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REVIEW

Combination therapy for extensively-drug resistant gram-negative bacteria

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ABSTRACT

Introduction: The ongoing crisis and emergence of extensively-drug resistant (XDR) gram-negative pathogens in the nosocomial setting is worrisome. The limited armamentarium in combination with the increasing resistance rates of last resort antibiotics has led clinicians to re-exploit existing antibiotic classes.

Areas covered: Current state of evidence concerning the administration of monotherapy versus combination therapy for the treatment of XDR gram-negative microorganism as well as salvage treatment are presented. Herein, the current knowledge concerning *in vitro* studies, animal models and clinical studies are discussed in detail.

Expert commentary: The efficacy of combination therapy in carbapenemase-producing K. pneumoniae is associated with reduced mortality in patients with septic shock and rapidly fatal underlying diseases. There is moderate evidence in support of the use of monotherapy for treating carbapenemase-producing Acinetobacter baumannii infections, however for septic shock patients, cancer patients and infections with an isolate with MIC in the upper limit of susceptibility combination therapy could be recommended. There are currently minimal and of low quality clinical evidence suggesting that combination treatment has no therapeutic advantage over monotherapy for XDR Pseudomonas aeruginosa infections. The *in vivo* validity of novel compounds and necessity for combination therapy is to be evaluated in future studies particularly for XDR infections.

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XDR; PDR; combination; monotherapy; carbapenemase producing gram-negative; *Klebsiella pneumoniae*; *Acinetobacter baumannii*; *Pseudomonas aeruginosa*; double carbapenem combination; colistin

1. Introduction

The increasing incidence of antimicrobial resistance among gram-negative bacteria possesses a serious threat in the management of nosocomial infections [1]. The emergence of extensively drug-resistant (XDR) and pan drug-resistant (PDR) gram-negative microorganisms [2], constitute a real threat almost all over the world, represented mainly by *Klebsiella pneumoniae, Acinetobacter baumannii*, and *Pseudomonas aeruginosa* [1,3]. Therefore, the World Health Organization (WHO) has identified antimicrobial resistance as one of the major problems for human health [4]. Infections caused by these organisms are associated with high mortality and particularly carbapenemase-producing *K. pneumoniae* (Cp-Kp) is associated with 23–75% mortality rates [5]. The lack of active antimicrobials in combination with the dry pipeline has led to exploiting existing antibiotic classes [1,6]. The administration of combination therapy for XDR gram-negative bacteria is a controversial issue with studies leading to conflicting conclusions [6]. The basic theoretical advantages of combination treatment consist of faster bacterial clearance, prevention in the development of bacterial resistance, and synergistic or additive effect with the primary objective being the improvement in mortality. On the other hand, potential side effects like increased toxicity and higher costs are possible drawbacks [7]. In this review, the current state of evidence regarding combination treatment versus monotherapy against XDR gram-negative microorganisms, including carbapenemase-producing *Klebsiella pneumoniae, Acinetobacter baumannii*, and *Pseudomonas aeruginosa* is examined.

2. General principles

Carbapenem resistance, mainly attributed to β-lactamases production is a resistance determinant of increasing clinical relevance in gram-negative bacteria, leading to a significant reduction of therapeutic options. Pathogens producing carbapenemases commonly encountered in clinical practice are *K. pneumoniae* and the non-fermenting gram-negative bacteria *P. aeruginosa* and *A. baumannii* [3,5,8]. The vast majority of acquired carbapenemases belong to three of the four known classes of β-lactamases, namely Ambler class A, Ambler class B (metallo-b-lactamases – MBLs), and Ambler class D (oxacillinases – OXAs) [9]. KPC-type class A carbapenemases have mainly spread in *K. pneumoniae*, although these enzymes have also been identified in *Klebsiella oxytoca, Enterobacter* spp., and *Pseudomonas* spp [3,5,8,9]. MBLs, primarily of the VIM, IMP, and NDM types, have been present mostly in Enterobacteriaceae, *P. aeruginosa*, and *A. baumannii* [8,9]. On the other hand, acquired OXA-type carbapenem-hydrolyzing class D β-lactamases are common among *A. baumannii* isolates, whereas OXA-48 has also been detected in *K. pneumoniae* isolates [8,9].

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Recently, a group of experts proposed a standardized international terminology with which to describe acquired resistance profiles in gram-negative pathogens. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and PDR was defined as non-susceptibility to all agents in all antimicrobial categories. Although the above definitions do not require resistance to carbapenems, the presence of carbapenemases is very common in XDR and PDR strains [2]. An XDR *K. pneumoniae* strain isolated in clinical practice would be likely susceptible to two or fewer antimicrobial classes from the following: polymyxins, tigecycline, fosfomycin or aminoglycoside, whereas for XDR *P. aeruginosa* isolates would be susceptible to polymyxins, aztreonam, fosfomycin, or aminoglycosides. On the other hand, for *A. baumannii* strains with an XDR profile susceptibility to sulbactam, tigecycline, polymyxins, or aminoglycosides would be reported. For example, *A. baumannii* strain susceptible only to colistin would be defined as XDR, whereas a carbapenems-resistant *K. pneumoniae* isolate susceptible to colistin, tigecycline, and fosfomycin would be categorized as MDR.

Various definitions for combination therapy have been proposed in different studies. Any combination of drugs, at least of one *in vitro* active drug or of at least two *in vitro* active drugs are some examples, leading to confounding results [10]. A reasonable compromise in most observational studies is focusing on adequate combination therapy, defined as combination therapy including at least two active antibiotics to which the bacteria is susceptible *in vitro*. However, it is important to emphasize that antibiotics inactive *in vitro* result in increased drug concentrations *in vivo* enough to overcome resistance and lead to a favorable outcome and 'adequate treatment' and will be discussed in the herein review [6,7,11].

