Journal of Global Antimicrobial Resistance 17 (2019) 250–259

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

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

Review

Clinical relevance of in vitro synergistic activity of antibiotics for multidrug-resistant Gram-negative infections: A systematic review

Konstantinos Z. Vardakas[a,b], Florentia Athanassaki[a], Vassiliki Pitiriga[c], Matthew E. Falagas[a,b,d,*]

[a] Alfa Institute of Biomedical Sciences (AIBS), Athens, Greece
[b] Department of Medicine, Henry Dunant Hospital Center, Athens, Greece
[c] Department of Microbiology, Henry Dunant Hospital Center, Athens, Greece
[d] Tufts University School of Medicine, Boston, MA, USA

ARTICLE INFO

Article history:
Received 27 July 2018
Accepted 9 January 2019
Available online 15 January 2019

Keywords:
Synergy
Gram-negative bacteria
Multidrug-resistant
Etest
Chequerboard
Time–kill

ABSTRACT

Objectives: The aim of this review was to investigate the outcomes of patients infected with multidrug-resistant (MDR) or extensively drug-resistant (XDR) Gram-negative bacteria following synergy-guided antibiotic combination therapy (SGACT).
Methods: A systematic review of PubMed and Scopus databases was performed. Published studies of any design reporting outcomes of patients with MDR Gram-negative bacteria treated with SGACT were included. Two reviewers independently assessed the relevancy and quality of the retrieved articles and extracted the available data.
Results: Nineteen reports (530 patients) were included. Eleven case reports/series described 26 cases of systemic infection due to MDR Gram-negative bacteria treated with SGACT. Five deaths were reported, two of which were attributed to the infection. Six studies (including one randomised controlled trial) provided comparative data for patients treated with SGACT and those treated with unguided combination therapy (UCT) or active monotherapy. In the pooled analysis of unadjusted data from these studies (504 patients), there was no difference between SGACT and UCT or monotherapy (OR = 0.47, 95% CI 0.21–1.04; I^2 = 52%). Analysis of adjusted data showed that SGACT was significantly associated with survival (OR = 0.44, 95% CI 0.20–0.98; I^2 = 54%).
Conclusion: These limited but promising findings warrant further investigation of SGACT in the outcome of patients with MDR Gram-negative infections in well-designed trials.

Contents

* Corresponding author. Present address: Alfa Institute of Biomedical Sciences (AIBS), 9 Neapoleos Street, 151 23 Marousi, Athens, Greece.
E-mail address: m.falagas@aibs.gr (M.E. Falagas).

https://doi.org/10.1016/j.jgar.2019.01.004

1. Introduction

Development of resistance to antibiotics is inevitable. Multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacterial infections have been associated with worse patient outcomes [1,2]. Concurrently, cases of infection due to isolates resistant to last-line antibiotics have been increasingly reported [3–5]. Although several new antibiotics have been approved or are under development, only a few have been shown to be variably active against MDR Gram-negative bacteria [6]. In addition, since most of them share similar mechanisms of action to already available antibiotics, the probability of development of resistance to the new compounds is likely if prolonged or repeated administration is required [7].

Combination antibiotic therapy has been proposed as a means to improve the outcomes of such patients. The number of observational studies suggesting better outcomes with combination therapy is increasing [8–12]. On the other hand, systematic reviews and meta-analyses as well as randomised controlled trials (RCTs) are less supportive [13–16]. More RCTs are currently under way (ClinicalTrials.gov ID NCT01732250 and NCT02134106). Several regimens have been employed in these studies for the treatment of infections due to various bacteria, with different susceptibility profiles and lack of microbiologically confirmed in vitro synergy.

Synergy may occur when antibiotics are combined, but this is neither universal nor predictable; interactions ranging from antagonism to >80% synergy have been reported in the literature [17,18]. Synergy depends on the tested antibiotics (classes, number and concentration), the bacterial species and its susceptibility profile (including the mechanism of resistance) as well as the test employed to evaluate it [19]. Furthermore, favourable in vitro reactions may not necessarily translate to improved clinical outcomes as the concentrations required for synergy may not be achievable in specific tissues or even in serum. This systematic review aimed to evaluate the clinical relevance of in vitro antibiotic synergy by summarising the available evidence from published clinical studies.

2. Methods

This review was registered at the PROSPERO database (No. CRD42017071704) and was reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The PubMed and Scopus databases were searched up to August 2017 for studies on patients with infections due to MDR or XDR Gram-negative bacteria who received treatment with a combination regimen with microbiologically proven in vitro synergy between the administered antibiotics [20]. The search terms used were [(resistance OR resistant) AND (synergy OR synergism) AND combination] AND (mortality OR clinical effectiveness OR clinical outcome). Only studies written in English were considered eligible. Reference lists of selected studies and relevant reviews were hand-searched. Conference abstracts were not searched.

Studies of any design (from case reports to RCTs) were eligible regardless of whether in vitro synergy testing preceded or followed antibiotic administration. However, studies evaluating in vitro synergy to guide informed, empirical, future treatment decisions were ineligible. The study was excluded if clinical data were missing. Any method used to study in vitro synergy (Etest, chequerboard, time–kill assay, etc.) could be employed. In vitro studies or animal model studies were ineligible. In case of comparative studies, the control group could receive either unguided combination therapy (UCT) or active monotherapy.

Synergy-guided antibiotic combination therapy (SGACT) was defined as combination of antibiotics guided (prospectively) or confirmed (retrospectively) by in vitro synergy testing. UCT was defined as any regimen (empirical or definitive) prescribed to patients without in vitro synergy tests. The definitions for synergy as well as clinical and microbiological effectiveness were based on those provided in the individual studies. In general, for the time–kill assays, synergism was defined as a >2-$\log_{10}$ decrease in colony count compared with the individual antibiotics. In the chequerboard and Etest methods, complete synergism of a combination treatment of antibiotics was defined as a fractional inhibitory concentration index (FICI) of $\leq$0.5 [21]. The outcomes were all-cause or infection-related mortality (at any given time point), clinical cure, microbiological cure or pathogen eradication, and development of resistance.

The meta-analysis was performed using Review Manager (RevMan) for Windows v.5.3 (The Nordic Cochrane Centre, the Cochrane Collaboration, Copenhagen, Denmark). Regarding unadjusted data, pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using a random-effects model. Statistical heterogeneity among studies was assessed by χ^2 test, with $P < 0.10$ indicating significant heterogeneity; I^2 was used to assess the degree of heterogeneity. Adjusted data were analysed using the inverse variance method of RevMan. If the adjusted effect size was not available and non-significant associations were reported, an adjusted effect size of 1 was imputed, and the standard error of the unadjusted analysis was used as the measure of dispersion [22]. Variables were categorised as having been adjusted for confounders if they were included in the multivariate model or if they had been included in a stepwise selection procedure (e.g. bivariate testing) but had not ended up in the final model.

3. Results

Fig. 1 shows the study selection process. A total of 19 reports fulfilled the eligibility criteria. Most of them (11) were case reports and case series. Patients requiring intensive care unit (ICU) services were mainly enrolled. *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most commonly isolated bacteria. Polymyxins were the main antibiotics included in the combination regimens; the antibiotics used in combination were mainly carbapenems and rifampicin, but several others were also used in variable proportions. Etest, chequerboard and time–kill assays were employed for synergy testing.

3.1. Case reports and series

Table 1 summarises the characteristics of 26 patients identified in case reports/series [23–33]. Complete demographic data were not available for all of the patients. History included various malignancies, surgical operations, congenital syndromes and metabolic diseases (diabetes). Bacteraemia was the most common type of infection (13 patients), followed by soft tissue or bone infections (7 patients). *Pseudomonas aeruginosa* ($n = 14$), followed by Enterobacteriaceae ($n = 7$) and *A. baumannii* ($n = 5$) were the isolated bacteria. Data on previous hospitalisation and antibiotic administration were commonly missing. Treatment failure of a previously administered regimen was the most common reason for in vitro microbiological studies (10/26; 38%). Time–kill assays and chequerboard method, often combined, were primarily performed. Colistin-based and double-carbapenem combinations were the most commonly employed regimens. Two and three patients died (mortality 19.2%) due to infection or to a complication soon after initiation of SGACT, respectively.

