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Polymyxin-resistant, carbapenem-resistant *Acinetobacter baumannii* is eradicated by a triple combination of agents that lack individual activity

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Objectives: The emergence of polymyxin resistance threatens to leave clinicians with few options for combatting drug-resistant *Acinetobacter baumannii*. The objectives of the current investigation were to define the *in vitro* emergence of polymyxin resistance and identify a combination regimen capable of eradicating *A. baumannii* with no apparent drug susceptibilities.

Methods: Two clonally related, paired, *A. baumannii* isolates collected from a critically ill patient who developed colistin resistance while receiving colistin methanesulfonate in a clinical population pharmacokinetic study were evaluated: an *A. baumannii* isolate collected before (03-149.1, polymyxin-susceptible, MIC 0.5 mg/L) and an isolate collected after (03-149.2, polymyxin-resistant, MIC 32 mg/L, carbapenem-resistant, ampicillin/sulbactam-resistant). Using the patient's unique pharmacokinetics, the patient's actual regimen received in the clinic was recreated in a hollow-fibre infection model (HFIM) to track the emergence of polymyxin resistance against 03-149.1. A subsequent HFIM challenged the pan-resistant 03-149.2 isolate against polymyxin B, meropenem and ampicillin/sulbactam alone and in two-drug and three-drug combinations.

Results: Despite achieving colistin steady-state targets of an AUC_{0-24} >60 mg·h/L and C_{avg} of >2.5 mg/L, colistin population analysis profiles confirmed the clinical development of polymyxin resistance. During the simulation of the patient's colistin regimen in the HFIM, no killing was achieved in the HFIM and amplification of polymyxin resistance was observed by 96 h. Against the polymyxin-resistant isolate, the triple combination of polymyxin B, meropenem and ampicillin/sulbactam eradicated the *A. baumannii* by 96 h in the HFIM, whereas monotherapies and double combinations resulted in regrowth.

Conclusions: To combat polymyxin-resistant *A. baumannii*, the triple combination of polymyxin B, meropenem and ampicillin/sulbactam holds great promise.

Introduction

Acinetobacter baumannii has emerged as a troubling nosocomial pathogen, largely due to its ability to acquire resistance mechanisms against commonly used antimicrobials.[1] While carbapenems were traditionally used as the agents of choice against MDR *A. baumannii*, the rising prevalence of carbapenem resistance has necessitated the use of polymyxin B or colistin (polymyxin E). However, *A. baumannii* strains have emerged that display high levels of resistance to polymyxins through lipid A modification or complete loss of lipopolysaccharides.[2] Even more worrisome are recent reports of plasmid-mediated polymyxin resistance

determinants (MCR-1) that are capable of transferring between Gram-negative species.[3]

One strategy for overcoming polymyxin resistance is the addition of antimicrobials that individually lack activity against polymyxin-resistant *A. baumannii* to create an active polymyxin combination regimen. Interestingly, a recently published study found that 19 patients developed colistin-resistant *A. baumannii* following treatment with colistin methanesulfonate (CMS). Subsequent treatment of ventilator-associated pneumonias (most commonly) using a triple combination of CMS, a carbapenem and ampicillin/sulbactam resulted in superior 30 day survival compared with other antibiotic regimens ($P = 0.03$).[4]

In the present study, a polymyxin-susceptible isolate collected prior to a patient's colistin therapy in an open-label CMS population pharmacokinetic study and a polymyxin-resistant isolate subsequently collected during colistin treatment were used to investigate polymyxin resistance *in vitro*. First, the polymyxin-susceptible isolate was exposed to the patient's clinical antibiotic regimen in a hollow-fibre infection model (HFIM) to mirror the *in vivo* evolution of polymyxin resistance. The polymyxin-resistant isolate collected from the patient was then used in a 14 day HFIM to profile the detailed pharmacodynamics and resistance suppression of a polymyxin B, meropenem and ampicillin/sulbactam triple combination against an *A. baumannii* strain resistant to all the investigated antimicrobials. Polymyxin B was selected over colistin for evaluation in the HFIM because polymyxin B has more favourable pharmacokinetics, which include administration of an active moiety and the lack of renal dose adjustments.[5]