In the present review, the existing evidence regarding the benefits and drawbacks of combination therapy for XDR gram-negative pathogens were thoroughly examined. Questions to be answered were: (1) Which combinations are found synergistic from *in vitro* and animals studies? (2) Is there evidence from clinical studies that these combinations are synergistic *in vivo* and in terms of clinical outcome? (3) Is there evidence that combination treatment for XDR gram negatives is always necessary for a successful clinical outcome or the necessity depends on risk factors for failure? The three most prevalent XDR gram negatives, consisting of carbapenemase-producing *K. pneumoniae, A. baumannii*, and *P. aeruginosa* are separately discussed in the herein review to highlight the advantage and disadvantages of combination therapy. It is known that XDR strains are susceptible only to last-line regimens i.e. colistin and due to accumulative reports on this antibiotic, most of the review focused on combinations with colistin. The conclusions derived are based mostly on low-quality evidence, as most reported studies are retrospective. However, the current situation reflects the real-life clinical practice, while pending the results of well-designed randomized trials to clarify the question of combination treatment for XDR gram-negative pathogens.

3. Enterobacteriaceae

Carbapenemase-producing *Klebsiella pneumoniae* (Cp-Kp) is the most prevalent and virulent strain belonging to the family of Enterobacteriaceae. Resistance to carbapenems in *K. pneumoniae* involves multiple mechanisms, including the production of carbapenemases (e.g. KPC, NDM, VIM, OXA-48 like), as well as alterations in outer membrane permeability mediated by the loss of porins and the upregulation of efflux systems [12]. Mortality rates in infections due to *K. pneumoniae* with carbapenemases often mount up to 75%, which are attributed to the lack of active antimicrobial agents and underlying comorbidities of patients [5]. Treatment options for Cp-Kp are limited to colistin, tigecycline, aminoglycosides, and fosfomycin, whereas ceftazidime-avibactam, a novel cephalosporin/β-lactamase inhibitor has been recently introduced to the therapeutic armamentarium [1]. In the present section, the focus will mainly be on *K. pneumoniae*-producing carbapenemase; however, a brief report on Enterobacteriaceae will also be presented.

3.1. In vitro *studies*

A variety of combinations of different antimicrobial agents have been evaluated mainly by time–kill curve studies and checkerboard techniques, while pharmacodynamic *in vitro* studies are being introduced in the newer years [13]. In a systematic review and meta-analysis of *in vitro* synergy of polymyxins and carbapenems, a synergy rate of 44% among 146 Cp-Kp isolates was observed with antagonism to be reported at a rate of 15%. Evaluation of carbapenem-resistant, colistin-susceptible *K. pneumoniae* found an overall synergy rate of 55% [14]. In a time–kill assay study, tigecycline alone and in combination with colistin and meropenem was tested against eight KPC-producing clinical strains of Enterobacteriaceae. The combination of tigecycline and colistin was more efficacious; however, the combination of tigecycline and meropenem was not synergistic against all strains [15]. In another 24-h time–kill experiment with VIM- and NDM-producing *K. pneumoniae* strains, synergistic or bactericidal activity was demonstrated for aztreonam, fosfomycin, meropenem, and rifampin in double-antibiotic combinations with colistin as well as for aztreonam, fosfomycin, and rifampin in triple-antibiotic combinations with meropenem and colistin, the combination of rifampin-meropenem-colistin being the most effective regimen [16]. Surprisingly, in the era of emerging PDR strains, Bulik and Nicolau [17], investigated the revolutionary combination of double carbapenems against KPC strains in the *in vitro* chemostat model, as well as in the *in vivo* thigh model and depicted enhanced efficacy of combination treatment over monotherapy in both models. The proposed theory indicates the administration of ertapenem, as a suicide substrate based on its increased affinity for KPCs, hindering subsequently doripenem or meropenem degradation in the environment of the targeted Cp-Kp strains [17].

Regarding *in vitro* pharmacodynamic/pharmacokinetic models, combination treatment with different scheme of antibiotics seems superior against monotherapy [18–21]. Particularly, in a dynamic time–kill experiment evaluating fosfomycin (FM) in combination with amikacin (AMK) or colistin

(CST) against KPC-producing *K. pneumoniae*, combination of FM/CST and FM/AMK indicated a substantial increase in bactericidal activity over monotherapies, although regrowth was observed. Furthermore, AMK and CST alone showed more bactericidal effect than FM [18]. Moreover, the pharmacodynamic (PD) activities of polymyxin B, rifampin, and meropenem alone and as polymyxin B-based double and triple combinations against polymyxin B-sensitive and polymyxin B-resistant KPC-producing *K. pneumoniae* isolates were evaluated in a static time-kill curve study. Additionally, humanized triple-drug regimens of polymyxin B, rifampin, and meropenem dosed as an extended 3-h infusion simulated over 48 h by using a one-compartment *in vitro* dynamic infection model were also examined suggesting monotherapy as ineffective against both strains, whereas bacterial activity with triple combination was sustained against both strains by 8 h and remained over 48 h [19]. Additionally, in a time–kill experiment and hollow-fiber infection model, it was demonstrated that colistin or fosfomycin monotherapy were ineffective and resulted in rapid proliferation of resistant subpopulation with regrowth by 24 h; however, the colistin/fosfomycin combination resulted in a rapid 6.15 log 10 cfu/mL reduction by 6 h and complete suppression of resistant subpopulations until 120 h [20]. Similarly, in a single-chamber *in vitro* model, fosfomycin and colistin sulfate in combination achieved increased bacterial killing and decreased the chance of emergence of resistance [21].