Eighteen patients were enrolled in a retrospective study evaluating the synergistic activity of vancomycin and colistin for

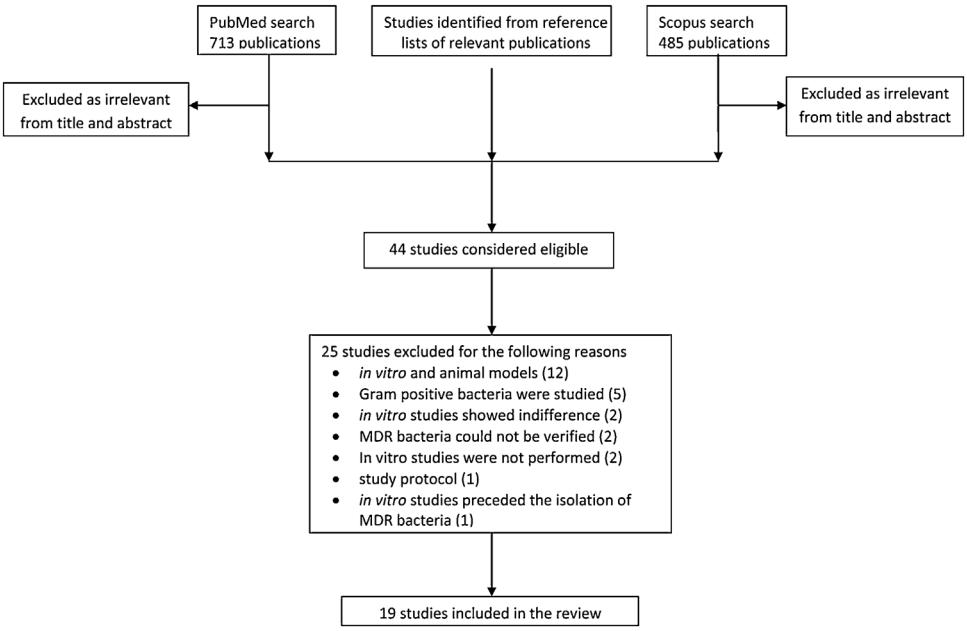

Fig. 1. Flow diagram of the study selection process. MDR, multidrug-resistant.

XDR *A. baumannii* [34]. Synergy was assessed by chequerboard and time–kill studies. Eight isolates demonstrated synergy between colistin and vancomycin (one colistin-susceptible and seven colistin-resistant) by the chequerboard method and all isolates (100%) by the time–kill assay, even at very low concentrations [0.125× the minimum inhibitory concentration (MIC)]. Mortality among patients receiving the microbiologically proven synergistic combination of vancomycin–colistin was 71.4% (5/7) [34].

In a recent case series, the effectiveness of the double-carbapenem regimen (ertapenem plus high-dose extended-infusion meropenem) was evaluated for the treatment of patients with carbapenem-resistant *K. pneumoniae* infections, including those with bacteraemia, who were not eligible for colistin treatment owing to nephrotoxicity concerns or colistin resistance [35]. Synergy was observed by chequerboard method (11/14) and time–kill assays (initial inoculum 5×10^5 CFU/mL; 12/14). Treatment was administered for a median of 15 days and clinical success was observed in 80% (12/15); mortality was low (1/15; 6.7%). Two patients suffered recurrence of the infection in the following 2 months. Nausea, hypernatremia and seizures were the observed adverse events [35].

3.2. Comparative studies

Table 2 summarises the characteristics of comparative studies. The outcome of 291 patients with XDR Gram-negative infection following treatment with polymyxins [polymyxin B (81%)] was evaluated in a retrospective multicentre study [36]. Patients were divided into three groups: those who received SGACT following request by an infectious diseases specialist ($n = 30$); those who received UCT ($n = 203$); and those receiving polymyxin monotherapy ($n = 58$). SGACT was further augmented by individualised pharmacokinetic/pharmacodynamic (PK/PD) recommendations by an infectious diseases pharmacist. Synergy was determined by the multiple-combination bactericidal test; outcomes of the synergistic tests were not reported. All isolates were susceptible to polymyxins. *Acinetobacter baumannii* was mainly isolated from patients in the UCT and monotherapy groups, and *P. aeruginosa* and *K. pneumoniae* from the SGACT group. Polymyxins were administered in combination with carbapenems (62%), followed by fluoroquinolones, aminoglycosides, aztreonam and rifampicin at similar proportions in both combination groups. More patients in the UCT group received a tigecycline combination. Patients in the SGACT group were more severely ill as judged by the Charlson comorbidity index and Acute Physiology and Chronic Health Evaluation (APACHE) II score, had more bacteraemic infections, and received polymyxin therapy sooner, for a longer time and at higher doses than the other two groups. In the multivariate analysis, monotherapy [adjusted odds ratio (aOR) = 8.49, 95% CI 1.56–46.05] and UCT (aOR = 5.75, 95% CI 1.25–25.73) were independent predictors of infection-related mortality. The Charlson comorbidity index and APACHE II score were also associated with mortality [36].

All cases managed with SGACT (39 patients) by the same group during the same study period (2009–2014) were included in a second publication [37]. *Pseudomonas aeruginosa* ($n = 20$), *A. baumannii* ($n = 13$) and *K. pneumoniae* ($n = 6$) were the implicated pathogens. A change in the empirical antibiotic regimen was recommended in 14/39 cases (36%) for being non-bactericidal in vitro and in 4/21 cases (19%) because an alternative regimen with better PK/PD target attainment could be achieved. In 7/39 cases (18%) the number of antibiotics used in the empirical combination regimen was reduced. Polymyxin B-based, two- or three-drug combination regimens were most commonly prescribed. Although a median delay of 4 days (range 2–47 days) in the time between culture result and request for synergy testing was observed, high clinical response (82.1%) and microbiological eradication (78.6%) were reported, resulting in a 30-day infection-related mortality of 15.4% [37].

In a prospective, single-centre study, intravenous (i.v.) tigecycline combined with extended-infusion imipenem was compared with imipenem/sulbactam for the treatment of patients with bacteraemic ventilator-associated pneumonia (VAP) due to OXA-51-producing XDR *A. baumannii* [38]. The tigecycline–imipenem combination was administered when previous treatment with imipenem/sulbactam had failed. The tigecycline and imipenem MICs for all isolates were ≥ 2 mg/L and >8 mg/L, respectively. Synergy between imipenem and tigecycline was evaluated by chequerboard microdilution and time–kill studies; synergy tests for the combination imipenem/sulbactam were not performed. All

Table 1
Case reports evaluating the clinical effectiveness of treatment with a combination of antibiotics (Abx) with confirmed in vitro synergy.