Methods

Bacterial isolates and clinical data for Patient 149

Two *A. baumannii* isolates (03-149.1 and 03-149.2) were collected from a 41-year-old patient (Patient 149) with multiple myeloma at the Siriraj Hospital in Bangkok, Thailand, who received CMS as part of his clinical care for treatment of pneumonia due to Gram-negative bacillus, as part of an open-label population pharmacokinetic study on CMS.[6] Patient 149 first presented with pneumonia due to *A. baumannii*; the polymyxin-susceptible isolate 03-149.1 was collected from an endotracheal aspirate and CMS therapy was subsequently initiated on day 1 and consisted of a 300 mg colistin base activity loading dose with a maintenance regimen of 75 mg every 12 h. On day 4, the patient's CMS dose escalated to 150 mg every 12 h, and 500 mg of meropenem every 12 h was started. On day 5, a second *A. baumannii* isolate was then collected from an endotracheal aspirate that was polymyxin resistant (03-149.2) and clonally identical to the first polymyxin-susceptible isolate (03-149.1) on PFGE. Figure 1(a) displays the timeline of antibiotic administration and isolates collected from Patient 149.

Antibiotics and susceptibility testing

Analytical grade polymyxin B was purchased from Sigma–Aldrich (St Louis, MO, USA). Fresh stock solutions of polymyxin B were prepared immediately prior to each experiment. Cation-adjusted Mueller–Hinton broth (CAMHB) (Difco, Detroit, MI, USA) supplemented with calcium (25 mg/L) and magnesium (12.5 mg/L) was used as the growth medium. MIC values were determined in quadruplicate according to the CLSI guidelines. Isolate 03-149.2 was resistant to all antibiotics tested, including colistin and polymyxin B (MICs 32 mg/L each), meropenem (MIC 64 mg/L) and ampicillin/sulbactam (MIC 32/16 mg/L), whereas 03-149.1 was susceptible to colistin and