3.2. Evidence from animal studies

The superiority of combination treatment against CRE has been illustrated in different animal models. In a rat model of sepsis, treatment with polymyxin B and tigecycline as well as triple combination with polymyxin B plus tigecycline plus meropenem demonstrated superiority to monotherapy in terms of survival and bacterial eradication [22]. A significant point to comment is that the combination of tigecycline and meropenem showed lower survival benefit and was antagonistic, with similar results observed *in vitro* [15,22]. Furthermore, the combination of tigecycline combined with colistin was found superior in a Galleria mellonella infection model against XDR Enterobacteriaceae-producing KPC, OXA-48, NDM, and VIM. It must be pointed out that antagonism with the above combination was found against *Serratia marcescens* [23]. On the other hand, in a murine thigh infection model caused by KPC isolates, combination of tigecycline plus colistin and tigecycline plus meropenem was found antagonistic in 33.3–44.4% and 44.4–55.5% of the strains, respectively. The best performance regarding bacterial eradication was observed with the combination of tigecycline with gentamycin or tigecycline with rifampicin [24]. Similarly, a murine sepsis model against OXA-48 failed to demonstrate superiority against the combination of tigecycline with colistin in parallel with previous study [25]. Therefore, since the reported studies in the animal models lead to conflicting conclusions, prospective randomized controlled clinical trials (RCT) in humans are essential to clarify the field.

3.3. Evidence from clinical studies

The benefit and drawbacks of combination treatment over monotherapy has been reported in several studies for infections caused by XDR Enterobacteriaceae; however, all studies due to high heterogeneity of study design and population have been characterized as very low-quality evidence, originating from classification and selection bias as well as unattended confounding and uncontrolled assignment of treatment regimens [10]. A systematic review of the literature of relevant studies was conducted using the PubMed database from 1970 up to August 2017 using keywords: 'Enterobacteriaceae,' 'K. pneumoniae,' 'combination therapy,' 'monotherapy,' 'carbapenemase,' 'XDR,' 'bloodstream infections,' 'sepsis,' 'outcome,' and 'mortality.' Original studies including at least 30 patients with infection caused by XDR Enterobacteriaceae were included. Studies reporting information on treatment options (monotherapy and combination treatment) as well as clinical outcomes and mortality were considered eligible for inclusion.

Based on the above criteria, 19 studies conducted from 2005 until 2016, mostly retrospective with the exception of five prospective observational cohort studies, were included for review. Randomized trials reporting on combination treatment (NCT01732250 and NCT01597973) are ongoing or have been recently completed, thus have not be included. A total of 3201 patients infected with carbapenemase-producing Enterobacteriaceae were included (Table 1) [26–44]. The majority of the isolates were CRE-*K.pneumoniae* (96% – 3087 patients) and the most prevalent carbapenemases reported were KPC in 90% (2338 isolates) followed by VIM, NDM, and OXA in 5, 3, and 0.1% accordingly, whereas a combination of KPC and VIM was reported in 1.9%. Thirteen studies exclusively included bloodstream infections (BSI) (83% – 2656 patients) [26–29,33,35–37,39,40,42–44], whereas six studies reported on a variety of infections (17%) including pneumonia, urinary tract infections (UTI), intra-abdominal infections (IAI), skin and soft-tissue infections (SSTI), surgical site infections (SSI) and other infections in 7, 6.8, 2, 0.3, 0.4, and 0.5%, respectively [30–32,34,38,41]. A large proportion were intensive-care unit (ICU) patients [26,31,38,39,43], but a significant number of patients were mixed from ICU as well as the surgical and medical wards [27–30,32–35,40–42,44]. A small portion of studies included patients with hematological malignancies [36,37]. Septic shock cases were reported in 15 out of 19 studies and ranged from 14 to 100% (Table 1).

The efficacy of different antibiotic regimens as part of definite treatment was assessed through the reviewed studies and a total of 2.972 patients with infections caused by CRE were compiled and analyzed. In more detail, 207 (7%) received inappropriate therapy (no drug was active *in vitro*), 1206 (40.5%) received monotherapy (one drug was active *in vitro*), and 1.559 (52.5%) received combination therapy (at least two drugs were active *in vitro* against the infecting organism). It should be noted that inclusion of a carbapenem in the combination treatment was also evaluated (635 patients). Mortality rates of infections caused by CRE according to treatment options are illustrated in Figure 1.

Combination therapy as an independent predictor of mortality was demonstrated in 11 studies [28,29,33,34,36–39,42–44],

Table 1. Clinical studies comparing monotherapy to combination therapy for infections caused by carbapenemase-producing Enterobacteriaceae [26–44].