Case	Reference	Age/ sex	History	Infection/ colonisation	Isolated organism	Days in hospital until isolation of MDR strain	Prior Abx use (days)	Reason for synergistic study of Abx combination	Synergy testing method	Synergy testing results[a,b]	Abx combination used after synergy test (days)	Outcome of infection
1	Piedra-Carrasco et al. [30]	36/F	Myeloid sarcoma	UTI, bacteraemia	XDR (KPC) *Klebsiella pneumoniae*	4	TZP (4d) TEC (4d) AMK (2d) TGC (2d)	Treatment failure	Etest, chequerboard, time–kill assay (initial inoculum 6×10^5 CFU/mL)	Synergy, bactericidal at 6 h, no re-growth 24 h	ETP + MEM (14d)	Cured
2	Oliva et al. [27]	61/F	Renal transplantation[c]	SSI, NP, bacteraemia	KPC *Escherichia coli*	22	MEM SXT LVX (ca. 12d)	High risk for nephrotoxicity with COL/AMK	Time–kill assay (initial inoculum 5×10^5 CFU/mL)	Bactericidal activity, no re-growth at 24 h	ETP + MEM (9d)	Improvement[d]
3	Oliva et al. [29]	75/F	Recent hip joint replacement	UTI, CR-BSI	PDR *K. pneumoniae*	15	RIF DAP (ca. 15d)	Definitive treatment of PDR infection	Time–kill assay (initial inoculum 5×10^5 CFU/mL)	Synergy, bactericidal, no re-growth at 24 h	ETP + MEM (21d) + COL (7d)	Cured[e]
4	Ceccarelli et al. [24]	3 mo/ NA[f]	Pierre–Robin syndrome	BSI (septic shock)	XDR *Acinetobacter baumannii*	NA	COL RIF (5d)	Treatment failure	Time–kill assay, chequerboard	Synergy, bactericidal, no re-growth at 24 h	VAN + COL + MEM (12d)	Cured[g,h] Late relapse (UTI)
5	Oliva et al. [28]	NA	Aortic endoprosthesis placement	BSI	PDR *K. pneumoniae*	NA	FOS + TGC (NA)	Treatment failure	Time–kill assay (initial inoculum 5×10^5 CFU/mL)	Synergy, bactericidal, maintained at 24 h	MEM + ETP (21d)	Cured
6	Oliva et al. [28]	NA	Aortobiiliac graft placement	BSI	PDR *K. pneumoniae*	2	MEM DAP (NA)	Treatment failure	Time–kill assay (initial inoculum 5×10^5 CFU/mL)	Synergy, bactericidal, maintained at 24 h	ETP + MEM (4d)	Improvement[i]
7	Oliva et al. [28]	NA	Arterial embolisation due to renal haematoma	BSI	PDR *K. pneumoniae*	1	TZP MEM TGC (NA)	Inadequate response	Time–kill assay (initial inoculum 5×10^5 CFU/mL)	Synergy, bactericidal, maintained at 24 h	MEM + ETP (24d)	Cured
8	Nakamura et al. [26]	NA	Aplastic anaemia	Pneumonia	MDR MBL (+) *Pseudomonas aeruginosa*	NA	NA	Evaluation of the effectiveness of Abx combination	BC plate	Synergistic activity[j]	ATM + AMK (11d)	Cured
9	Nakamura et al. [26]	NA	None	Vertebral osteomyelitis	MDR MBL (+) *P. aeruginosa*	NA	NA	Evaluation of the effectiveness of Abx combination method	BC plate	Synergistic activity[j]	ATM + AMK (16w)	Cured
10	Nakamura et al. [26]	NA	None	Pyelonephritis	MDR MBL (+) *P. aeruginosa*	NA	NA	Evaluation of the effectiveness of Abx combination	BC plate	Synergistic activity[j]	PIP + AMK (14d)	Cured
11	Nakamura et al. [26]	NA	Leukaemia	Prostatitis	MDR MBL (+) *P. aeruginosa*	NA	NA	Evaluation of the effectiveness of Abx combination	BC plate	Synergistic activity[j]	ATM + COL (8w)	Cured
12	Nakamura et al. [26]	NA	Leukaemia	Septonasal abscess	MDR MBL (+) *P. aeruginosa*	NA	NA	Evaluation of the effectiveness of Abx combination	BC plate	Synergistic activity[j]	ATM + ARB[k] (14d)	Cured
13	Nakamura et al. [26]	NA	Ovarian cancer	CA-UTI	MDR MBL (+) *P. aeruginosa*	NA	NA	Evaluation of the effectiveness of Abx combination	BC plate	Synergistic activity[j]	ATM + CIP (2d)	Death
14	Ceccarelli et al. [23]	65/ M	Cerebral haemorrhage and hydrocephalus	BSI, pneumonia	KPC *K. pneumoniae*	47	COL, MEM, RIF (6d); COL, FOS (5d)	Treatment failure	Time–kill assay (initial inoculum 5×10^5 CFU/mL)	Synergy maintained at 24 h	ETP + DOR (28d)	Cured
15	Lee et al. [25]	67/F	Lymphoma	BSI, VAP	MDR *A. baumannii*	23	MEM LVX (3d)	Treatment failure	Etest	Additive (FICI = 0.56)	MEM + SUL (14d)	Cured[l]
16	Lee et al. [25]	39/ M	Trauma, hypovolaemic shock	BSI, VAP	MDR *A. baumannii*	10	ATM, VAN (3d)	Treatment failure	Etest	Additive (FICI = 0.56)	MEM + SUL (10d)	Cured
17	Lee et al. [25]	40/F	Hyperosmolar coma, septic shock, *Staphylococcus aureus* fasciitis, ARF	CR-BSI	MDR *A. baumannii*	NA	NA	NA	Etest	Additive (FICI = 0.75)	IPM + SUL (NA)	Cured
18	Lee et al. [25]	41/F	Postpartum hypovolaemic shock	CR-BSI	MDR *A. baumannii*	6	ATM, VAN (NA)	Treatment failure	Etest	Additive (FICI = 0.56)	IPM + SUL (NA)	Cured
19	Tascini et al.[m] [32]	88/F	Diabetes 2	DFI with osteomyelitis	MDR *P. aeruginosa*	NA	NA	NA	Chequerboard	Synergy[n]	COL + RIF (50d)	Cured
20	Tascini et al.[m] [32]	53/ M	Diabetes 2	DFI	MDR *P. aeruginosa*	NA	NA	NA	Chequerboard	Synergy[n]	COL + RIF (70d)	Unchanged

Table 1 (*Continued*)

Case	Reference	Age/ sex	History	Infection/ colonisation	Isolated organism	Days in hospital until isolation of MDR strain	Prior Abx use (days)	Reason for synergistic study of Abx combination	Synergy testing method	Synergy testing results[a,b]	Abx combination used after synergy test (days)	Outcome of infection
21	Tascini et al.[m] [32]	69/F	Diabetes 1	DFI	MDR *P. aeruginosa*	NA	NA	NA	Chequerboard	Synergy[n]	COL + RIF (40d)	Death
22	Tascini et al. [32]	67/ M	Diabetes 2	DFI with osteomyelitis	MDR *P. aeruginosa*	NA	CAZ (40d); IPM (15d); FEP (30d)	Treatment failure	Chequerboard	COL + RIF, FICI = 0.36 COL + RIF + IPM[o], FICI = 0.25	COL + RIF + IPM (42d)	Cured
23	Tascini et al. [31]	40/ M	AIDS, wasting syndrome, cerebral toxoplasmosis, MDR *P. aeruginosa* urethritis	CR-BSI	MDR *P. aeruginosa*	NA	CAZ AMK (ca. 10d)	Treatment failure	Chequerboard Time–kill assay (initial inoculum NA)	FICI = 0.31 Time–kill assay Bactericidal, prolonged for 12 h	COL (1 MIU q12 h) + RIF (600 mg q24 h) (14d)	Cured
24	Tascini et al. [31]	45/ M	CVID	Bronchitis and pneumonia	MDR *P. aeruginosa*	NA	NA	Evaluation of microbiological and clinical activity of combination treatment	Chequerboard Time–kill assay (initial inoculum NA)	FICI = 0.56 Time–kill assay Bactericidal, prolonged for 12 h	COL + RIF (14d) INH COL[m]	Cured
25	Tascini et al. [31]	40/ NA	Head trauma	VAP	MDR *P. aeruginosa*	NA	MEM (NA)	Evaluation of microbiological and clinical activity of combination treatment	Chequerboard	FICI = 0.56, partially synergistic	COL + RIF (10d) INH COL[m]	Improvement
26	Tascini et al. [31]	61/ NA	Non-Hodgkin's lymphoma	Multiple abscesses of lungs, perineum and gluteus	MDR *P. aeruginosa*	NA	NA	Evaluation of microbiological and clinical activity of combination treatment	Chequerboard	FICI = 0.53, partially synergistic	COL + RIF + AMK (28d)	Cured

AIDS, acquired immune deficiency syndrome; ARF, acute renal failure; BC plate, breakpoint chequerboard plate; BSI, bloodstream infection; CA-UTI, catheter-associated urinary tract infection; CR-BSI, catheter-related bloodstream infection; CVID, common variable immune deficiency; d, days; DFI, diabetic foot infection; FICI, fractional inhibitory concentration index; MBL, metallo-β-lactamase; MDR, multidrug-resistant; MIU, million international units; mo, months; NA, not available; NP, nosocomial pneumonia; PDR, pandrug-resistant; q12 h, every 12 h; q24 g, every 24 h; SSI, surgical site infection; UTI, urinary tract infection; VAP, ventilator-associated pneumonia; w, weeks; XDR, extensively drug-resistant.

