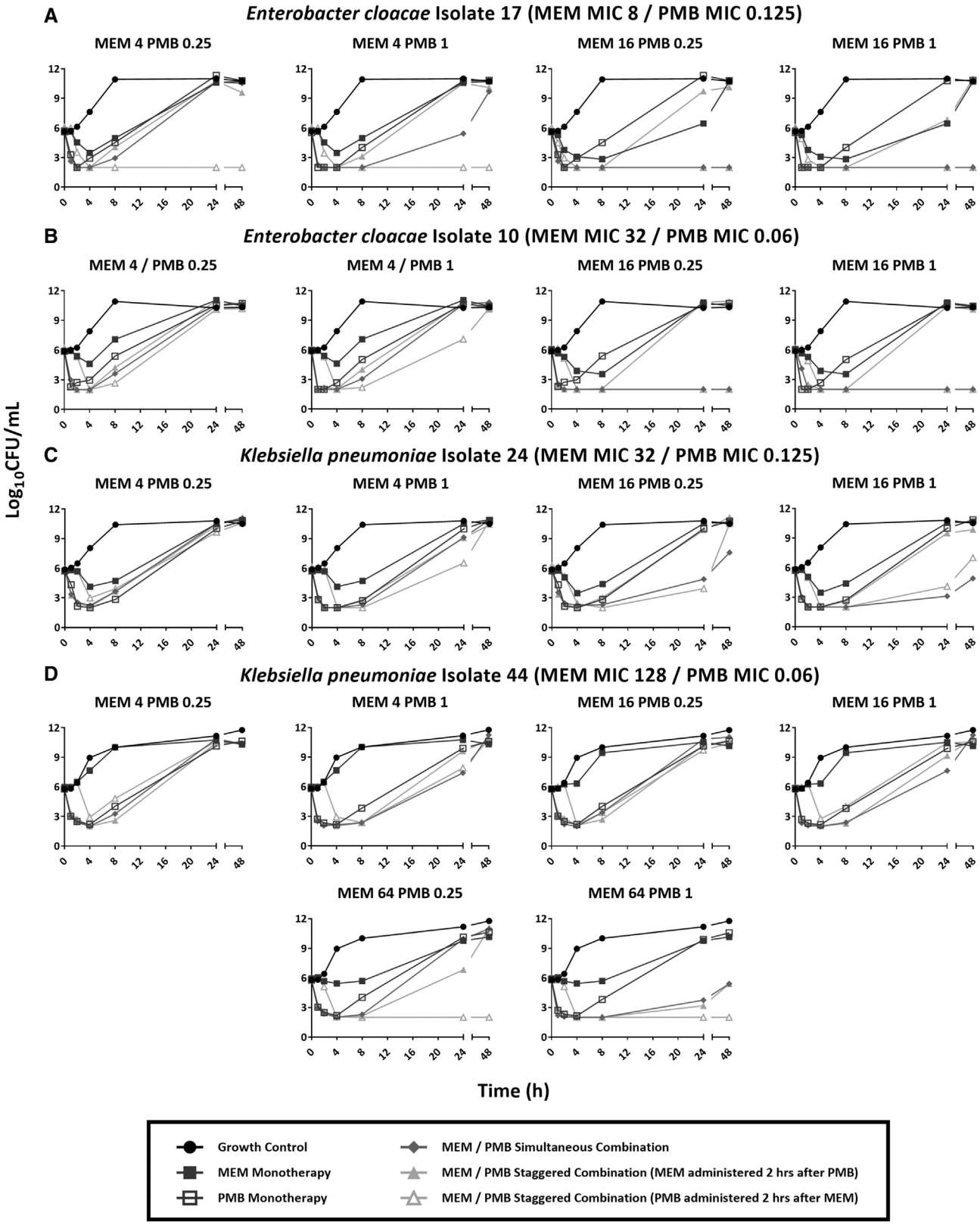

Fig. 1. Time-kill curves of meropenem and polymyxin B against carbapenem-resistant *Enterobacteriaceae*. Curves represent geometric means of separate-day duplicate time-kill experiments conducted over 48 h. Antibiotic concententrations in mg/L are subtitled above their corresponding graph. Solid circles are antibiotic-free growth control, solid squares are meropenem alone, hollow squares are polymyxin B alone, solid diamonds are simultaneous administration of meropenem and polymyxin B, solid triangles are meropenem administered 2 h after polymyxin B, and hollow triangles are polymyxin B administered 2 h after meropenem.