First author -reference[a]	Study period	Study design	Number of Pts	Pathogens	Site of infection	Pts characteristics	Type enzyme	Treatment options[b] (number of survivors/all patients)	Mortality rate	Comments and OR (95% CI)
Mouloudi [26]	2007–2008	Prospective	37	*K. pneumoniae*	BSI	ICU population APACHE II: 23 (4–36) Septic shock: NR	KPC:19 VIM:18	**Monotherapy**: 8/20 **Combination**: 8/17	In-hospital mortality: 56% for MBL group 79% for KPC group	In univariate analysis, no difference between monotherapy and combination treatment OR: 1.2 (0.4–3.8)
Zarkotou [27]	2008–2010	Prospective	53	*K. pneumoniae*	BSI • Primary:23 • CRBSI:12 • Pneumonia:7 • UTI: 6 • SSTI:4 • CNS:1	Mixed population APACHE II: 21 ± 7.3 Severe sepsis or septic shock: 39.6%	KPC	**Inappropriate**: 7/11 **Monotherapy**: 8/15 **Combination**: 20/20 - Carb-com: 4/4 - Non-Carb com:16/16	Overall: 52.8% D14: 28.3%	In univariate analysis, combination schemes were associated with lower infection mortality ($p = 0.0001$) In multivariate analysis combination therapy was not an independent predictor of survival OR: NR
Qureshi [28]	2005–2009	Retrospective	41	*K. pneumoniae*	BSI • Primary:6 • CRBSI:13 • Pneumonia:10 • UTI:7	Mixed population APACHE II Monotherapy: 21.3 ± 8.7 Combination: 17.4 ± 6.7 Septic shock: NR	KPC	**Inappropriate**: 2/7 **Monotherapy**: 8/19 **Combination**: 13/15 - Carb-com: 7/9 - Non-Carb com: 6/6	D28: 39%	In multivariate analysis, combination therapy was independent predictor of survival OR: 0.07 (0.009–0.71)
Tumbarello [29]	2010–2011	Retrospective	125	*K. pneumoniae*	BSI • UNK:75 • CRBSI:13 • Pneumonia:28 • UTI:25	Mixed population CCI: 2 APACHE II: Non-survivors: 40 ± 22 Survivors: 24 ± 15 Septic shock: 14%	KPC	**Monotherapy**: 21/46 **Combination**: 52/79 - Carb-com: 29/37 - Non-Carb com:23/42	D30: 41.6%	In multivariate analysis, combination therapy with TGC+CST+MEM was independent predictor of survival OR: 0.11 (0.02–0.69)
Capone [30]	2010–2011	Prospective	91	*K. pneumoniae*	BSI:34 UTI:29 Pneumonia:14 SSTI:11 IAI:3	Mixed population CCI:5 APACHE II:15 Septic shock:16.5%	KPC: 89 VIM: 3	**Monotherapy**: 21/26 **Combination**: 37/54	In hospital mortality: 25.8%	In univariate and multivariate analysis, combination therapy was not associated with mortality ($p = 0.70$)
Kontopidou [31]	2009–2010	Retrospective	127	*K. pneumoniae*	BSI:69 Primary:30 CRBSI:39 VAP:35 UTI:13 IAI:6 SSI:4	ICU population APACHE II: 18.2 Septic shock: NR	KPC: 52 VIM: 22 KPC+VIM: 6	**Inappropriate**: 13/20 **Monotherapy**: 35/65 **Combination**: 25/42 - Carb-com: 1/1 - Non-Carb com:24/41	D14: 25.2%	In a logistic regression analysis, combination therapy was not superior to monotherapy in terms of survival benefit OR: NR
De Oliviera [32]	2009–2013	Retrospective	118	*K. pneumoniae*: 108 *S. marcescens*: 6 *E. cloacae*: 3 *E. coli*: 1	CRBSI:51 UTI:26 Pneumonia:8 SSI:10 IAI:13 Other:10	Mixed population APACHE II-survivors:16 Non-survivors:18 Septic shock: NR	KPC	**Monotherapy**: 36/57 **Combination**: 29/61 - Carb-com: 23/54 - Non-Carb com: 6/7	D30: 45%	In bivariate analysis, combination therapy was not superior to monotherapy in terms of survival benefit OR: 1.93 (1.15 – 3.22)

(*Continued*)

Table 1. (Continued).

