European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:1925–1931
https://doi.org/10.1007/s10096-019-03628-6

ORIGINAL ARTICLE

Comparative serum bactericidal activity of meropenem-based combination regimens against extended-spectrum beta-lactamase and KPC-producing *Klebsiella pneumoniae*

Paolo Gaibani[1] · Donatella Lombardo[1] · Michele Bartoletti[2,3] · Simone Ambretti[1] · Caterina Campoli[2] · Maddalena Giannella[2,4] · Sara Tedeschi[2] · Matteo Conti[4] · Rita Mancini[4] · Maria Paola Landini[1,3,5] · Maria Carla Re[1,3] · Pierluigi Viale[2,3] · Russell E. Lewis[2,4]

Received: 13 May 2019 / Accepted: 30 June 2019 / Published online: 6 July 2019

Abstract

Combination therapies are frequently used in the treatment of multidrug-resistant *Klebsiella pneumoniae* infection without consensus regarding which combination is the most effective. We compared bactericidal titres from sera collected from critically ill patients receiving meropenem plus tigecycline ($n = 5$), meropenem plus colistin ($n = 5$), or meropenem, colistin and tigecycline ($n = 5$) against *K. pneumoniae* isolates that included ESBL-producing ($n = 7$) and KPC-producing strains ($n = 14$) with varying sensitivity patterns to colistin and tigecycline. Meropenem concentrations (C_{min}) were measured in all samples by LC-MS/MS, and indexed to respective pathogen MICs to explore differences in patterns of bactericidal activity for two versus three drug combination regimens. All combination regimens achieved higher SBTs against ESBL (median reciprocal titre 128, IQR 32–256) versus KPC (4, IQR 2–32) strains. Sera from patients treated with meropenem-colistin yielded higher median SBTs (256, IQR 64–512) than either meropenem-tigecycline (32, IQR 8–256; $P < 0.001$). The addition of tigecycline was associated with a lower probability of achieving a reciprocal SBT above 8 when meropenem concentrations were below the MIC ($P = 0.04$). Although the clinical significance is unknown, sera from patients receiving tigecycline-based combination regimens produce lower serum bactericidal titres against ESBL or KPC-producing *K. pneumoniae*. SBTs may represent a useful complimentary endpoint for comparing pharmacodynamics of combinations regimens for MDR *Enterobacteriaceae*.

Keywords *Klebsiella pneumoniae* · Serum bactericidal assay · ESßL · KPC · Tigecycline

Introduction

In the last decade, carbapenem resistance among *Enterobacteriaceae* is now increasing globally, especially among *K. pneumoniae* [1]. Treatment of KPC-producing *K. pneumoniae* (KPC-Kp) infection represents a challenge to clinicians due to its broad antibiotic resistance. The poor outcomes of patients infected with KPC-Kp are directly attributable to the lack of effective remaining antibiotic treatment options [1]. Several multicenter observational studies have suggested that antimicrobial combination strategy improves the survival of patients infected with KPC-Kp [2–4]. Yet, many questions remain regarding which combination of agents to use, the effectiveness of combination therapies against isolates with high-level resistance, how to modify the regimens in a patient who is not clinically responding or with relapsing infection, and how combine novel antimicrobial drugs [5–7].

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10096-019-03628-6) contains supplementary material, which is available to authorized users.

✉ Paolo Gaibani
paolo.gaibani@unibo.it

[1] Operative Unit of Clinical Microbiology, Regional Reference Centre for Microbiological Emergencies, S.Orsola-Malpighi University Hospital, 9 via G. Massarenti, 40138 Bologna, Italy

[2] Department of Medical Sciences and Surgery, Operative Unit of Infectious Diseases, S.Orsola-Malpighi University Hospital, Bologna, Italy

[3] University of Bologna, Bologna, Italy

[4] Central Laboratory, S. Orsola-Malpighi University Hospital, Bologna, Italy

[5] Present address: Istituto Ortopedico Rizzoli, Bologna, Italy

Although the clinical use was first described over 70 years ago, the serum bactericidal titre (SBT) assay remains perhaps the only direct methods for measuring antimicrobial pharmacodynamics that takes into account the susceptibility of the pathogen, patient pharmacokinetics, drug protein binding, and synergistic or antagonistic interactions of antibiotic combinations [8]. However, SBT tests are rarely performed for patients with KPC-Kp bloodstream infection (BSI), and no data available regarding which combination of drugs routinely achieves the highest SBT against a variety of isolates. To address this knowledge gap, we performed SBT tests using sera collected from 15 patients undergoing routine therapeutic drug monitoring (TDM) for meropenem during treatment of KPC-Kp BSI. Instead of performing SBT assays for the patient's respective isolate, we tested each serum sample against 21 well-characterised *K. pneumoniae* strains with varying susceptibility to meropenem, colistin and tigecycline. We then analysed the relationship between meropenem serum concentration/MIC ratio for each sample-isolate combination and the observed SBT activity.