Antibiotics: AMK, amikacin; ARB, arbekacin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; COL, colistin; DAP, daptomycin; DOR, doripenem; ETP, ertapenem; FEP, cefepime; FOS, fosfomycin; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; MIN, minocycline; PIP, piperacillin; RIF, rifampicin; SAM, ampicillin/sulbactam; SUL, sulbactam; SXT, trimethoprim/sulfamethoxazole; TEC, teicoplanin; TGC, tigecycline; TZP, piperacillin/ tazobactam; VAN, vancomycin.

[a] On the basis of FICIs, the results were categorised as follows: synergistic, FICI $\leq$ 0.5; partially synergistic, 0.5 < FICI < 1; additive, FICI = 1; indifferent, 1 < FICI $\leq$2; and antagonistic, FICI >2.

[b] Bactericidal activity was defined as a $\geq$3-$\log_{10}$ CFU/mL reduction in the initial bacterial count at each time point, whereas synergy was defined as a $\geq$2-$\log_{10}$ decrease in CFU/mL between the combinations and its most active constituent after 24 h.

[c] Disease (onset after transplant).

[d] Death due to massive bleeding at the level of surgical anastomosis 6 days following treatment initiation.

[e] After 48 h of therapy, the patient remained febrile and blood cultures grew PDR *K. pneumoniae*. After 96 h she became afebrile. Laboratory analyses showed a reduction of the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Blood and urine cultures did not grow any organism. After 7 days of therapy, COL was stopped because of visual hallucinations, whereas the double-carbapenem regimen was continued for an additional 14 days in the absence of adverse events. The patient was discharged in good condition; she was afebrile with an ESR of 31 mm/h and CRP of 0.9 mg/dL. She was then transferred to the orthopaedic department for a new hip prosthesis replacement.

[f] Index case: a newborn with severe sepsis caused by MDR and COL-susceptible *A. baumannii*. Based on the favourable outcome of the index case, three subsequent cases (Patients 6, 7 and 8) were treated with the combination COL + VAN + MEM.

[g] The patient was initially treated for 5 days with intravenous (i.v.) COL (6 mg/kg, equivalent to 75 000 IU/kg/day in three divided doses, following a loading dose of 6 mg/kg) and RIF (10 mg/kg/day). However, due to worsening of the clinical condition, VAN (40 mg/kg/day in three divided doses) and MEM (60 mg/kg/day in three divided doses) were added to COL, whereas RIF was stopped owing to hepatic toxicity.

[h] A prompt clinical response was observed and the patient completed a 17-day treatment course. Subsequently, the patient developed a late-onset uncomplicated UTI caused by the same MDR *A. baumannii*.

[i] Acute heart failure 4 days following treatment initiation.

[j] Combination activity was measured by the BC plate method. It is difficult to define and assess the synergistic effect of the BC plate method without using the FICI or the time–kill curve. Therefore, in this study combination activity was defined as inhibition in the culture well being observed with the antibiotic combination in comparison with inhibition by a single agent.

[k] ARB is characterised by its effectiveness against methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa*. Patient 12 was treated with ATM and ARB because of a combined MRSA infection. In this study, the BC plate does not include ARB in any of the drug evaluating combination effects.

[l] The patient died due to the lymphoma 14 days after completion of therapy.

[m] INH COL, inhaled colistin.

[n] In two patients it was possible to demonstrate in vitro synergistic activity of the combination COL and RIF. This combination showed full synergistic activity against two strains of MDR *P. aeruginosa* (FICI $\leq$ 0.5). The study does not clarify who are the two patients of the total of three.

[o] Although the combination COL + RIF displayed full bacteriostatic synergism with an FICI of 0.36, samples taken from the DFI were still positive for MDR *P. aeruginosa* despite having administered the combination of the two antibiotics. Thus, IPM was added to the existing regimen in order to improve the efficacy of therapy. The synergy test methods used proved full synergism (FICI = 0.25) of the combination of three antibiotics and, after 6 weeks of this multiple antibiotic regimen, the DFI was sterilised and the osteomyelitis was cured.

Table 2
Characteristics and outcomes of studies evaluating the clinical effectiveness of treatment with a combination of antibiotics (Abx) with confirmed in vitro synergy.

Reference	Study design	Study place/ period	Study scope	Characteristics	Days in hospital until isolation of MDR strain	Infection/ colonisation	Isolated organism	Synergy testing method	Synergy testing	Abx	Mortality
de Maio Carrillho et al. [40]	SC, prospective	Brazil, 2011–2012	Evaluation of in vitro activities and clinical outcomes of Abx combination	NA	NA	Pneumonia, UTI, BSI, SSTI	COL-resistant Enterobacteriaceae	Time–kill assay (initial inoculum NA)	Synergy for 14/19 isolates	COL + AMK + TGC COL + AMK + TGC + Carb. COL + AMK + Carb. COL + TGC + Carb. COL + AMK COL +TGC	6/14 (42.9%) vs. 4/13 (30.8%)[a]
Bremmer et al. [39][b]	SC, retrospective	USA/ 2009–2013	Correlation between in vitro synergy and clinical outcomes	Age (NA) APACHE II, 20 vs. 20.5	NA	Pneumonia BSI[c]	XDR *Acinetobacter baumannii*	Chequerboard[d] Time–kill assay (initial inoculum 10^6 CFU/mL)	Synergy No re-growth at 24 h for several combinations	MNO + COL DOR + TGC TGC + COL DOR + COL DOR + COL + TGC	3/8 (37.5%) vs. 6/10 (60.0%)
Cai et al. [36]	MC, retrospective	Singapore/ 2009–2014	Correlation between in vitro synergy and clinical outcomes	Age[e,f], 59 (21–92) years vs. 58 (16–87) years APACHE II[e,f], 16 (5–31) vs. 14 (0–29)	NA	HAP, UTI, SSTI, BSI	XDR *A. baumannii* (75%) XDR *Pseudomonas aeruginosa* (20%) XDR *Klebsiella pneumoniae* (5%)	Time–kill assay (initial inoculum NA)	Synergy proven for 30 isolates	POL + 1 or more antibiotics[g]	4/30 (13.3%) vs. 50/203 (24.6%)[e]
Jean et al. [38]	Prospective	Taiwan/ 2013	Correlation between in vitro synergy and clinical outcomes	Age[f], 75 (45–88) years vs. 77 (40–86) years APACHE II[f], 30 (19–45) vs. 29 (20–45)	12 (6–17) vs. 11 (6–16)	VAP	XDR *A. baumannii*	Chequerboard Time–kill assay (initial inoculum NA)	Synergy for 24/28 isolates	TGC + IPM vs. SUL + IPM	4/28 (14.3%) vs. 36/56 (64.3%)
Aydemir et al. [42]	SC, open RCT	Turkey/ 2011–2012	Efficacy of COL + RIF vs. COL for CRAB VAP	Adults, age[f] 58 ± 23 years vs. 63 ± 17 years APACHE II[f], 20.1 ± 6.8 vs. 18.0 ± 4.9	12.8 ± 7.4 vs. 19.6 ± 18.2	VAP with bacteraemia	CRAB	Chequerboard, FICI	Synergy for all isolates	COL + RIF vs. COL	13/21 (61.9%) vs. 16/22 (72.7%)[e,g]
Jang et al. [41]	SC, retrospective	Korea/ 2006–2007	Correlation between in vitro synergy and clinical outcomes	Age[d], 57.0 ± 16.6 years vs. 62.5 ± 17.5 years APACHE II[d], 26.7 ± 6.8 vs. 27.8 ± 7.6	NA	VAP	MDR *A. baumannii*	Double-disk diffusion	Synergy	COL + CSL COL + SAM COL + MNO COL + SXT	11/19 (57.9%) vs. 14/22 (63.6%)[h]

APACHE, Acute Physiology and Chronic Health Evaluation; BSI, bloodstream infection; CRAB, carbapenem-resistant *A. baumannii*; FICI, fractional inhibitory concentration index; HAP, hospital-acquired pneumonia; MC, multicentre; MDR, multidrug-resistant; NA, not available; SC, single-centre; SSTI, skin and soft-tissue infection; UTI, urinary tract infection; VAP, ventilator-associated pneumonia; XDR, extensively drug-resistant.
Antibiotics: AMK, amikacin; Carb., carbapenem; COL, colistin; CSL, cefoperazone/sulbactam; DOR, doripenem; IPM, imipenem; MNO, minocycline; POL, polymyxin; RIF, rifampicin; SAM, ampicillin/sulbactam; SUL, sulbactam; TGC, tigecycline; SXT, trimethoprim/sulfamethoxazole.