First author -reference[a]	Study period	Study design	Number of Pts	Pathogens	Site of infection	Pts characteristics	Type enzyme	Treatment options[b] (number of survivors/all patients)	Mortality rate	Comments and OR (95% CI)
Daikos [33]	2009–2010	Retrospective	205	*K. pneumoniae*	BSI • Primary:83 • CRBSI:22 • Pneumonia:43 • UTI:19 • SSTI:6 • IAI:29 • CNS:3	Mixed population survivors: CCI:1 McCabe and Jackson -rapidly fatal: 15 Non-survivors CCI:2 McCabe and Jackson -rapidly fatal: 28 Septic shock:19%	KPC: 163 VIM: 42 KPC+VIM:36	**Inappropriate**: 8/12 **Monotherapy**: 40/72 **Combination**: 75/103 - Carb-com: 25/31 - Non-Carb com: 50/72	D30: 40%	In cox proportional hazard model, combination therapy was an independent predictor of survival in patients with rapidly fatal underlying disease and patients with septic shock OR:0.08 (0.01–0.52) OR: 0.22 (0.05–1.0)
Tumbarello [34]	2010–2013	Retrospective	661	*K. pneumoniae*	BSI:447 Pneumonia:85 UTI:82 IAI:42 Other:5	Mixed population CCI ≥3: 51% APACHE II >15: 73% Septic shock: 15%	KPC	**Monotherapy**: 189/307 **Combination**: 247/354 - Carb-com: 156/213 - Non-Carb com: 91/141	D14: 34.1% In hospital mortality: 41.1%	In multivariate analysis, combination therapy was independent predictor of survival OR: 0.52 (0.35–0.77)
Gomez-Simmonds [35]	2006–2013	Retrospective	141	*K. pneumoniae*	BSI • CRBSI:10 • Pneumonia:19 • UTI:31 • IAI:32 • SSTI:7	Mixed population CCI ≥4: 55% PBS≥4:43% Septic shock:31%	KPC (33 isolated only tested)	**Monotherapy**: 50/68 **Combination**: 45/73 - Carb-com: 22/33 - Non-Carb com: 23/40	D30: 33%	In a univariate analysis, combination therapy was not superior to monotherapy in terms of survival benefit ($p = 0.1$)
Trecarichi [36]	2010–2014	Prospective	161	*K. pneumoniae*	BSI • UNK:102 • CRBSI:22 • Pneumonia:19 • UTI:17	Neutropenic pts with hematological diseases CCI:2 Septic shock: 30%	NR	**Monotherapy**: 8/40 **Combination**: 69/109 - Carb-com: 45/77 - Non-Carb com: 24/32	D21: 52.2%	In multivariate analysis, combination therapy was independent predictor of survival OR: 0.32 (0.19–0.54)
Tofas [37]	2010–2014	Retrospective	50	*K. pneumoniae*	BSI • BSI primary:41 • CRBSI:5 • Pneumonia:2 • UTI:2	Neutropenic pts with hematological diseases CCI:2 Septic shock: 26%	KPC: 48 VIM: 2	**Inappropriate**: NR/4 **Monotherapy**: 5/10 **Combination**: 19/30	D14: 50%	In cox proportional hazard model, combination therapy was independent predictor of survival OR: 3.95 (1.23–12.65)
Falcone [38]	2010–2014	Retrospective	111	*K. pneumoniae*	BSI:53 CRBSI:25 Pneumonia:52 UTI:25 SSTI:18 IAI:12	ICU population APACHE II: 20.7 ± 5.6 SAPS: 45.2 ± 16.2 Septic shock: 100%	KPC	**Inappropriate**: 0/3 **Monotherapy**: 10/44 **Combination**: 57/64	D30: 39.6%	In multivariate analysis, combination therapy was independent predictor of survival OR: 0.08 (0.02–0.21)
Papamiditriou-Olivgeris [39]	2012–2015	Retrospective	139	*K. pneumoniae*	BSI • Primary: 88 • CRBSI:46 • IAI:4 • VAP:1	ICU population APACHE II: 17.9 ± 6 SAPS: 39.7 ± 11.4 SOFA: 8.8 ± 3.2 Septic shock: 58%	KPC:128 KPC+VIM:7 VIM:3 NDM:1	**Inappropriate**: 19/44 **Monotherapy**: 39/57 **Combination**: 31/38	D30: 36%	In multivariate analysis, combination therapy was independent predictor of survival OR: 0.31 (0.12–0.76) No specific data on antibiotic treatment

(*Continued*)

Table 1. (Continued).

First author -reference[a]	Study period	Study design	Number of Pts	Pathogens	Site of infection	Pts characteristics	Type enzyme	Treatment options[b] (number of survivors/all patients)	Mortality rate	Comments and OR (95% CI)
Satlin [40]	2013	Retrospective	131	*K. pneumoniae*: 97 *Enterobacter spp*: 5 *E. coli*: 3 *M.morganii*:1	BSI • CRBSI:8 • Pneumonia:11 • UTI:25 • IAI:51 • SSTI:7 • UNK:19	Mixed population Septic shock: 38%	KPC: 97 OXA:1 NDM:1	**Inappropriate**: 3/12 **Monotherapy**: 34/55 **Combination**: 21/43 - Carb-com: 6/16 - Non-Carb com: 15/27	D30: 38% (monotherapy) D30: 49% (combination)	In multivariate analysis, no difference between monotherapy and combination treatment OR: 1.56 (0.63–3.88)
Alexander [41]	2013–2014	Retrospective	256	*K. pneumoniae*:222 *E.*cloacae:15 *E.coli*:7 *P.mirabilis*:5 *S.*marcescens:4 *K.oxytoca*:2 *C.freundii*:1	cUTI/AP:75 HABP:21 VAP:20 BSI:140	Mixed population CCI: 3 APACHE II: 21.9 Septic shock: 29%	KPC:167 MBL:5 OXA:1 UNK:50 (*K. pneumoniae*)	**Monotherapy**: 37/52 **Combination**: 66/115	D28: 28.1%	In multivariate analysis, no difference between monotherapy and combination OR: 1.6 (0.8–3.2)
Gutiérrez-Gutiérrez [42]	2004–2013	Retrospective	437	*K. pneumoniae*:375 *E.cloacae*:28 *E.coli*:17 *E.aerogenes*:13 *Citrobacter spp*:3 *S.marcescens*:1	BSI	Mixed population Pitt score: 2 CCI: 2 Severe sepsis-Septic shock: 50%	KPC:329 OXA:69 MBL:39	**Inappropriate**: 37/94 **Monotherapy**:123/208 **Combination**: 88/135 - Carb-com: 23/37 - Non-Carb com: 65/98	D30: 38%	In multivariate analysis, combination therapy was an independent predictor of survival in patient with high mortality score 0R: 0.56 (0.34–0.91)
Machuca [43]	2012–2016	Prospective	104	*K. pneumoniae*	BSI • Primary:38 • Pneumonia:39 • UTI:27	ICU, hematology CCI: 4 Septic shock: 46%	KPC	**Monotherapy**: 14/32 **Combination**: 18/72	D30: 30.8%	In multivariate analysis, combination treatment was an independent predictor of survival in patients with septic shock OR: 0.14 (0.03–0.67)
Cristina [44]	2013–2014	Retrospective	213	*K. pneumoniae*	BSI	Mixed population CCI: 2 Septic shock: 20%	NR	**Monotherapy**: 10/13 **Combination**: 113/135 - Carb-com: 107/123 - Non-Carb com: 6/12	D15: 26%	In multivariate analysis, combination treatment was an independent predictor of survival in patients treated with combination therapy including a carbapenem OR: 0.11 (0.03–0.43)