Materials and methods

Test isolates

Non-replicate *K. pneumoniae* blood culture isolates ($n = 21$) were collected between March 2011 and April 2012 from hospitalised patients. The genetic relationship between *K. pneumoniae* strains was investigated by multilocus sequence typing (MLST) and characterization of carbapenemase and ESBL genes was performed by PCR and DNA sequencing, as previously described [9].

Ethics statement

This non-interventional study was performed at S. Orsola-Malpighi Hospital, University of Bologna—a tertiary 1420-bed hospital with approximately 72,000 yearly inpatient admissions. Sera samples were collected from March 2013 to November 2014 as part of routine TDM of meropenem performed for patients with KPC-Kp infection. The study design was approved by the Institutional Research Committee in accordance with principles outlined in the Declaration of Helsinki.

Antimicrobial treatments

All patients had a confirmed diagnosis of KPC-Kp bacteraemia or intraabdominal infection. Per institutional protocol, meropenem was administered as a 2-g loading dose followed by 4–6 g per day administered by extended infusion (over 8 h) adjusted for patient renal function. Colistin was administered intravenously at a loading dose of 9 million international units (MIU) followed by a 9-MIU daily dose administered in two divided doses, titrated for renal function. Tigecycline was administered at standard intravenous doses (100-mg loading dose followed by 50 mg twice daily).

Serum sample collection

Meropenem trough (C_{min}) serum samples were collected immediately prior to the start of the next meropenem infusion between days 2 and 5 of therapy, and samples were split to perform SBT assays in parallel. Peak SBT activity was not specifically analysed due to the administration strategy of meropenem, and expected significant interpatient variability in active colistin formation rates (time to C_{max}) among patients with varying renal function.

Serum bactericidal assays

SBTs were performed according to CLSI-M21A guidelines with minor modifications [10]. Samples were not heat treated to inactivate protein prior to testing. Briefly, serum samples were twofold serially and incubated with bacteria inoculum of 5×10^5 CFU per mL at 37 °C for 24 h. Then, 20 μL of each well was subcultured to 180 μL of MacConkey broth and incubated at 37 °C. After 24 h, the serum bactericidal titre (SBT) results were determined by visual inspection and defined as the highest dilution of serum that completely inhibited sub-cultured bacterial growth.

Antibiotic concentration monitoring

Serum antibiotic concentrations were measured by means of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system composed of an HPLC (Prominence, Shimadzu, JAP) coupled with a triple quadrupole mass spectrometer (API-4000, AB-SCIEX, USA). Briefly, 0.1 mL of plasma was spiked with 30 μL of an internal standard solution (1 μg/mL deuterated compounds in methanol-H_2O 50–50% *v*/*v*) and, after 5-min rest, deproteinised/extracted by the addition of 0.3 mL of acetonitrile. After thoroughly vortexing for 1 min, the samples were centrifuged at 18000*g* for 10 min at 48 °C. A volume of 0.1 mL of the supernatant was diluted with 0.9 mL of a mixture of chromatographic phase A (see below) and 10 μL of the diluted supernatant was injected on column by the autosampler of the LC-MS/MS system.

Chromatography was performed in binary gradient mode at constant flow rate (0.5 mL/min) onto a phenyl-hexil column (Luna 100 × 2.0 mm, 5 μm, Phenomenex, USA). Mobile phase A was composed by 95% *v*/*v* of a solution of 1 mM

ammonium acetate, 1 mM acetic acid in LC-MS grade water and 5% *v/v* of LC-MS grade methanol. Mobile phase B was 95% *v/v* of methanol and 5% 1 mM ammonium acetate, 1 mM acetic acid in water. The binary concentration gradient was the following, according to phase B composition: 0% for 1 min, from 0 to 100% in 3 min, 100% for 1 min then 0% for 1 min. Total run time was 5 min. Compounds were ionised in positive electrospray ionization (ESI+) mode and detected by means of two specific mass-to-charge ratio (m/z) transitions for each compound in multiple reaction monitoring (MRM) mode.