[a] Data for the control group refer to the sum of unguided combination therapy and monotherapy.

[b] Group 1 patients demonstrated microbiological clearance by follow-up culture (4/5) or presumed microbiological eradication (3/3). Group 2 patients demonstrated microbiological clearance by follow-up cultures (1/5) or presumed microbiologic eradication (2/5). There were no differences in the acute physiology and chronic health evaluation II (APACHE II) score, the Charlson score, or the time to targeted therapy between two groups.

[c] Patients who had pneumonia ($n = 17$) or bacteraemia ($n = 1$) and received 48 h of an antibiotic combination analysed by the chequerboard method.

[d] By the chequerboard method, the MNO—COL and DOR–COL combinations displayed synergy against 6.6% and 5.3% of the isolates, respectively. The best triple combination was DOR–COL–MNO, which displayed synergy against 6.6% of the isolates. TGC—COL was the only combination that did not display a synergistic effect against any isolate. The combination was antagonistic against 13.2% of the isolates tested. Additionally, TGC—COL was the only combination not to inhibit growth in any of the serum-achievable concentration (SAC) wells in all isolates tested. MNO—COL and DOR–COL–MNO inhibited growth in at least one SAC well in all isolates tested. Next, time–kill assays were performed with all of the isolates against which synergistic activity was demonstrated by the chequerboard method.

[e] Data for validated and non-validated combination therapy are presented. The values for the monotherapy group were age 63 (16–93) years, APACHE II score 12 (0–23) and mortality 13/58 (22.4%). Infection-related mortality was reported in this study.

[f] Mean (range).

[g] Other antibiotics included carbapenems, fluoroquinolones, β-lactam/β-lactamase inhibitors, aminoglycosides, cephalosporins, aztreonam, rifampicin and tigecycline.

[h] In-hospital.

but four of the tested isolates showed synergy or additivity at concentrations well below the MICs by both methods. The characteristics of patients in the compared groups were not different. Mortality was lower in patients receiving the tigecycline–imipenem combination [4/28 (14.3%) vs. 36/56 (64.3%); $P < 0.001$]. Microbiological eradication in the tigecycline–imipenem combination group was low (8/28; 28.6%), but none of the patients developed a subsequent VAP episode. Breakthrough bacteraemia, shock and treatment with sulbactam/imipenem (aOR = 8.56, 95% CI 2.81–29.75) were independent predictors of mortality [38].

Synergy between colistin, minocycline, tigecycline, doripenem (in several combinations of two or three drugs) was evaluated against 76 XDR *A. baumannii* isolates in a retrospective study by the chequerboard method and time–kill assay [39]. Minocycline–colistin and minocycline–colistin–doripenem combinations showed synergy or additivity more frequently at the various concentrations tested. The colistin–tigecycline combination was the single combination not to demonstrate synergy against any of the isolates; in addition, antagonism was also observed with this combination. The outcome of 18 patients with pneumonia or bacteraemia due to XDR *A. baumannii* treated with a combination of these antibiotics was retrospectively evaluated. Patients were divided in two groups: Group 1 included patients who received a combination that inhibited growth in one or more of the test wells by the chequerboard method, whilst patients in Group 2 received a combination that demonstrated growth in all wells. Colistin–tigecycline was the most commonly prescribed regimen (9/18) and was the commonest combination regimen prescribed in Group 2 (9/10). Clinical effectiveness [4/8 (50%) vs. 3/10 (30%); $P = 0.63$] and 30-day all-cause mortality [3/8 (38%) vs. 6/10 (60%); $P = 0.63$] did not differ between the two groups, whereas microbiological effectiveness was higher for patients in Group 1 [7/8 (88%) vs. 3/10 (30%); $P = 0.02$] [39].

A prospective study evaluated the in vitro synergy of antibiotics against colistin- and carbapenem-resistant Enterobacteriaceae (26 *K. pneumoniae* and 1 *Enterobacter* spp.) as well as the outcomes of patients from whom these bacteria were isolated [40]. Combination therapy was given to 19 patients; in 14 cases synergy between the administered antibiotics was observed. No difference in mortality was observed between patients receiving SGACT and those who did not [6/14 (42.9%) vs. 3/5 (60.0%)]. Monotherapy was administered only in patients with urinary tract infection; mortality was 12.5% (1/8). Dialysis and shock were predictors of mortality.

In a retrospective, single-centre study, i.v. colistin was administered either alone or in combination following in vitro synergy testing requested by the attending physician for the treatment of 41 patients with MDR *A. baumannii* VAP [41]. Inhaled colistin was also administered to a non-specified number of patients. Of 28 tested isolates, 2 were resistant to colistin. A double-disk diffusion test was used to test for synergy. There were no differences in baseline characteristics between the SGACT and monotherapy groups. Colistin was administered for a longer period in the combination group. There was no difference in clinical effectiveness [10/19 (52.6%) vs. 8/22 (36.4%); $P = 0.36$] or microbiological eradication [6/19 (31.6%) vs. 6/22 (27.3%); $P = 0.76$] between SGACT and colistin monotherapy. ICU [10/19 (52.6%) vs. 14/22 (63.6%); $P = 0.54$], in-hospital [11/19 (57.9%) vs. 14/22 (63.6%); $P = 0.76$] and VAP-related [7/19 (36.8%) vs. 12/22 (54.5%); $P = 0.41$] mortality were not significantly different between the two groups [41].

The aim of an open-label, single-centre RCT was to compare the clinical and microbiological effectiveness and safety of the combination of i.v. colistin and rifampicin versus i.v. colistin alone administered for the treatment of patients with VAP due to carbapenem-resistant, colistin-susceptible *A. baumannii* [42]. Synergy between colistin and rifampicin was studied by the chequerboard microdilution method and was confirmed using the FICI. Colistin MICs alone and following combination with rifampicin were not provided, but synergy was reported to be confirmed for all isolates. The study enrolled only one-half of the initially planned population (43 instead of 88). The Sequential Organ Failure Assessment (SOFA) score was higher in the combination group (8.2 ± 2.9 vs. 6.5 ± 2.6; $P = 0.04$). There was no difference in clinical [11/21 (52.4%) vs. 9/22 (40.9%); $P = 0.65$] or microbiological [15/21 (71.4%) vs. 13/22 (59.1%); $P = 0.60$] efficacy between the two groups, although the time to microbiological clearance was significantly shorter in the combination group (3.1 ± 0.5 days vs. 4.5 ± 1.7 days; $P = 0.03$). The in-hospital [13/21 (61.9%) vs. 16/22 (72.7%); $P = 0.67$] and VAP-related [8/21 (38.1%) vs. 14/22 (63.6%); $P = 0.17$] mortality were also not statistically different.

3.3. Pooled analysis

Pooled analysis of unadjusted data from these studies (504 patients) showed that there was no difference between SGACT and UCT or monotherapy (OR = 0.47, 95% CI 0.21–1.04) (Fig. 2). Statistical heterogeneity was significant ($P = 0.06$, $I^2 = 52\%$). Analysis of the adjusted data showed that SGACT was significantly associated with survival (OR = 0.44, 95% CI 0.20–0.98; $I^2 = 54\%$) (Fig. 3). The small number of available studies precluded the conduct of meaningful subgroup analyses.