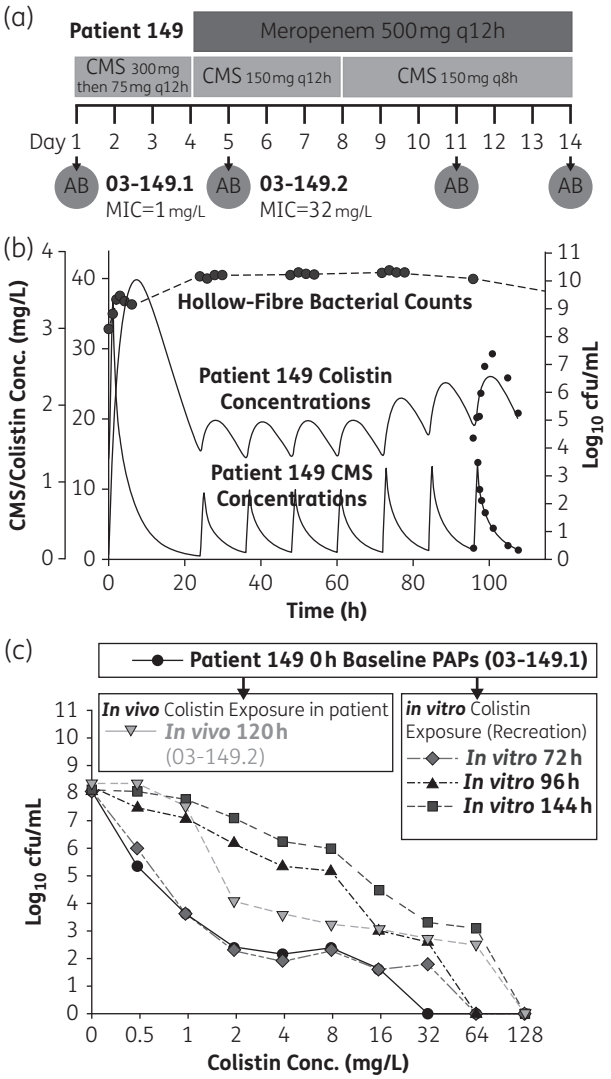

Figure 1. (a) Patient 149's treatment course involving CMS regimens combined with meropenem. Two endotracheal isolates of *A. baumannii* (AB) were collected from Patient 149. The first isolate was collected prior to colistin therapy and was polymyxin susceptible (03-149.1), whereas the second isolate was collected during colistin treatment and was polymyxin resistant (03-149.2). (b) Patient 149's measured CMS and colistin concentrations are shown as filled circles, whereas lines represent concentrations predicted by a population pharmacokinetic model in the CMS/colistin clinical study by Garonzik *et al.*[6] To completely simulate Patient 149's colistin pharmacokinetics and meropenem regimen (see panel a), a subsequent HFIM was used to recapitulate the patient's colistin profile against the polymyxin-susceptible 03-149.1 and the total bacterial population quantified in the HFIM is shown. (c) The *A. baumannii* collected throughout the HFIM were investigated using colistin PAPs to observe the stepwise increase in colistin resistance during *in vitro* colistin exposure to the polymyxin-susceptible (03-149.1) isolate, which was compared with the *in vivo* derived colistin-resistance isolate (03-149.2), which occurred in Patient 149. q8h, every 8 h; q12h, every 12 h.

polymyxin B (MICs 0.5 mg/L each), but resistant to meropenem (MIC 64 mg/L) and ampicillin/sulbactam (MIC 32/16 mg/L).

Population analysis profiles (PAPs)

To compare the relative susceptibilities of the two clinical isolates, 10^8 cfu/mL inocula of the polymyxin-susceptible (03-149.1) and polymyxin-resistant (03-149.2) isolates were used to determine colistin, polymyxin B, meropenem and ampicillin/sulbactam PAPs. In brief, serial dilutions of the 10^8 cfu/mL inocula were performed and then subsequently plated onto Mueller–Hinton agar (MHA) containing 0–128 mg/L drug. Following 48 h of incubation at 37°C, colonies were enumerated to quantify populations with varying degrees of antibiotic susceptibility.

Simulating Patient 149's colistin regimen in the HFIM

An HFIM was used to simulate the exact clinical regimen that Patient 149 received by using a population pharmacokinetic model to generate the patient's colistin profile from day 0 to day 5 of clinical treatment. Using Patient 149's specific half-life of 19 h, the colistin profile of a 300 mg CMS loading dose followed by a CMS maintenance dose of 75 mg every 12 h that escalated to 150 mg every 12 h on day 4 was simulated (colistin fC_{max} = 3.6 mg/L on day 1, fC_{max} = 1.8 mg/L on day 3 and fC_{max} = 2.3 mg/L on day 5). On day 4 of the HFIM, 500 mg of meropenem every 12 h was simulated using a 2 h half-life and fC_{max} of 23 mg/L. Beginning with a starting inoculum of 10^8 cfu/mL 03-149.1, samples were periodically withdrawn from the HFIM and used for viable cell counts on MHA, as well as colistin PAPs on MHA containing 0–128 mg/L colistin.