Calibration was performed by means of a 7-point curve (linear fitting) run in triplicate. As controls, three pooled human plasmas of known concentration were processed at the beginning, in the middle and at the end of each analytical session. Intra- and inter-day precision (C.V.%) and accuracy (BIAS %) were all below 10% of assigned values. Lower limit of quantitation (LOQ) was better than 0.1 μg/mL for each compound.

Statistical analysis

The distribution of log-transformed reciprocal SBT titres produced by each combination regimen against ESBL and KPC-producing strains was compared by median and interquartile range (IQR) box-whisker plots and the ANOVA/ Kruskal-Wallis test with Tukey-Kramer test for pairwise comparisons. Continuous variables were compared by two-tailed Student's *T* test with Welch correction for unequal variances. Categorical ratios were analysed using the chi-square test. The relationship of SBT titres for each combination indexed to sample meropenem C_{min}/MIC ratio for the respective isolate were analysed using density plots and nonparametric density shading to assess the frequency of SBT titres above and below a predefined reciprocal titre of 8, and at meropenem C_{min} above or below the MIC.

Results

Patients population

We enrolled 15 patients during the study period. Thirteen patients had a KPC bacteraemia while two patients had KPC intra-abdominal infection treated with combination antimicrobial therapy and surgical source control (Table 1). The majority of patients (10/15) had a severe sepsis or a septic shock. The median (IQR) age was 60 (52–74), 69% of patients were male. Five out of 15 patients (33%) were solid organ recipients while 3/15 (20%) of them had a chronic renal failure. The median (IQR) estimated clearance of creatinine, calculated with Cockcroft-Gault equation at the day of serum collection, was 48 (31–86). The median (IQR) daily dose of meropenem was 4 (4–6) grams. Samples for SBT and TDM of antimicrobials were obtained after a median (IQR) of 3 (2–6) days of antimicrobial treatment obtaining a median (IQR) C_{min} of meropenem of 36 (21–47) mg/L.

Table 1 Demographics, comorbidities, clinical characteristics and outcome of 15 patients treated with three combination regimens

Characteristics	Total, $n = 15$
Age (years) (median (IQR))	60 (52–74)
Sex, male	6 (69)
Comorbidities, no. (%)	
Chronic renal failure	3 (20)
COPD	2 (13)
Liver cirrhosis	3 (20)
Solid malignancies	3 (20)
Solid organ transplant	5 (33)
Liver transplant	4 (26)
Kidney transplant	1 (7)
Charlson score (median (IQR))	4 (4–6)
Infection information, no. (%)	
Site of infection	
Primary bloodstream infection	3 (20)
Secondary bloodstream infection	10 (60)
Intra-abdominal source	6 (40)
Urinary tract source	1 (7)
Lower respiratory tract source	3 (20)
Intra-abdominal	2 (13)
Infection severity	
Sepsis	5 (33)
Severe sepsis	3 (20)
Septic shock	7 (47)
Treatment	
Meropenem + colistin + tigecycline	5 (33)
Meropenem + colistin	5 (33)
Meropenem + tigecycline	5 (33)
Outcome, no. (%)	
30-day mortality	2 (13)
In-hospital mortality	2 (13)
Relapse	1 (6)

Phenotypic and genotypic characterization of multidrug-resistant *K. pneumoniae*

Genotypic and phenotypic characteristics of *K. pneumoniae* isolates used in this study are shown in Table 2. Nearly all (6/7) of ESBL producers were susceptible to meropenem, while median MIC of ceftazidime was 128 mg/L (IQR 128–≥256 mg/L). Only one of the seven ESBL producers was resistant to colistin. Analysis of β-lactamase gene sequences revealed that all KPC-producing strains harboured bla_{KPC-3}. Analysis of *mgrB* sequence revealed that 85.7% (6/7) of colistin-resistant KPC producers were disrupted by insertion sequence (IS*5*-like) element. MLST showed that

Table 2 Phenotypic and genotypic characteristics of 21 multidrug-resistant *K. pneumoniae* strains