4. Discussion

The currently available body of evidence suggests that SGACT may reduce the mortality of patients with MDR Gram-negative bacterial infections compared with UCT or monotherapy. Besides improvement in clinical outcomes, SGACT could reduce antibiotic consumption, with further potential impact on antimicrobial resistance and healthcare costs. These findings are limited by the observational nature of the studies, the small number of patients

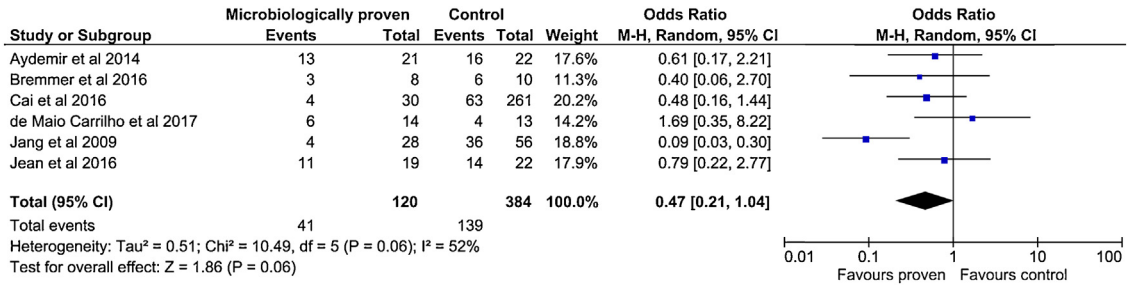

Fig. 2. Forest plot for unadjusted mortality among patients with multidrug-resistant Gram-negative infections treated with synergy-guided antibiotic combination therapy and unguided combination therapy or monotherapy. Squares, odds ratio; horizontal lines, 95% confidence interval; diamond, pooled odds ratio. CI, confidence interval.

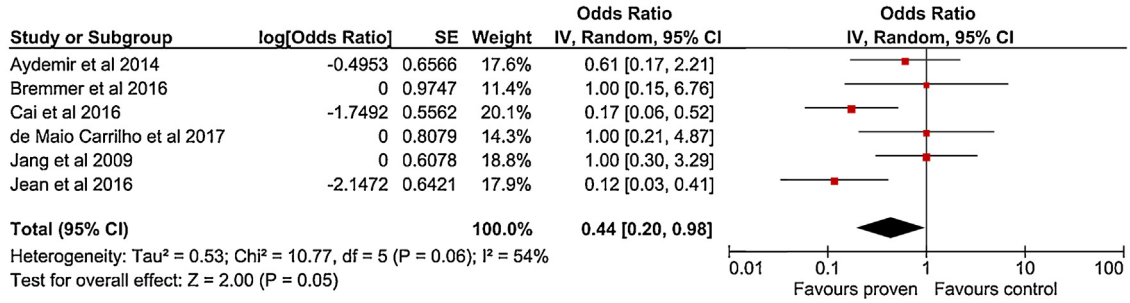

Fig. 3. Forest plot for adjusted mortality among patients with multidrug-resistant Gram-negative infections treated with synergy-guided antibiotic combination therapy (SGACT) and unguided combination therapy (UCT) or monotherapy. Squares, odds ratio; horizontal lines, 95% confidence interval; diamond, pooled odds ratio. Footnote. In this analysis, data for SGACT versus UCT were introduced only for the study by Cai et al. (2016). CI, confidence interval.

receiving treatment based on synergistic tests (120 in the 'comparative' studies), the variable site and severity of infections, the treatment received by the control populations (monotherapy instead of combination), the timing when treatment was provided, the different mortality endpoints (infection-related, in-hospital, etc.), and the various tests performed and criteria used for treatment decisions, as treatment for several patients was based on proof of additive and not synergistic effects.

The role of antibiotic combination therapy in the treatment of hospital-acquired or requiring hospitalisation community-associated bacterial infections is a long-standing issue of research and debate, but in most cases a clear benefit in favour of combination therapy has not been established [43–46]. Despite this fact, combination regimens are commonly proposed in treatment guidelines, especially when the infection is considered severe, risk factors for MDR bacteria are accumulating, or first-line agents (β-lactams or fluoroquinolones) cannot be employed. The rationale behind this approach is multidimensional: combination regimens provide a broader coverage for potential pathogens, increase the probability of achieving bactericidal antibiotic concentrations of at least one active antibiotic at the infection site, may decrease the probability of development of resistance even when heteroresistance is a concern, and offer the potential of synergy [47,48].

Introducing SGACT in routine clinical practice could be problematic due to the time required to perform the tests and the number of combinations to be tested. Selection of the most appropriate testing method could be also difficult. Time–kill assays appear to be the most commonly used method with which the outcomes of other synergistic methods are compared [49]. Although a standardised protocol for it has been provided by the Clinical and Laboratory Standards Institute (CLSI), there is no true gold-standard method for synergy testing; in addition, different initial inocula may be used in individual studies. With limited exceptions, several studies have shown that no two methods have produced comparable results [49]. Although chequerboard and time–kill assays were mostly used, studies have reported variable intermethod agreement [50,51]. Therefore, it might be difficult to identify the method that would correlate more accurately with clinical outcome. This complicates the interpretation of the outcome of the current review and meta-analysis, since inconsistent testing methods have been applied.

Besides the performed method, the synergistic effect also depends on the underlying mechanism of resistance and antibiotic MICs [52,53]. For example, for the double-carbapenem combination (use of ertapenem as a suicide substrate owing its higher affinity to carbapenemases), synergy may not be observed in cases of KPC-producing Enterobacteriaceae but can be observed in NDM-, VIM- or OXA-producing isolates [52]. Furthermore, the meropenem MIC might affect synergy (up to 128 μg/mL may be a viable option) [53]. Respectively, disruption of efflux pumps or increased membrane permeability in *A. baumannii* isolates in the presence of polymyxin B may lead to an increased intracellular concentration of minocycline and thus to a synergistic interaction [54]. In contrast to this, the combination of tigecycline and colistin may be indifferent and in some cases antagonistic [39]. It has been reported that addition of tigecycline to colistin was associated with poor clinical efficacy compared with other regimens [39,55]. Unfortunately, we could not compare the efficacy of antibiotic combinations in relation to resistance mechanism owing to lack of relevant data in the individual studies.

In vitro synergy testing before antibiotic prescription could be the first step to improve patient outcomes. However, administration of adequate antibiotic doses and exploitation of their PK/PD properties should not be underestimated, especially in septic patients [56,57]. In a meta-analysis, we found that colistin combination treatment was associated with lower mortality when high colistin doses (>6 MIU, unadjusted to renal function) were administered (OR = 0.80, 95% CI 0.69–0.93) but not when lower doses were employed [61]. In the single available study that explored both dosing and synergy, patients who received SGACT received higher doses, sooner and for a longer duration than comparator groups [36]. Although only SGACT was associated with survival, the contribution of adequate antibiotic administration and exploitation of PK/PD properties of antibiotics deserve further study. Therefore, implementation of a multidisciplinary approach may be required to achieve the desired outcomes. This has been shown in other settings, e.g. the use of rapid diagnostic tests in bacteraemic patients was associated with improved clinical outcomes only when they were accompanied by antibiotic stewardship programmes [58]. Finally, SGACT may be useful in de-escalation of therapy, especially when more than two antibiotics are used, with important consequences on antimicrobial consumption and antimicrobial stewardship programmes.

In several of the case reports as well as in a subpopulation of the cohort studies, an additive effect was associated with a favourable outcome, denoting that additive effects may be adequate. Since separate data for additive and synergistic effects were lacking, further studies are required to define the potential role of additivity and synergy in patient outcome. Finally, the potential benefit from the introduction of technological advances on the performance of antimicrobial susceptibility and synergy testing (3D printing, Xact test) should be explored further [59,60].

In conclusion, the limited available data suggest that SGACT may be associated with survival in patients with MDR Gram-negative bacterial infections. However, its role is still not clear owing to the small number and the observational nature of studies. Given the complexity in the mechanisms of synergy, accurate predictions based on clinical judgement or experience seems risky and specific in vitro synergy testing is probably inevitable. However, adoption of synergy testing would be costly and

laborious. Although SGACT cannot be currently recommended as a routine clinical practice, these early promising findings warrant further investigation in well-designed prospective trials.

Funding

None.

Competing interests

MEF has participated on advisory boards for AstraZeneca, InfectoPharm, Tetraphase, Shionogi and Xellia, has received lecture honoraria from Cipla, Merck and Pfizer, and has received research support from Shionogi, Tetraphase and Helperby. All other authors declare no competing interests.

Ethical approval

Not required.