Antibiotic combinations against polymyxin-resistant, carbapenem-resistant 03-149.2 in the HFIM

To identify a combination of antibiotics that maintains activity against the Patient 149's polymyxin-resistant strain (03-149.2), HFIM experiments were conducted over 14 days as described previously.[7] Polymyxin B was utilized instead of colistin due to the superior kinetics of polymyxin B, which allow rapid target attainment.[5] Meropenem and ampicillin/sulbactam were also investigated due to the reported success of a polymyxin, meropenem and ampicillin/sulbactam combination against polymyxin-resistant *A. baumannii*.[4] On the day of each experiment, overnight cultures of 03-149.2 were used to achieve a 10^8 cfu/mL inoculum in CAMHB. Bacterial samples were collected at 0, 1, 2, 3, 4, 6, 24, 26, 28, 30, 48, 50, 52, 54, 72, 74, 76, 78, 96, 144, 192, 240, 288 and 336 h for viable cell counting. As polymyxin hetero-resistance has been frequently reported in *A. baumannii*,[8] real-time polymyxin PAPs were conducted every 24–48 h using 0.5, 1, 2 and 10 mg/L polymyxin B.

A dose-optimized polymyxin B regimen was simulated based on a recent population pharmacokinetics study in 24 adult patients that received physician-selected, intravenous polymyxin B regimens ranging from 0.45 to 3.38 mg/kg/day.[5,9] The simulated polymyxin B half-life was 8 h and unbound drug concentrations were calculated using a protein binding level of 58%. The concentration–time profiles of an FDA approved dose of meropenem[10] and a high dose of ampicillin/sulbactam that has been utilized in patients[11] were also simulated in the HFIM. Both meropenem and ampicillin/sulbactam were administered as 3 h prolonged infusions with a 1.5 h half-life. During combination regimens, the half-lives of multiple agents were maintained as described previously.[12] The following regimens were simulated in the HFIM: alone; as double combinations (polymyxin B + meropenem, polymyxin B + ampicillin/sulbactam and meropenem + ampicillin/sulbactam); and as a triple combination of all three agents [3.33 mg/kg polymyxin B at time 0 h (fC_{max} = 3.61 mg/L) then 1.43 mg/kg every 12 h (fC_{max} = 2.41 mg/L, $fAUC_{ss}$ = 35.9 mg·h/L, where ss means steady-state), 2 g of meropenem every 8 h (fC_{max} = 54.8 mg/L) and 8 g/4 g of ampicillin/sulbactam every 8 h (fC_{max} = 132/70.2 mg/L)].

Pharmacokinetics

Polymyxin B concentrations were determined by an LC–single quadrupole MS (LC–MS) method[13] with good reproducibility (coefficients of variation ≤10%) and accuracy (observed concentrations were ≤10.0% from target concentrations). Meropenem concentrations were quantified by LC–MS/MS using an Agilent 1200 and Agilent 6430 (Santa Clara, CA, USA). The meropenem calibration curve was linear, with R^2 >0.999 and good reproducibility (coefficient of variation ≤3.57%) and accuracy (99.7%–109.4%). The limit of quantification was 0.05 mg/L.[14] Sulbactam concentrations were analysed on a triple quadrupole LC–MS/MS (Agilent 6460 series). Chromatographic separation used an Agilent XDB-C18 column (particle size 1.8 μm; 50 × 4.6 mm) with a gradient elution (water and acetonitrile, both containing 0.1% formic acid). Mass transitions were *m/z* 234–124 for sulbactam.

Results

The PAPs for isolates 03-149.1 and 03-149.2 are shown for colistin, polymyxin B, meropenem and ampicillin/sulbactam in Figure S1 (available as Supplementary data at *JAC* Online). Although only minor differences between isolates were noted for subpopulations with reduced susceptibilities to meropenem and ampicillin/sulbactam, 03-149.2 contained subpopulations capable of growing on 0.5, 1 and 2 mg/L colistin that were over 10^3, 10^5 and $10^{1.5}$ times more abundant than 03-149.1. For each isolate, colistin and polymyxin B PAPs were relatively similar.