Isolate	Sequence type	MIC (mg/L)				Resistance mechanism		
		Meropenem	Colistin	Tigecycline	Ceftazidime	*blaKPC*	ESβL	Colistin
Kp1	512	32	0.064	2	≥256	bla_{KPC-3}	bla_{SHV}	NA
Kp2	512	128	0.032	2	≥256	bla_{KPC-3}	bla_{SHV}	NA
Kp3	258	32	0.064	2	≥256	bla_{KPC-3}	bla_{TEM}-bla_{SHV}	NA
Kp4	512	128	0.064	2	≥256	bla_{KPC-3}	bla_{SHV}	NA
Kp5	258	16	0.125	2	≥256	bla_{KPC-3}	bla_{TEM}-bla_{SHV}	NA
Kp6	512	128	0.064	2	≥256	bla_{KPC-3}	bla_{SHV}	NA
Kp7	512	64	0.064	4	≥256	bla_{KPC-3}	bla_{SHV}	NA
Kp8	258	16	16	2	≥256	bla_{KPC-3}	bla_{TEM}-bla_{SHV}	IS*5*-like element
Kp9	258	32	64	2	≥256	bla_{KPC-3}	bla_{TEM}-bla_{SHV}	IS*5*-like element
Kp10	258	16	32	2	≥256	bla_{KPC-3}	bla_{TEM}-bla_{SHV}	IS*5*-like element
Kp11	512	64	32	16	≥256	bla_{KPC-3}	bla_{TEM}-bla_{SHV}	IS*5*-like element
Kp12	258	32	64	2	≥256	bla_{KPC-3}	bla_{SHV}	IS*5*-like element
Kp13	258	16	32	2	≥256	bla_{KPC-3}	bla_{TEM}-bla_{SHV}	IS*5*-like element
Kp14	258	64	128	2	≥256	bla_{KPC-3}	bla_{SHV}	ND
Kp15	15	0.064	0.064	0.5	128	NA	bla_{TEM}-bla_{SHV} $bla_{CTX-M15}$	NA
Kp16	512	1	0.064	4	128	NA	bla_{SHV}	NA
Kp17	15	2	16	2	128	NA	bla_{SHV} $bla_{CTX-M15}$	ND
Kp18	147	0.125	0.064	2	256	NA	bla_{TEM}-bla_{SHV}	NA
Kp19	37	8	0.064	2	>256	NA	bla_{SHV} $bla_{CTX-M15}$	NA
Kp20	15	0.032	0.032	2	128	NA	bla_{SHV} $bla_{CTX-M15}$	NA
Kp21	37	2	0.25	2	256	NA	bla_{TEM}-bla_{SHV} $bla_{CTX-M15}$	NA

ND not detected, *NA* not assessed

KPC-producing *K. pneumoniae* belonged to the clonal complex 258 (CC258). Several sequence types (STs) were identified among ESBL producers including ST15 (3/7, 42.8%), ST37 (2/7, 28.5%), ST512 (1/7, 14.3%) and ST147 (1/7, 14.3%).

SBT of meropenem-based combination regimens

All treatment regimens exhibited bactericidal activity against ESBL-producing strains (Fig. 1a). Overall, sera collected from meropenem-colistin-treated patients produced significantly higher reciprocal SBTs (median 256, IQR 64–512) than patients treated with either meropenem-tigecycline (median 32, IQR 8–256; $P < 0.001$) or meropenem-colistin-tigecycline (median 64, IQR 32–128; $P < 0.05$).

Compared with ESBL-producing strains, reciprocal SBTs were significantly lower for all combinations against KPC-Kp (median 128, IQR 32–256 vs. 4, IQR 2–32; $P < 0.001$). However, sera collected from patients receiving meropenem plus colistin still achieved higher median reciprocal SBTs against KPC-Kp (24, IQR 8–64) compared with meropenem-tigecycline (1, IQR 1–2) or the three-drug combination (8, IQR 2–64; Fig. 1b). When analysed in relation to colistin susceptibility status of KPC-KP strains, colistin resistance was associated with a 4/5-fold decrease in the SBTs for colistin-based combinations (Supplementary Fig. S1).

Meropenem serum concentrations

When analysed in relation to the MIC of test isolates, only 2% of samples (2/105) were found to have a meropenem C_{min} below the MIC of ESBL-producing test isolates (median ratio 40.9, IQR 10.9–535) (Table 3**)**. In contrast for KPC-producing strains, meropenem concentrations were below the MIC in 120/210 (57.1%) of serum samples. Interestingly, a higher proportion of sera contained meropenem concentrations below the pathogen MIC when tested against colistin-susceptible (73/105) versus colistin-resistant 44% (47/105) strains ($P = 0.003$). The median meropenem C_{min}/MIC for KPC-producing strains was 0.44 (IQR 0.31–1.75) for colistin-susceptible versus 1.15 (IQR 0.52–2.25) for colistin-resistant strains.