References

[1] Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. Emerg Infect Dis 2014;20:1170–5.
[2] Vardakas KZ, Rafailidis PI, Konstantelias AA, Falagas ME. Predictors of mortality in patients with infections due to multi-drug resistant Gram negative bacteria: the study, the patient, the bug or the drug? J Infect 2013;66:401–14.
[3] Matthaiou DK, Michalopoulos A, Rafailidis PI, Karageorgopoulos DE, Papaioannou V, Ntani G, et al. Risk factors associated with the isolation of colistin-resistant Gram-negative bacteria: a matched case–control study. Crit Care Med 2008;36:807–11.
[4] Sun Y, Cai Y, Liu X, Bai N, Liang B, Wang R. The emergence of clinical resistance to tigecycline. Int J Antimicrob Agents 2013;41:110–6.
[5] Vardakas KZ, Legakis NJ, Triarides N, Falagas ME. Susceptibility of contemporary isolates to fosfomycin: a systematic review of the literature. Int J Antimicrob Agents 2016;47:269–85.
[6] Falagas ME, Mavroudis AD, Vardakas KZ. The antibiotic pipeline for multi-drug resistant Gram negative bacteria: what can we expect? Expert Rev Anti Infect Ther 2016;14:747–63.
[7] Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 2010;74:417–33.
[8] Bass SN, Bauer SR, Neuner EA, Lam SW. Impact of combination antimicrobial therapy on mortality risk for critically ill patients with carbapenem-resistant bacteremia. Antimicrob Agents Chemother 2015;59:3748–53.
[9] Petrosillo N, Giannella M, Antonelli M, Antonini M, Barsic B, Belancic L, et al. Clinical experience of colistin–glycopeptide combination in critically ill patients infected with Gram-negative bacteria. Antimicrob Agents Chemother 2014;58:851–8.
[10] Qureshi ZA, Paterson DL, Potoski BA, Kilayko MC, Sandovsky G, Sordillo E, et al. Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae*: superiority of combination antimicrobial regimens. Antimicrob Agents Chemother 2012;56:2108–13.
[11] Santimaleeworagun W, Wongpoowarak P, Chayakul P, Pattharachayakul S, Tansakul P, Garey KW. Clinical outcomes of patients infected with carbapenem-resistant *Acinetobacter baumannii* treated with single or combination antibiotic therapy. J Med Assoc Thai 2011;94:863–70.
[12] Tumbarello M, Trecarichi EM, De Rosa FG, Giannella M, Giacobbe DR, Bassetti M, et al. Infections caused by KPC-producing *Klebsiella pneumoniae*: differences in therapy and mortality in a multicentre study. J Antimicrob Chemother 2015;70:2133–43.
[13] Al-Shaer M, Nazer LH, Kherallah M. Rifampicin as adjunct to colistin therapy in the treatment of multidrug-resistant *Acinetobacter baumannii*. Ann Pharmacother 2014;48:766–71.
[14] Durante-Mangoni E, Signoriello G, Andini R, Mattei A, De Cristoforo M, Murino P, et al. Colistin and rifampicin compared with colistin alone for the treatment of serious infections due to extensively drug-resistant *Acinetobacter baumannii*: a multicenter, randomized clinical trial. Clin Infect Dis 2013;57:349–58.
[15] Falagas ME, Lourida P, Poulikakos P, Rafailidis PI, Tansarli GS. Antibiotic treatment of infections due to carbapenem-resistant Enterobacteriaceae: systematic evaluation of the available evidence. Antimicrob Agents Chemother 2014;58:654–63.
[16] Paul M, Carmeli Y, Durante-Mangoni E, Mouton JW, Tacconelli E, Theuretzbacher U, et al. Combination therapy for carbapenem-resistant Gram-negative bacteria. J Antimicrob Chemother 2014;69:2305–9.
[17] Hong DJ, Kim JO, Lee H, Yoon EJ, Jeong SH, Yong D, et al. In vitro antimicrobial synergy of colistin with rifampicin and carbapenems against colistin-resistant *Acinetobacter baumannii* clinical isolates. Diagn Microbiol Infect Dis 2016;86:184–9.
[18] Michail G, Labrou M, Pitiriga V, Manousaka S, Sakellaridis N, Tsakris A, et al. Activity of tigecycline in combination with colistin, meropenem, rifampin, or gentamicin against KPC-producing Enterobacteriaceae in a murine thigh infection model. Antimicrob Agents Chemother 2013;57:6028–33.
[19] Zusman O, Avni T, Leibovici L, Adler A, Friberg L, Stergiopoulou T, et al. Systematic review and meta-analysis of in vitro synergy of polymyxins and carbapenems. Antimicrob Agents Chemother 2013;57:5104–11.
[20] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81.
[21] Hall MJ, Middleton RF, Westmacott D. The fractional inhibitory concentration (FIC) index as a measure of synergy. J Antimicrob Chemother 1983;11:427–33.
[22] Vardakas KZ, Falagas ME. Colistin versus polymyxin B for the treatment of patients with multidrug-resistant Gram-negative infections: a systematic review and meta-analysis. Int J Antimicrob Agents 2017;49:233–8.
[23] Ceccarelli G, Falcone M, Giordano A, Mezzatesta ML, Caio C, Stefani S, et al. Successful ertapenem–doripenem combination treatment of bacteremic ventilator-associated pneumonia due to colistin-resistant KPC-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2013;57:2900–1.
[24] Ceccarelli G, Oliva A, d'Ettorre G, D'Abramo A, Caresta E, Barbara CS, et al. The role of vancomycin in addition with colistin and meropenem against colistin-sensitive multidrug resistant *Acinetobacter baumannii* causing severe infections in a paediatric intensive care unit. BMC Infect Dis 2015;15:393.
[25] Lee NY, Wang CL, Chuang YC, Yu WL, Lee HC, Chang CM, et al. Combination carbapenem–sulbactam therapy for critically ill patients with multidrug-resistant *Acinetobacter baumannii* bacteremia: four case reports and an in vitro combination synergy study. Pharmacotherapy 2007;27:1506–11.
[26] Nakamura I, Yamaguchi T, Tsukimori A, Sato A, Fukushima S, Mizuno Y, et al. Effectiveness of antibiotic combination therapy as evaluated by the breakpoint checkerboard plate method for multidrug-resistant *Pseudomonas aeruginosa* in clinical use. J Infect Chemother 2014;20:266–9.
[27] Oliva A, Cipolla A, Gizzi F, D'Abramo A, Favaro M, De Angelis M, et al. Severe bloodstream infection due to KPC-producer *E. coli* in a renal transplant recipient treated with the double-carbapenem regimen and analysis of in vitro synergy testing: a case report. Medicine (Baltimore) 2016;95:e2243.
[28] Oliva A, D'Abramo A, D'Agostino C, Iannetta M, Mascellino MT, Gallinelli C, et al. Synergistic activity and effectiveness of a double-carbapenem regimen in pandrug-resistant *Klebsiella pneumoniae* bloodstream infections. J Antimicrob Chemother 2014;69:1718–20.
[29] Oliva A, Mascellino MT, Cipolla A, D'Abramo A, De Rosa A, Savinelli S, et al. Therapeutic strategy for pandrug-resistant *Klebsiella pneumoniae* severe infections: short-course treatment with colistin increases the in vivo and in vitro activity of double carbapenem regimen. Int J Infect Dis 2015;33:132–4.
[30] Piedra-Carrasco N, Miguel L, Fàbrega A, Viñado B, Campany D, Mir A, et al. Effectiveness of a double-carbapenem regimen in a KPC-producing *Klebsiella pneumoniae* infection in an immunocompromised patient. Microb Drug Resist 2018;24:199–202.
[31] Tascini C, Gemignani G, Ferranti S, Tagliaferri E, Leonildi A, Lucarini A, et al. Microbiological activity and clinical efficacy of a colistin and rifampin combination in multidrug-resistant *Pseudomonas aeruginosa* infections. J Chemother 2004;16:282–7.
[32] Tascini C, Gemignani G, Palumbo F, Leonildi A, Tedeschi A, Lambelet P, et al. Clinical and microbiological efficacy of colistin therapy alone or in combination as treatment for multidrug resistant *Pseudomonas aeruginosa* diabetic foot infections with or without osteomyelitis. J Chemother 2006;18:648–51.
[33] Tascini C, Menichetti F, Gemignani G, Palumbo F, Leonildi A, Tedeschi A, et al. Clinical and microbiological efficacy of colistin therapy in combination with rifampin and imipenem in multidrug-resistant *Pseudomonas aeruginosa* diabetic foot infection with osteomyelitis. Int J Low Extrem Wounds 2006;5:213–6.
[34] Leite GC, Oliveira MS, Perdigao-Neto LV, Rocha CK, Guimaraes T, Rizek C, et al. Antimicrobial combinations against pan-resistant *Acinetobacter baumannii* isolates with different resistance mechanisms. PLoS One 2016;11:e0151270.
[35] Oliva A, Gizzi F, Mascellino MT, Cipolla A, D'Abramo A, D'Agostino C, et al. Bactericidal and synergistic activity of double-carbapenem regimen for infections caused by carbapenemase-producing *Klebsiella pneumoniae*. Clin Microbiol Infect 2016;22:147–53.
[36] Cai B, Cai Y, Liew YX, Chua NG, Teo JQ, Lim TP, et al. Clinical efficacy of polymyxin monotherapy versus nonvalidated polymyxin combination therapy versus validated polymyxin combination therapy in extensively drug-resistant Gram-negative bacillus infections. Antimicrob Agents Chemother 2016;60:4013–22.
[37] Cai Y, Chua NG, Lim TP, Teo JQ, Lee W, Kurup A, et al. From bench-top to bedside: a prospective in vitro antibiotic combination testing (iACT) service to guide the selection of rationally optimized antimicrobial combinations against extensively drug resistant (XDR) Gram negative bacteria (GNB). PLoS One 2016;11:e0158740.
[38] Jean SS, Hsieh TC, Hsu CW, Lee WS, Bai KJ, Lam C. Comparison of the clinical efficacy between tigecycline plus extended-infusion imipenem and sulbactam plus imipenem against ventilator-associated pneumonia with pneumonic extensively drug-resistant *Acinetobacter baumannii* bacteremia, and correlation of clinical efficacy with in vitro synergy tests. J Microbiol Immunol Infect 2016;49:924–33 Erratum in: J Microbiol Immunol Infect 2018;51:157.
[39] Bremmer DN, Bauer KA, Pouch SM, Thomas K, Smith D, Goff DA, et al. Correlation of checkerboard synergy testing with time–kill analysis and