The pharmacokinetic profiles of Patient 149's CMS and colistin concentrations were constructed by applying a population pharmacokinetic model to plasma samples taken before and after the eighth dose of CMS (Figure 1b).[6] Following Patient 149's seventh dose of CMS, a colistin trough of 1.58 mg/L was recorded and a colistin peak concentration of 2.67 mg/L was observed after the eighth CMS dose (model-projected AUC_{ss} 62.5 mg·h/L). To track the development of polymyxin resistance *in vitro*, the isolate taken from Patient 149 prior to CMS treatment (03-149.1) was exposed to a simulation of Patient 149's colistin regimen in an HFIM. The simulated colistin regimen was incapable of reducing bacterial counts in the HFIM, with a total population that exceeded 10^{10} cfu/mL by 24 h. The addition of meropenem on the fourth day of treatment was also incapable of conferring additional killing over colistin alone. Colistin PAPs conducted on samples taken from the HFIM revealed that the emergence of colistin resistance in the HFIM was delayed, with counts on MHA imbued with >1 mg/L colistin remaining below 10^3 cfu/mL by 72 h, followed by growth on MHA containing ≤8 mg/L colistin that exceeded 10^5 cfu/mL at 96 h. The *in vitro*-derived resistance profiles generated from the HFIM versus the *in vivo*-derived resistance profile in Patient 149 are strikingly similar (Figure 1c).

Clinical polymyxin B, meropenem and ampicillin/sulbactam combinations were simulated in 14 day HFIMs against 03-149.2 to assess the combinatorial pharmacodynamics of agents that lack individual activity against resistant *A. baumannii* isolates (Figure 2). When used alone, none of the antimicrobials was able to cause a noticeable reduction in bacterial counts (Figure 2b–d). Similarly, ampicillin/sulbactam in combination with either polymyxin B or meropenem was unable to achieve >0.5 $\log_{10}$ cfu/mL reduction in total counts (Figure 2f and g). In contrast, the polymyxin B and meropenem combination reduced counts by 4.2 $\log_{10}$ cfu/mL at 6 h, followed by gradual regrowth over 66 h until observed counts