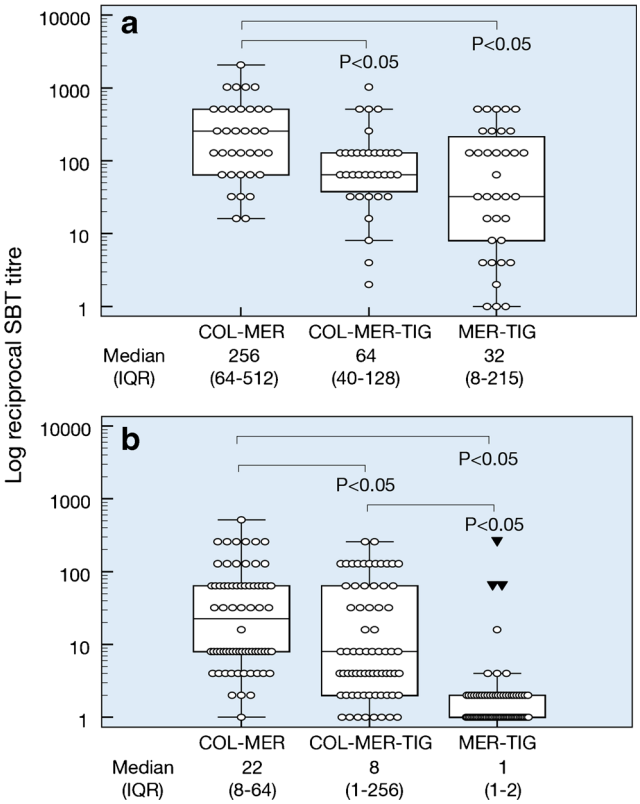

Fig. 1 Box and whisker plots of log-transformed reciprocal SBT troughs for *K. pneumoniae* isolates. (**a**) ESBL-positive *K. pneumoniae*, $n = 105$ samples. Median SBT 128 (IQR 32–256); (**b**) KPC-positive *K. pneumoniae*, $n = 210$ samples; median SBT 4 (IQR 2–32). Black triangles represent values that are above the upper value plus three times the IQR. Groups were compared by ANOVA with Tukey-Kramer test for pairwise comparisons

Pharmacodynamic analysis of antimicrobial combinations

Different patterns of SBT activity were observed for the three meropenem-based combinations when indexed to meropenem C_{min}/MIC (Fig. 2). A significantly higher proportion of samples from patients receiving the meropenem-colistin combination (Fig. 2a) produced reciprocal SBTs in excess of 8 compared with meropenem-colistin-tigecycline (Fig. 2b) or meropenem-tigecycline-treated patients (Fig. 2c), $P = 0.0001$ all comparisons. The difference in the proportion of samples achieving reciprocal titres greater than 8 was especially notable when analysis is restricted to samples with meropenem C_{min}/MIC ratios less than 1. In these samples, 60% meropenem-colistin samples (27/45 samples) versus 46% of meropenem-colistin-tigecycline (14/30) and 0% (0/47) of meropenem-tigecycline samples produced reciprocal SBTs above 8 ($P < 0.01$ all comparisons).

Discussion

Clinicians have few tests beyond the MIC to guide combination antimicrobial therapy for multidrug-resistant pathogens. The revival and clinical validation of SBT testing for multidrug-resistant (MDR) Gram-negative pathogens may be the most straightforward for identifying patients at risk for treatment failure due to suboptimal antimicrobial regimens. However, the distribution of typical SBTs for common combination regimens used in the treatment of MDR Gram-negative bacteria is not well defined. To address this need, we compared the serum bactericidal activity for common meropenem combination regimens against ESBL and KPC-producing *K. pneumoniae* strains representing common genotypes and phenotypes of extended-spectrum beta-lactamase (ESBL) and KPC producers isolated from patients with BSI in our region.

Our analysis demonstrated that sera collected from patients receiving a combination of meropenem-colistin achieved significantly higher serum bactericidal titres against a collection of not only ESBL producers, but also against KPC-producing *K. pneumoniae*, including both colistin-sensitive and resistant strains. Notably, patients who received tigecycline as part of their combination regimen had lower SBTs irrespective of meropenem or colistin sensitivity, even when used in combination with both drugs as a part of a three-drug regimen. This raises the question of whether tigecycline antagonises activity of either meropenem or colistin, or both, and if antagonism that is observed depends on full or residual activity of the respective drugs during the treatment of MDR strains.