clinical outcomes of extensively drug-resistant *Acinetobacter baumannii* respiratory infections. Antimicrob Agents Chemother 2016;60:6892–5.
[40] de Maio Carrillho CM, Gaudereto JJ, Martins RC, de Castro Lima VA, de Oliveira LM, Urbano MR, et al. Colistin-resistant Enterobacteriaceae infections: clinical and molecular characterization and analysis of in vitro synergy. Diagn Microbiol Infect Dis 2017;87:253–7.
[41] Jang HJ, Kim MN, Lee K, Hong SB, Lim CM, Koh Y. The comparative efficacy of colistin monotherapy and combination therapy based on in vitro antimicrobial synergy in ventilator-associated pneumonia caused by multi-drug resistant *Acinetobacter baumannii*. Tuberc Respir Dis 2009;67:212–20.
[42] Aydemir H, Akduman D, Piskin N, Comert F, Horuz E, Terzi A, et al. Colistin vs. the combination of colistin and rifampicin for the treatment of carbapenem-resistant *Acinetobacter baumannii* ventilator-associated pneumonia. Epidemiol Infect 2013;141:1214–22.
[43] Paul M, Dickstein Y, Schlesinger A, Grozinsky-Glasberg S, Soares-Weiser K, Leibovici L. β-Lactam versus β-lactam–aminoglycoside combination therapy in cancer patients with neutropenia. Cochrane Database Syst Rev 2013(6)CD003038.
[44] Vardakas KZ, Samonis G, Chrysanthopoulou SA, Bliziotis IA, Falagas ME. Role of glycopeptides as part of initial empirical treatment of febrile neutropenic patients: a meta-analysis of randomised controlled trials. Lancet Infect Dis 2005;5:431–9.
[45] Vardakas KZ, Tansarli GS, Bliziotis IA, Falagas ME. β-Lactam plus aminoglycoside or fluoroquinolone combination versus β-lactam monotherapy for *Pseudomonas aeruginosa* infections: a meta-analysis. Int J Antimicrob Agents 2013;41:301–10.
[46] Vardakas KZ, Trigkidis KK, Falagas ME. Fluoroquinolones or macrolides in combination with β-lactams in adult patients hospitalized with community acquired pneumonia: a systematic review and meta-analysis. Clin Microbiol Infect 2017;23:234–41.
[47] Ni W, Wei C, Zhou C, Zhao J, Liang B, Cui J, et al. Tigecycline–amikacin combination effectively suppresses the selection of resistance in clinical isolates of KPC-producing *Klebsiella pneumoniae*. Front Microbiol 2016;7:1304.
[48] Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, et al. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2006;50:2946–50.
[49] Doern CD. When does 2 plus 2 equal 5? A review of antimicrobial synergy testing. J Clin Microbiol 2014;52:4124–8.
[50] Bonapace CR, Bosso JA, Friedrich LV, White RL. Comparison of methods of interpretation of checkerboard synergy testing. Diagn Microbiol Infect Dis 2002;44:363–6.
[51] Bonapace CR, White RL, Friedrich LV, Bosso JA. Evaluation of antibiotic synergy against *Acinetobacter baumannii*: a comparison with Etest, time–kill, and checkerboard methods. Diagn Microbiol Infect Dis 2000;38:43–50.
[52] Fredborg M, Sondergaard TE, Wang M. Synergistic activities of meropenem double and triple combinations against carbapenemase-producing Enterobacteriaceae. Diagn Microbiol Infect Dis 2017;88:355–60.
[53] Oliva A, Scorzolini L, Cipolla A, Mascellino MT, Cancelli F, Castaldi D, et al. In vitro evaluation of different antimicrobial combinations against carbapenemase-producing *Klebsiella pneumoniae*: the activity of the double-carbapenem regimen is related to meropenem MIC value. J Antimicrob Chemother 2017;72:1981–4.
[54] Bowers DR, Cao H, Zhou J, Ledesma KR, Sun D, Lomovskaya O, et al. Assessment of minocycline and polymyxin B combination against *Acinetobacter baumannii*. Antimicrob Agents Chemother 2015;59:2720–5.
[55] Cheng A, Chuang YC, Sun HY, Sheng WH, Yang CJ, Liao CH, et al. Excess mortality associated with colistin–tigecycline compared with colistin–carbapenem combination therapy for extensively drug-resistant *Acinetobacter baumannii* bacteremia: a multicenter prospective observational study. Crit Care Med 2015;43:1194–204.
[56] Abdul-Aziz MH, Lipman J, Mouton JW, Hope WW, Roberts JA. Applying pharmacokinetic/pharmacodynamic principles in critically ill patients: optimizing efficacy and reducing resistance development. Semin Respir Crit Care Med 2015;36:136–53.
[57] Vardakas KZ, Rellos K, Triarides NA, Falagas ME. Colistin loading dose: evaluation of the published pharmacokinetic and clinical data. Int J Antimicrob Agents 2016;48:475–84.
[58] Vardakas KZ, Anifantaki FI, Trigkidis KK, Falagas ME. Rapid molecular diagnostic tests in patients with bacteremia: evaluation of their impact on decision making and clinical outcomes. Eur J Clin Microbiol Infect Dis 2015;34:2149–60.
[59] Smith KP, Kirby JE. How inkjet printing technology can defeat multidrug-resistant pathogens. Future Microbiol 2016;11:1375–7.
[60] van Belkum A, Halimi D, Bonetti EJ, Renzi G, Cherkaoui A, Sauvonnet V, et al. Meropenem/colistin synergy testing for multidrug-resistant *Acinetobacter baumannii* strains by a two-dimensional gradient technique applicable in routine microbiology. J Antimicrob Chemother 2015;70:167–72.
[61] Vardakas KZ, Mavroudis AD, Georgiou M, Falagas ME. Intravenous colistin combination antimicrobial treatment vs. monotherapy: a systematic review and meta-analysis. Int J Antimicrob Agents 2018;51(4):535–47.