meropenem was administered first and at the highest meropenem concentration (MEM 64 mg/L) was bactericidal activity maintained throughout 48 h. Regrowth was observed by 8 h for the remaining combinations against this isolate except for the simultaneous MEM 64 / PMB 1 mg/L and the polymyxin first MEM 64 / PMB 1 mg/L curves where regrowth was observed by 24 h (Fig. 1, panel D).

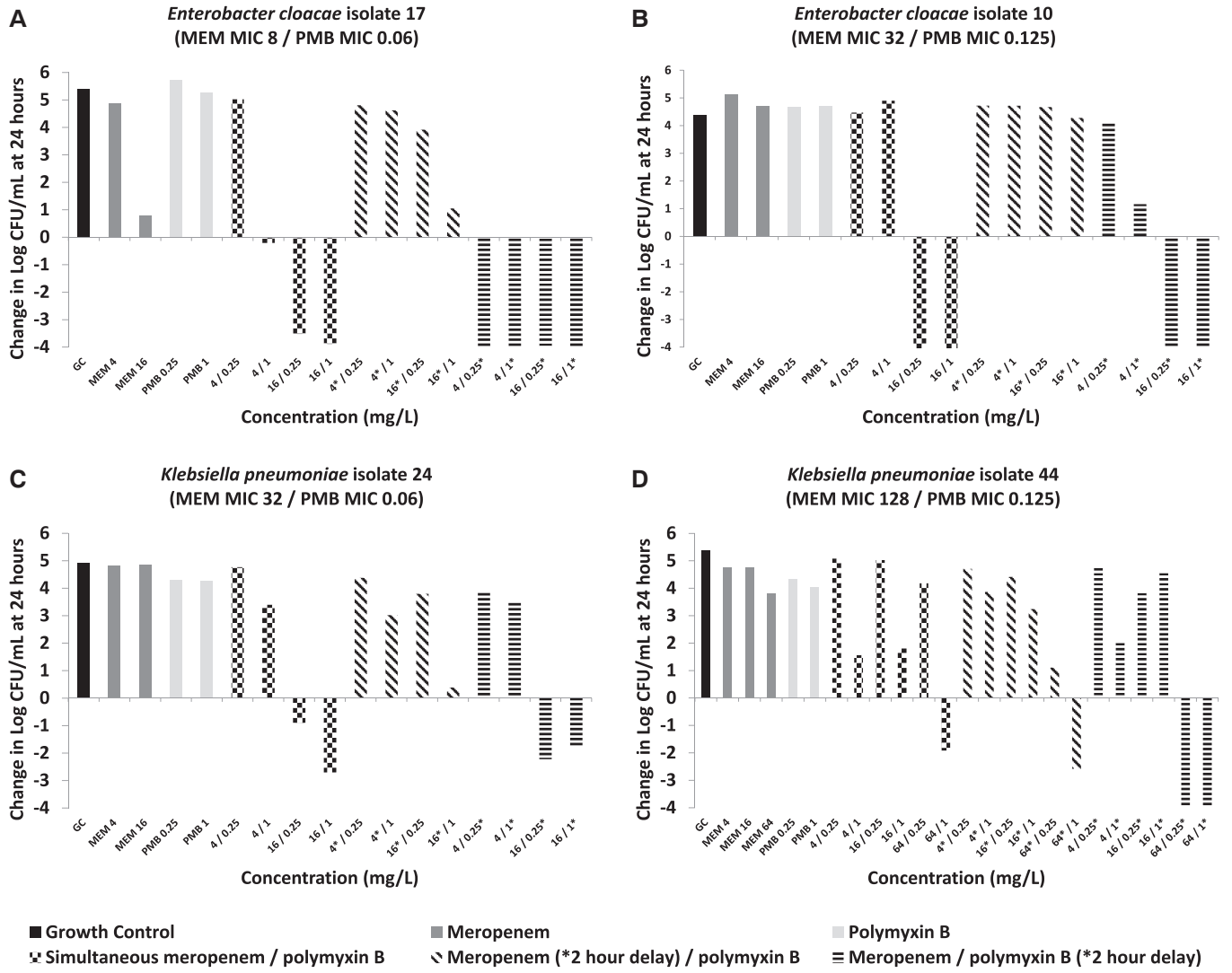

Fig. 2. Change in colony count after 24 h of meropenem and polymyxin B against carbapenem-resistant *Enterobacteriaceae*. Bars represent the change in geometric mean colony counts from time 0 to time 24 h. Black bars are antibiotic-free growth control, dark gray bars are meropenem alone, light gray bars are polymyxin B alone, checkered bars are simultaneous meropenem and polymyxin B, diagonal striped bars are meropenem administered 2 h after polymyxin B, and horizontal striped bars are polymyxin B adminstered 2 h after meropenem.

Although we are not the first to employ staggered administration techniques with combination antimicrobials, this study is the first to look at the sequencing of a carbapenem and a polymyxin against CRE. Lewis et al. (1998) demonstrated polyene-azole antagonism when fluconazole was administered prior to amphotericin B in a dynamic in vitro model; Zelenitsky et al. (2004) demonstrated significantly improved (6-fold) activity with simultaneous or beta-lactam-first staggering of ceftazidime and either ciprofloxacin or tobramycin against *P. aeruginosa*. Based on our data, a carbapenem should be given prior to a polymyxin antimicrobial when these 2 agents are used in combination. Polymyxins should not be administered first, and neither agent should be administered as monotherapy. However, some clinical data with *A. baumannii* have suggested no difference in clinical failure with colistin monotherapy compared to colistin combinations with meropenem, rifampin, or fosfomycin (Paul et al., 2018; Perez and Bonomo, 2018). Data from another clinical trial of colistin with meropenem against extensively drug resistant gram-negative bacilli are anxiously awaited (NCT01597973, ClinicalTrials.gov).