Published literature cannot answer that question as pharmacodynamic interactions of tigecycline with meropenem or colistin for KPC-Kp provide mixed answers. Both in vitro and

Table 3 Trough plasma level (C_{min}) of meropenem measured in serum samples collected from 15 patients treated with different combination therapies

Therapy	No. of patients	Median daily dose, grams (IQR)	Median estimated glomerular filtrate rate, mL/min[a] (IQR)	Median meropenem concentration, mg/L (IQR)
Meropenem-tigecycline	5	4.0 (4.0–4.0)	63.5 (44.4–77.8)	28.0 (21.3–33.7)
Meropenem-colistin	5	4.0 (3.5–6.0)	56.0 (35.4–94.5)	21.7 (8.4–81.7)
Meropenem-colistin-tigecycline	5	4.0 (3.5–6.0)	23.8 (14.5–80.5)	46.2 (44.9–46.9)

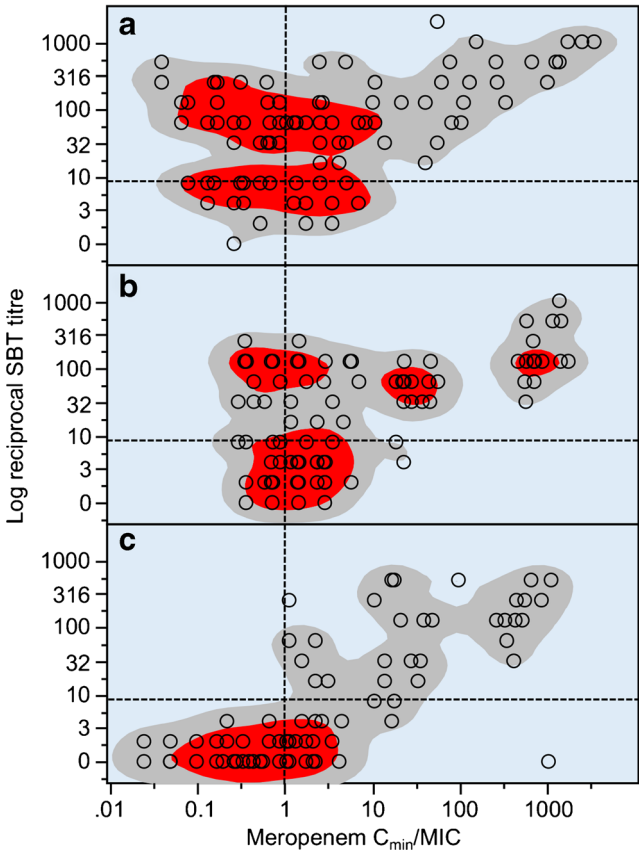

Fig. 2 Density plots of observed SBTs by meropenem combination indexed to meropenem C_{min}/MIC ratio. The vertical dashed line separates sera samples with meropenem C_{min}/MIC ratios > 1. Horizontal dashed lines indicate a 1:8 SBT titre threshold. Nonparametric density shading indicates area that contains 90% of data points (light grey) and 50% of data points (dark grey). When tested against the isolate bank, sera samples collected from patients receiving meropenem-colistin (a) produced significantly higher SBT titres than either meropenem-tigecycline-colistin, $P = 0.001$ (b); or meropenem-tigecycline $P < 0.0001$ (c). For sample-isolate combinations with a meropenem C_{min}/MIC concentrations of < 1 (left of dotted line), the frequency of SBT titres exceeding 1:8 (left upper quadrant) was significantly lower in meropenem-tigecycline-colistin, $P = 0.04$; and meropenem-tigecycline group, $P < 0.01$ (c) versus meropenem-colistin

in vivo studies have suggested that a combination of meropenem plus tigecycline is antagonist indifferent or at least not consistently synergistic [9, 12–15]. In this context, bacteriostatic antibiotics are known to potentially antagonise bactericidal activity of many antibiotic in vitro [11]. On the other hand, observational studies have reported that meropenem-based combinations that frequently include tigecycline in combination with colistin are associated with improved survival from KPC-Kp BSIs, especially when meropenem MICs are less than 8 mg/L [2, 3]. However, these case-control studies can be biased by comparisons of combination therapy against monotherapy regimens that are widely recognised to be inferior for BSIs (i.e. aminoglycoside, tigecycline for BSI, or lower dose colistin monotherapy). Moreover, the numerous combinations and medically complex patient populations analysed in these observational studies limit statistical power required to differentiate which combination regimen is superior [16, 17]. This highlights another potential role for SBT assay as a possible surrogate endpoint for future prospective observational or randomised controlled trials evaluating antimicrobial combination therapy of KPC-Kp infection.