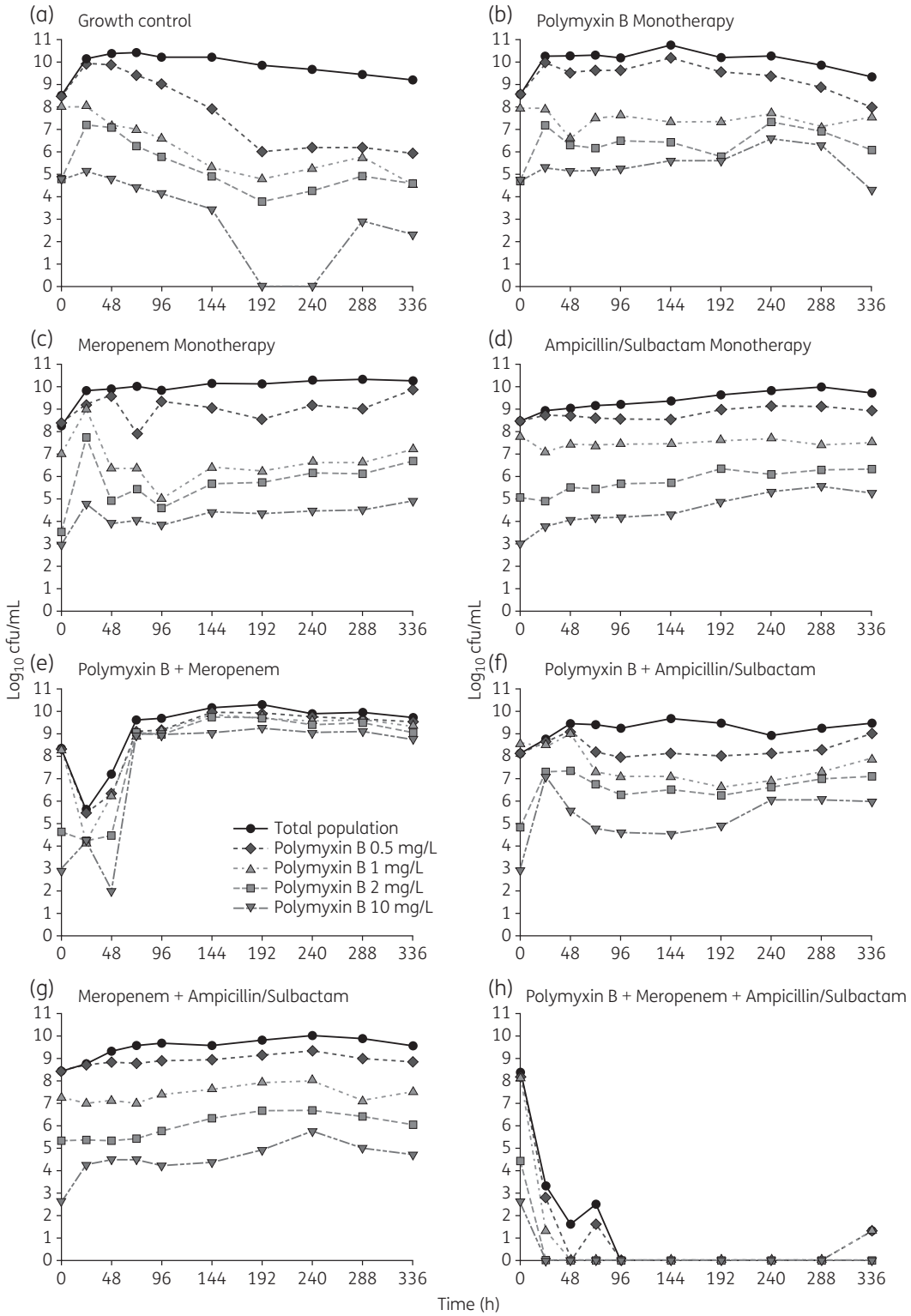

Figure 2. Clinical regimens of polymyxin B, meropenem and ampicillin/sulbactam were simulated in 14 day HFIMs against an *A. baumannii* isolate (03-149.2) resistant to all three agents from Patient 149. MICs: colistin and polymyxin B, 32 mg/L each; meropenem, 64 mg/L; and ampicillin/sulbactam, 32/16 mg/L. Polymyxin B, meropenem and ampicillin/sulbactam were investigated alone (b–d), as double combinations (e–g) and as a triple combination of all three agents (h). The simulated antibiotic regimens consisted of 3.33 mg/kg polymyxin B at time 0 h (fC_{max} = 3.61 mg/L) then 1.43 mg/kg every 12 h (fC_{max} = 2.41 mg/L), 2 g of meropenem every 8 h (fC_{max} = 54.8 mg/L) and 8 g/4 g of ampicillin/sulbactam every 8 h (fC_{max} = 132/70.2 mg/L). Circles represent the total population of *A. baumannii* plated on drug-free agar, whereas the other symbols correspond to *A. baumannii* that grew on agar containing polymyxin B (0.5, 1, 2 and 10 mg/L).

were $>10^9$ cfu/mL by 72 h (Figure 2e). The combination of all three agents achieved a similar 4.2 $\log_{10}$ cfu/mL reduction at 6 h, but the bacterial burden continued to decline until no viable cells were detected, beginning at 96 h (Figure 2h). Surprisingly, proliferation of polymyxin-resistant subpopulations was only observed during the polymyxin B and meropenem combination with $\sim10^3$ cfu/mL (0.001% of initial population) of *A. baumannii* growing on 10 mg/L polymyxin B at baseline and $>10^8$ cfu/mL (10% of final population) being detected at 336 h.