The mechanism explaining why polymyxin B administered first results in less killing activity of the combination has yet to be determined. As previously mentioned, we anticipated improved combination antimicrobial activity based on the idea that polymyxins disrupt the outer membrane of bacteria, improving permeability of other compounds (Zavascki et al., 2007). Instead, polymyxin B may be decreasing bacterial growth and metabolism without improving beta-lactam activity on cell wall synthesis (Zelenitsky et al., 2004). Alternatively, polymyxin B may have increased beta-lactamase concentration in the growth media by improving the permeability of beta-lactamase out of viable bacterial cells or causing its release upon cell death. Increased extracellular beta-lactamase may result in decreased beta-lactam concentrations, but data suggest a 30% increase in permeability of meropenem in the presence of polymyxin B after accounting for increased extracellular beta-lactamase. However, that data involved polymyxin B administered 20 min prior to meropenem (X. Tao et al., presented at ASM Microbe 2018, Atlanta, GA, 8 June 2018).

Although there is not a clear benefit to administering a carbapenem first compared to simultaneously in isolates exhibiting carbapenem MICs of 32 mg/L, a benefit is observable at lower and higher carbapenem MICs (8 and 128 mg/L). Additional data are needed to confirm these findings and to determine optimal staggering time since only a 2-h delay was evaluated in the present study. Additional data are also needed to determine the mechanism for the decreased antimicrobial activity observed when polymyxin B was administered first relative to simultaneously or when meropenem was administered first.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Transparency

Declarations of interest: none.

Acknowledgments

We thank Chase N. Ayres and Thien-Nam T To, PharmD candidates on a research elective rotation, and Eric R. Gregory, PharmD for excellent technical assistance.

The data and results of this study were presented at ASM Microbe 2018. Poster: AAR04-527.

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