AP: acute pyelonephritis; APACHE: acute physiology and chronic health evaluation; BSI: blood stream infection; Carb com: carbapenem containing combination; Charlson comorbidity index; 95% CI: 95% confidence interval; CNS: central nervous system; CRBSI: catheter-related blood stream infection; CST: colistin; cUTI: complicated urinary tract infection; D: day; IAI: intra-abdominal infection; HABP: hospital-acquired bacterial pneumonia; ICU: intensive care unit; KPC: Klebsiella pneumoniae carbapenemase; MBL: metallo-beta-lactamase; MEM: meropenem; NDM: New Delhi metallo-beta-lactamase; NR: not reported; Non-Carb com: combination not containing a carbapenem; OR: odd ratio; OXA: oxacillinase; PBS: Pitt bacteremia score; pts: patients; SAPS: simplified acute physiology score; SOFA: sequential organ failure assessment; SSI: surgical site infection; SSTI: skin and soft-tissue infection; TGC: tigecycline; UNK: unknown; UTI: urinary tract infection; VAP: ventilator-associated pneumonia; VIM: Verona integron-encoded metallo-β-lactamase.

[a]Articles presented based on year of publication

[b]Treatment options based on definite treatment. Inappropriate therapy was defined as no drug *in vitro* active; Monotherapy as one drug active *in vitro*; and combination therapy as two or more drugs active *in vitro*; combination treatment was subanalyzed (whenever reported) if combination treatment included a carbapenem (MIC ≤8 mg/L) (carb-com) or not (Non-carb com).

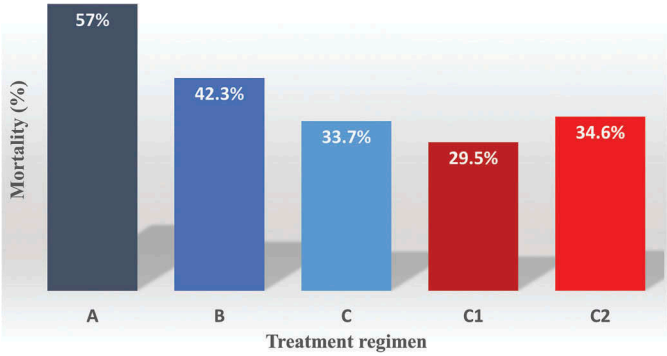

Figure 1. Mortality rates of 2.972 patients with infections caused by CP-Kp according to treatment regimen. Column A: Inappropriate therapy (no drug was active *in vitro*); Column B: Monotherapy (one drug was active *in vitro*); Column C: Combination therapy (two or more drugs were active *in vitro*); Column C1: combination therapy with two or more *in vitro*-active drugs, including a carbapenem (MIC ≤ 8mg/L); Column C2: Combination therapy with two or more *in vitro*-active drugs not including a carbapenem [26–44].

whereas in the remaining studies superiority of combination therapy was not depicted in multivariate analysis [26,27,30–32,35,40,41] (Table 1). It should be emphasized that combination treatment has a distinct statistically significant therapeutic advantage over monotherapy in high-risk and more severely ill patients [33,34,42]. In a retrospective study with 205 patients with BSI caused by Cp-Kp, monotherapy was associated with significantly higher mortality rate and high dose and prolonged infusion carbapenem-containing combinations (provided that MIC of meropenem of isolates was ≤8 mg/L) was strongly associated with survival in patients with rapidly fatal underlying disease and septic shock [33]. Similar findings were observed in the largest retrospective cohort from Italy including 661 adults, with the majority of infections being BSI (n = 447). Combination therapy with at least two active *in vitro* drugs was associated with lower mortality (30.2 vs. 38.4%, p = .03), whereas a favorable impact on survival was observed when a combination included a high-dose meropenem and the isolate had a meropenem MIC of ≤8 mg/L. The protective effect of combination treatment was noted in patients with BSI, lower respiratory infections, APACHE III score of >15, septic shock at infection onset, whereas indication for monotherapy was illustrated in non-bacteremic UTI infections caused by Cp-Kp [34]. In parallel, the Increment project with 437 BSI caused by CRE from 26 tertiary hospitals in 10 countries, concluded that combination therapy was associated with lower mortality than monotherapy in the high-mortality-score stratum (48 vs. 62%, p = 0.02). In patients at lower risk of mortality, no clear survival benefit of combinations over monotherapy was demonstrated [42]. The INCREMENT CPE mortality score was utilized in the previous study for the assessment of the stratum and the effect of combination therapy. Severe sepsis or septic shock (5 points), a Pitt bacteremia score of at least 6 (4 points), a Charlson comorbidity index score of at least 2 (3 points), a source of BSI other than urinary or biliary tract (3 points) were calculated and high mortality score was indicated from 8 to 15 [45]. Furthermore, combination therapy has also been proven to provide survival benefit in hematological patients with febrile neutropenia and Cp-Kp BSI in two retrospective studies with 161 and 50 patients suffering from hematological malignancies and aplastic anemia, respectively [36,37]. Similarly, in ICU patients with septic shock treatment with combination therapy was strongly correlated with survival [38,39,43]. In a study with 111 ICU patients with KPC-Kp infections (resistance rates to colistin: 51.3%), mainly BSI (47.7%) and pneumonia (46.8%) complicated with septic shock, combination treatment including two or more antibiotics displaying *in vitro* activity was found superior when treatment was commenced within 24 h. It must be also highlighted that a prompt control of a removable source of infection was associated with a favorable outcome [38]. Similarly, in a Greek study with 139 ICU patients who developed Cp-Kp bacteremia, multivariate analysis revealed that administration of combination treatment in septic shock patients was identified as a predictor of survival, whereas concomitant corticosteroid administration, isolates carrying a bla_{KPC} gene and SOFA score at onset of infection were predictors of mortality [39]. Last, in a prospective cohort study including 104 ICU and hematological patients with bacteremia due to Colistin-resistant Cp-Kp with high-level meropenem resistance (MIC ≥64 mg/L), the impact of targeted combination therapy was independently associated with reduced mortality in the subgroup of patients with septic shock [43].