A limitation of the study is that concentrations of polymyxins and tigecycline are unknown in the samples. Therefore, it is possible that the low SBTs observed in tigecycline-treated patients may be related to an unknown pharmacokinetic interaction or lower rates/concentration of active colistin formation rather than an antagonistic interaction. However, lower meropenem exposures could not explain the lower SBTs observed in patients receiving tigecycline in combination with meropenem and colistin, and median concentration was higher (46 mg/L, IQR 44.9–46.9) in patients receiving the three-drug versus meropenem-tigecycline (28, IQR 21.3–33.70) or meropenem-colistin (21.7, IQR 8.4–81.7). At the same time, it is possible that antagonism effect of tigecycline to the double combination of meropenem and colistin was related to the low serum tigecycline levels [18, 19], thus resembling a concentration-dependent interaction [20].

In conclusion, we found that sera collected from patients receiving meropenem-colistin achieved higher SBTs against ESBL and KPC-producing *K. pneumoniae* isolates compared with sera from meropenem-tigecycline or meropenem-colistin-tigecycline-treated patients. The addition of tigecycline to meropenem-based combinations was associated with a reduction in bactericidal activity, particularly when meropenem concentrations in the sample were below the MIC. Further studies are required to establish the relationship of SBT activity and the probability of clinical response or survival from *K. pneumoniae* BSI, and whether antagonistic effects are clinically relevant.

Acknowledgements We would like to thank the patients, nurses, and medical residents and technicians who donated their time and energy to complete this study.

Funding This work was partially supported by the Italian Ministry of Health (Ricerca Finalizzata, Giovani Ricercatori, GR-2018-12367572) and the Emilia-Romagna region.

Compliance with ethical standards

Conflict of interest Russell Lewis has received research funding support from Gilead Inc., Pfizer, and served on speakers or advisory boards for Merck & Co. Inc., and Gilead.

All others declare no interests.

Ethical approval The study design was approved by the Institutional Research Committee.