Discussion

Strains of *A. baumannii* are emerging that are capable of mounting resistance mechanisms against nearly all of the antimicrobials available in the global armamentarium. Here, we profile the time course of polymyxin resistance during exposure to CMS of a patient who received this antimicrobial together with meropenem in an open-label population pharmacokinetic study. Despite achieving the colistin steady-state targets proposed by Garonzik *et al.*[6] and Nation *et al.*[15] of an AUC_{0-24} >60 mg·h/L and C_{avg} of >2.5 mg/L, the emergence of polymyxin resistance was confirmed by colistin PAPs of 03-149.1 and 03-149.2. Previous metabolomic and structural analyses of Patient 149's polymyxin-resistant isolate (03-149.2) identified the modification of lipid A with phosphoethanolamine,[16] suggesting that polymyxin resistance in 03-149.2 is mechanistically similar to resistance conferred by the MCR-1 phosphoethanolamine transferase plasmid.[3] However, an important limitation of the present study is the simulation of colistin plasma concentrations, which likely resulted in much greater colistin exposure than would be expected for peripheral tissues such as the lungs. Due to the uncertainty of how to optimally dose agents in polymyxin combinations at the time of the clinical pharmacokinetic study, Patient 149 also received a non-traditional adjunctive meropenem dose of 500 mg every 12 h; however, a recent investigation has suggested that high carbapenem exposure is necessary to prevent the emergence of resistance.[14]

In agreement with the clinical observation made by Qureshi *et al.*[4] that colistin exposure may result in colistin-resistant *A. baumannii* strains, exposure of a colistin-susceptible *A. baumannii* isolate (03-149.1) to a clinical colistin regimen in the HFIM resulted in the gradual emergence of polymyxin resistance. The *in vitro* HFIM-generated profiles demonstrated a heterogeneously resistant profile that was similar to that of the 03-149.2 isolate after *in vivo* colistin exposure in Patient 149. The common bacterial signatures of polymyxin resistance derived from the HFIM and that in the actual patient are striking, providing new insight into the emergence of polymyxin resistance. Although the amplification of polymyxin resistance was most pronounced after a low-dose meropenem regimen was initiated, the causality of the sudden shift in resistant subpopulations remains unknown; however, the polymyxin B and meropenem combination was also the only combination that demonstrated the proliferation of polymyxin resistance against 03-149.2, suggesting that concomitant carbapenem use that fails to achieve microbiological eradication may exacerbate the emergence of polymyxin resistance. The simulated colistin and meropenem regimens were unable to achieve killing, due to the development of colistin resistance by day 4. Two randomized clinical trials are currently evaluating colistin monotherapy versus colistin in combination with meropenem to better determine the potential advantages of combination therapy in Gram-negative bacteria with nominal polymyxin susceptibility (NCT01732250 and NCT01597973).

Against polymyxin-resistant *A. baumannii*, the encouraging performance of the triple combination of polymyxin B, meropenem and ampicillin/sulbactam is consistent with a recent study that investigated the treatment of colistin-resistant *A. baumannii* infections.[4] Out of 17 patients, 7 received triple therapy with ampicillin/sulbactam, a carbapenem and colistin, whereas the 10 other patients received either ampicillin/sulbactam monotherapy, a double combination of a carbapenem and colistin, or a triple combination utilizing colistin, tigecycline and either rifampicin or ampicillin/sulbactam. All of the patients receiving the triple combination of colistin, a carbapenem and ampicillin/sulbactam survived for at least 30 days following their diagnoses, whereas 6 out of 10 patients receiving the other treatments died ($P = 0.03$). The authors concluded that the promising results of their study require further validation.[4] In the current study, the triple combination was the only regimen capable of eradicating the polymyxin-resistant *A. baumannii* isolate, further supporting the clinical utility of the combination. Considering the lack of an immune component to the *in vitro* HFIM, *in vivo* systems that benefit from granulocyte-mediated bacterial clearance may have an increased likeliness of bacterial eradication during combination therapy.