Information regarding exact therapeutic options was available from 12 studies [26–29,31–33,35,36,38,40,43]. The antimicrobial regimens mostly administrated in the monotherapy arm were polymyxins, tigecycline, aminoglycosides fosfomycin as well as carbapenems [26–36,40,42]. Regarding the combination treatments, a heterogeneity of double- and triple-combination regimens was administrated. The antimicrobial agents most commonly administrated in double or triple combinations were colistin plus tigecycline, carbapenem plus colistin [27–33,35,36,40,44] and carbapenem with colistin and tigecycline accordingly [27,29,31–33,35,36]. Furthermore, a wide variety of different combination schemes of colistin, tigecycline, gentamicin, amikacin, or fosfomycin as well as conditionally carbapenems in double and triple combinations were also administrated [26–44]. Dosing of the antibiotics was reported only in six studies [29,31–33,35,37,43]. However, the major question on which combination regimen is superior is far from being clear. Only one study by Tumbarello et al. [29] stated a survival benefit with a specific antibiotic combination consisting of colistin plus tigecycline and meropenem in the multivariate analysis [29]. As illustrated in Figure 1, the combination therapy with two or more *in vitro* active drugs, including a carbapenem demonstrated the lowest mortality rate of 29.5%.

Moreover, indications of administration of high-dose meropenem in infections caused by Cp-Kp are apparent; however, the exact level of carbapenem MIC prohibiting meropenem administration is quite questionable [29,33,34]. It has been reported that high-dose carbapenems-based combination therapy may be a reasonable treatment option against Cp-Kp, provided that (1) MIC of the infecting organism is ≤8 mg/L, (2) carbapenem is given in combination with another active compound, i.e. colistin, tigecycline, and aminoglycoside; and (3) carbapenem is given in high dose (2 g q8h) and prolonged infusion (3 h) [29,33]. Recently, a *post hoc* analysis of 595 patients with Cp-Kp BSI from Italy with 77% of the isolates exhibiting a carbapenem MIC ≥16 mg/L, evaluated the impact

of high-dose carbapenems-based combination therapy on mortality. The multivariate analysis marked the administration of carbapenem as a protective factor and the benefit was also observed for strains with carbapenem MIC ≥16 mg/L. A major drawback of the study is the lack of stratification of carbapenem MIC hindering the exact MIC level where high-dose carbapenems may be administrated safely [46]. However, based on pharmacokinetic/PD (PK/PD) models using high dose and prolonged infusion of meropenem in patients with BSI caused by Cp-Kp, a target of meropenem MIC up to 32–64 mg/L is possible to be achieved [47,48]. The administration of carbapenem-based regimens in cases of meropenem MICs >8 mg/L might be considered for MICs up to 32–64 mg/L, provided that TDM is available to monitor optimal drug exposure [49].

Ceftazidime-avibactam (CAZ-AVI), a novel cephalosporin/β-lactamase inhibitor combination, recently launched into market has been evaluated in a retrospective study including 109 ICU patients with Cp-Kp bacteremia. Thirteen patients received CAZ-AVI (five concomitantly received aminoglycoside for a median of 4 days) and clinical success at 30 days was reported significantly higher among patients receiving CAZ-AVI compared to comparators i.e. carbapenem and aminoglycoside, carbapenem and colistin being 85, 48, and 40%, respectively [50]. However, the small sample size and the emergence of resistance in microbiological failures [51] render the elucidation of the true efficacy of CAZ-AVI in the era of MDR–XDR pathogens rather indistinct and further randomized trials are urgently needed.

In conclusion, treatment of CRE infections with combination therapy is indicated in patients with severe underlying diseases and septic shock and is associated with benefit in terms of survival; however, in low-risk BSIs and non-bacteremic urinary and abdominal infections, monotherapy seems an adequate therapeutic choice.