References

1. Pitout JDD, Nordmann P, Poirel L (2015) Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother 59:5873–5884. https://doi.org/10.1128/AAC.01019-15
2. Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, S panu T, Ambretti S, Ginocchio F, Cristini F, Losito AR, Tedeschi S, Cauda R, Bassetti M (2012) Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase–producing *K. pneumoniae*: importance of combination therapy. Clin Infect Dis 55:943–950. https://doi.org/10.1093/cid/cis588
3. Daikos GL, Tsaousi S, Tzouvelekis LS, Anyfantis I, Psichogiou M, Argyropoulou A, Stefanou I, Sypsa V, Miriagou V, Nepka M, Georgiadou S, Markogiannakis A, Goukos D, Skoutelis A (2014) Carbapenemase-producing *Klebsiella pneumoniae* bloodstream infections: lowering mortality by antibiotic combination schemes and the role of carbapenems. Antimicrob Agents Chemother 58:2322–2328. https://doi.org/10.1128/AAC.02166-13
4. Falcone M, Russo A, Iacovelli A, Restuccia G, Ceccarelli G, Giordano A, Farcomeni A, Morelli A, Venditti M (2016) Predictors of outcome in ICU patients with septic shock caused by Klebsiella pneumoniae carbapenemase-producing *K. pneumoniae*. Clin Microbiol Infect 22:444–450. https://doi.org/10.1016/j.cmi.2016.01.016
5. Giannella M, Trecarichi EM, Giacobbe DR, De Rosa FG, Bassetti M, Bartoloni A, Bartoletti M, Losito AR, Del Bono V, Corcione S, Tedeschi S, Raffaelli F, Saffioti C, Spanu T, Rossolini GM, Marchese A, Ambretti S, Cauda R, Viscoli C, Lewis RE, Viale P, Tumbarello M (2018) Italian study group on resistant infections of the Società Italiana Terapia Antinfettiva (ISGRI-SITA). Effect of combination therapy containing a high-dose carbapenem on mortality in patients with carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection. Int J Antimicrob Agents 51:244–248. https://doi.org/10.1016/j.ijantimicag.2017.08.019
6. Tumbarello M, Trecarichi EM, Corona A, De Rosa FG, Bassetti M, Mussini C, Menichetti F, Viscoli C, Campoli C, Venditti M, De Gasperi A, Mularoni A, Tascini C, Parruti G, Pallotto C, Sica S, Concia E, Cultrera R, De Pascale G, Capone A, Antinori S, Corcione S, Righi E, Losito AR, Digaetano M, Amadori F, Giacobbe DR, Ceccarelli G, Mazza E, Raffaelli F, Spanu T, Cauda R, Viale P (2019) Efficacy of ceftazidime-avibactam salvage therapy in patients with infections caused by *Klebsiella pneumoniae* carbapenemase-producing K. pneumoniae. Clin Infect Dis 68:355–364. https://doi.org/10.1093/cid/ciy492
7. Watkins RR, Deresinski S (2015) Is combination therapy for carbapenem-resistant *Klebsiella pneumoniae* the new standard of care? Expert Rev Anti-Infect Ther 13:405–407. https://doi.org/10.1586/14787210.2015.1018825
8. Stratton CW (1988) Serum bactericidal test. Clin Microbiol Rev 1:19–26
9. Gaibani P, Lombardo D, Lewis RE, Mercuri M, Bonora S, Landini MP, Ambretti S (2014) In vitro activity and post-antibiotic effects of colistin in combination with other antimicrobials against colistin-resistant KPC-producing *Klebsiella pneumoniae* bloodstream isolates. J Antimicrob Chemother 69:1856–1865. https://doi.org/10.1093/jac/dku065
10. Clinical Laboratory Standards Insititute (1999) M21A-methodology for the serum bactericidal test: approved guideline. Clinical Laboratory Standards Institute, Wayne
11. Ocampo PS, Lazar V, Papp B, Arnoldini M, Abel zur Wiesch P, Busa-Fekete R, Fekete G, Pál C, Ackermann M, Bonhoeffer S (2014) Antagonism between bacteriostatic and bactericidal antibiotics is prevalent. Antimicrob Agents Chemother 58:4573–4582
12. Bi S, Yao X, Huang C, Zheng X, Xuan T, Sheng J, Xu K, Zheng B, Yang Q (2019) Antagonistic effect between tigecycline and meropenem: successful management of KPC-producing *Klebsiella pneumoniae* infection. Infection. https://doi.org/10.1007/s15010-019-01274-w
13. Pournaras S, Vrioni G, Neou E, Dendrinos J, Dimitroulia E, Poulou A, Tsakris A (2011) Activity of tigecycline alone and in combination with colistin and meropenem against Klebsiella pneumoniae carbapenemase (KPC)-producing *Enterobacteriaceae* strains by time–kill assay. Int J Antimicrob Agents 37:244–247. https://doi.org/10.1016/j.ijantimicag.2010.10.031
14. Wiskirchen DE, Koomanachai P, Nicasio AM, Nicolau DP, Kuti JL (2011) In vitro pharmacodynamics of simulated pulmonary exposures of tigecycline alone and in combination against *Klebsiella pneumoniae* isolates producing a KPC carbapenemase. Antimicrob Agents Chemother 55:1420–1427. https://doi.org/10.1128/AAC.01253-10
15. Michail G, Labrou M, Pitiriga V, Manousaka S, Sakellaridis N, Tsakris A, Pournaras S (2013) Activity of tigecycline combinations with colistin, meropenem, rifampin and gentamicin against KPC-producing *Enterobacteriaceae* in a murine thigh model. Antimicrob Agents Chemother 57:6028–6033. https://doi.org/10.1128/AAC.00891-13
16. Falagas ME, Vardakas KZ, Tsiveriotis KP, Triarides NA, Tansarli GS (2014) Effectiveness and safety of high-dose tigecycline-containing regimens for the treatment of severe bacterial infections. Int J Antimicrob Agents 44:1–7. https://doi.org/10.1016/j.ijantimicag.2014.01.006
17. Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, Pascual A (2018) Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and CARBAPENEMASE-producing Enterobacteriaceae. Clin Microbiol Rev 31:e00079–e00017. https://doi.org/10.1128/CMR.00079-17
18. Xie J, Roberts JA, Alobaid AS, Roger C, Wang Y, Yang Q, Sun J, Dong H, Wang X, Xing J, Lipman J, Dong Y (2017) Population Pharmacokinetics of Tigecycline in Critically Ill Patients with Severe Infections. Antimicrob Agents Chemother 61:e00345–e00317. https://doi.org/10.1128/AAC.00345-17
19. Dixit D, Madduri RP, Sharma R (2014) The role of tigecycline in the treatment of infections in light of the new black box warning. Expert Rev Anti-Infect Ther 12:397–400. https://doi.org/10.1586/14787210.2014.894882
20. Meletiadis J, Stergiopoulou T, O'Shaughnessy EM, Peter J, Walsh TJ (2007) Concentration-dependent synergy and antagonism within a triple antifungal drug combination against Aspergillus species: analysis by a new response surface model. Antimicrob Agents Chemother 51:2053–2064