In conclusion, the triple combination of polymyxin B, meropenem and ampicillin/sulbactam demonstrated sustained bactericidal activity against a polymyxin-resistant *A. baumannii* isolate in a 14 day HFIM. It is also noteworthy that the polymyxin B plus meropenem combination amplified polymyxin B resistance in 03-149.2 despite high levels of resistance at baseline, which highlights the risk of being too conservative with the design of combination regimens. Considering the high inoculum, these results encouragingly suggest that polymyxin-resistant *A. baumannii* responsible for high-burden infections may be treated with agents to which the organism is nominally resistant, as indicated by MIC testing. Due to the intrinsic activity of sulbactam against *A. baumannii*, the triple combination pursued in the current study may not have applications outside of *Acinetobacter* species. Fortunately, new β-lactam/β-lactamase combinations such as ceftolozane/tazobactam and ceftazidime/avibactam may be viable options for some strains of MDR *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, whereas the introduction of new antimicrobials may eventually expand the number of therapeutic options against *A. baumannii*.

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Transparency declarations

None to declare.

Disclaimer

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Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (https://academic.oup.com/jac).

References

1 Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 2008; **21**: 538–82.

2 Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Front Microbiol* 2014; **5**: 643.

3 Liu YY, Wang Y, Walsh TR *et al*. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 2016; **16**: 161–8.

4 Qureshi ZA, Hittle LE, O'Hara JA *et al*. Colistin-resistant *Acinetobacter baumannii*: beyond carbapenem resistance. *Clin Infect Dis* 2015; **60**: 1295–303.

5 Sandri AM, Landersdorfer CB, Jacob J *et al*. Population pharmacokinetics of intravenous polymyxin B in critically ill patients: implications for selection of dosage regimens. *Clin Infect Dis* 2013; **57**: 524–31.

6 Garonzik SM, Li J, Thamlikitkul V *et al*. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrob Agents Chemother* 2011; **55**: 3284–94.

7 Lenhard JR, Brown T, Rybak MJ *et al*. Sequential evolution of vancomycin-intermediate resistance alters virulence in *Staphylococcus aureus*: pharmacokinetic/pharmacodynamic targets for vancomycin exposure. *Antimicrob Agents Chemother* 2015; **60**: 1584–91.

8 Hawley JS, Murray CK, Jorgensen JH. Colistin heteroresistance in *Acinetobacter* and its association with previous colistin therapy. *Antimicrob Agents Chemother* 2008; **52**: 351–2.

9 Tsuji BT, Landersdorfer CB, Lenhard J *et al*. The paradoxical effect of polymyxin B: high drug exposure amplifies resistance in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2016; **60**: 3913–20.

10 Kaye KS, Pogue JM, Tran TB *et al*. Agents of last resort: polymyxin resistance. *Infect Dis Clin North Am* 2016; **30**: 391–414.

11 Betrosian AP, Frantzeskaki F, Xanthaki A *et al*. High-dose ampicillin-sulbactam as an alternative treatment of late-onset VAP from multidrug-resistant *Acinetobacter baumannii*. *Scand J Infect Dis* 2007; **39**: 38–43.

12 Blaser J. In-vitro model for simultaneous simulation of the serum kinetics of two drugs with different half-lives. *J Antimicrob Chemother* 1985; **15** (Suppl A): 125–30.

13 Cheah SE, Bulitta JB, Li J *et al*. Development and validation of a liquid chromatography-mass spectrometry assay for polymyxin B in bacterial growth media. *J Pharm Biomed Anal* 2014; **92**: 177–82.

14 Lenhard JR, Bulitta JB, Connell TD *et al*. High-intensity meropenem combinations with polymyxin B: new strategies to overcome carbapenem resistance in *Acinetobacter baumannii*. *J Antimicrob Chemother* 2016; **72**: 153–65.

15 Nation RL, Garonzik SM, Li J *et al*. Updated US and European dose recommendations for intravenous colistin: how do they perform? *Clin Infect Dis* 2016; **62**: 552–8.

16 Mahamad Maifiah MH, Cheah SE, Johnson MD *et al*. Global metabolic analyses identify key differences in metabolite levels between polymyxin-susceptible and polymyxin-resistant *Acinetobacter baumannii*. *Sci Rep* 2016; **6**: 22287.