3.4. Salvage treatment

The combination of two carbapenems (ertapenem 1 g every 24 h followed by meropenem 2 g every 8 h with prolonged infusion of 3 h) is a revolutionary and salvage approach suggested by Bulik and Nicolau, as a unique and potential treatment challenge against XDR and PDR KPC-producing *K. pneumoniae*. The explanation of the observed synergistic effect was based on the assumption that ertapenem partially inactivates the carbapenemase activity, permitting meropenem to express its effect [17]. *In vitro* experiments have been proved promising against serine-β-lactamase producers (KPC, OXA-48), including not only the combination of meropenem plus ertapenem, but also with imipenem plus meropenem and imipenem and ertapenem. However, the double carbapenem combination (DCC) has not been found, at least *in vitro*, active against *K. pneumoniae* strains producing NDM enzymes [52]. Lack of synergy between two carbapenems has also been addressed in *in vitro* and animal studies [53,54]. Interestingly, an accumulative number of cases in humans demonstrated the effectiveness of the dual carbapenems for KPC infections [55–59]. First *in vivo* experience was a successful report of three patients, two of them with bacteremia [55]. Current clinical experience with DCC includes 211 patients (58% BSI). Overall clinical success was 70% and all-cause mortality 26%. However, 33.2% of patients received additional active *in vitro* antibiotics, mainly colistin precluding the evaluation of the efficacy of DCC [38,44,55–59]. The largest cohort of patients administered exclusively DCC (without other concomitant active *in vitro* antimicrobials) as salvage and exclusive therapy for XDR and PDR Cp-Kp infections included 27 patients. Complicated UTIs (cUTI) were the most common infections (59.3%) followed by BSI (48.2%). A successful clinical outcome was noted in 77.8%, while a successful microbiological outcome in 74.1% patients. It is of great significance to mention that the subgroup of 14 patients with PDR infections had a successful clinical and microbiological outcome in 78.5%, whereas among patients with severe sepsis or septic shock, a successful outcome was noted in 81.8% [56]. In another study, 32 patients with carbapenem-resistant *K. pneumoniae* received ertapenem and high-dose meropenem as exclusive treatment (18 patients), whereas 14 patients received DCC plus colistin. Triple-combination therapy was used more frequently in patients presenting with septic shock. A successful clinical outcome of 75% was observed, whereas relapse and death in 6.2 and 18.7% of cases accordingly [59]. Furthermore, in a case–control study, DCC was administrated in 48 critically ill patients and compared to 96 control patients treated with standard treatment (i.e. colistin, tigecycline, aminoglycoside) for documented infections by Cp-Kp. The 28-day mortality was significantly higher in the arm of standard treatment compared to DCC group (47.9 vs. 29.2%, $p = 0.04$) and in multivariate analysis, DCC regimen was associated with reduction in mortality (OR: 0.33, 0.13–0.87) [58]. Despite the promising results, including PDR cases where DCC was administrated particularly as salvage therapy, a randomized prospective clinical study comparing DCC without concomitant antibiotics with standard of care in critically ill patients seems to be indicated.

4. Acinetobacter baumannii

Resistance to carbapenems is mainly due to production of β-lactamases in *Acinetobacter baumannii* (AB). Carbapenem-hydrolyzing class D β-lactamases are the most commonly identified carbapenemases in *A. baumannii*. However, the involvement of efflux pump systems and porin modifications has also been reported [60]. Treatment options for carbapenem-resistant XDR AB infections include colistin, tigecycline, aminoglycosides, sulbactam. Selected strains could be susceptible to co-trimoxazole and minocycline but rarely to tetracyclines, either as monotherapy or in combination [61].

4.1. In vitro *studies*

Seventy studies and 31 conference proceedings on colistin/polymyxin B combinations against MDR/XDR AB including a high number of isolates (1484) were recently reviewed and subjected to meta-analysis by Ni et al. [62]. Relevant papers for the years 2008–2014 and proceeding of ICAAC, IDSA, and ESCMID conferences for the years 2006–2013 were included.

With the application of the time-kill methodology, synergy was obtained in 84.9% of 273 strains from 26 studies after the combination of colistin with a carbapenem, without any significant difference between colistin-R and colistin-S strains, whereas combinations suppressed the emergence of colistin resistance. More specifically, in 100 AB strains harboring at 79% rate bla_{OXA-23}-like enzymes, of which 84% were being resistant to meropenem and 78% to imipenem and only one strain to colistin, combination with carbapenems showed an additive effect with a 2.6–2.8-fold decrease in the MIC of colistin, without bacterial regrowth following a 24-h incubation [63]. It was pointed out that colistin/carbapenems combinations could represent a promising strategy at clinical level possibly permitting lower doses of colistin in order to reduce the incidence of nephrotoxicity.

Interestingly, colistin with levofloxacin exhibited at a rate of 84% synergistic effect against AB [64]. Promising results at a rate of 57.2% in 280 isolates from 22 studies were also obtained in the presence of rifampicin [62]. In a very recent systemic review and meta-analysis, the interactions of colistin with rifampicin against MDR AB strains were examined [65]. Seventeen studies from 1998 until 2014 as well as ECCMID posters printed from 2007 to 2014 including 448 strains were eligible. In eleven the time-kill and in six the checkerboard methods were applied. MIC90 for both rifampicin and colistin were ≤4 μg/ml. Results demonstrated synergy in 63% of the strains, with partial synergy and additive effect in 7 and 3%, respectively, without any antagonistic effect. Regarding combinations of colistin with tigecycline, testing of 135 strains from 13 studies yielded synergy in 41.6% [62]. However, it should be pointed out that combination results were in general inferior whenever polymyxin-B was used [62].

Recently the PD of tigecycline alone and in combination with colistin against four clinical XDR AB isolates, resistant to carbapenems but susceptible to both colistin and tigecycline in an *in vitro* PD model was studied [66]. When tigecycline or colistin monotherapy levels were below mutant prevention concentration (MPC), tigecycline and colistin MICs increased 4–32- and >16-fold, respectively. However, no loss in susceptibility to tigecycline was found with combination therapy indicating that at the PK/PD level a combination of high-dose tigecycline (100 mg 12 hourly) and colistin could be an effective therapy to prevent the emergence of resistance to tigecycline.

Interestingly, a synergy rate of 70.8% has been obtained in nine studies after combining colistin with linezolid or various glycopeptides [13,67]. The latter observation was attributed to the permeability effect of colistin on the outer membrane of AB allowing the entrance of the large glycopeptide molecule into the cell.

As pointed out by Ni et al. [62], it should be seriously considered that there is a great discordance between different testing methods, since in their review and meta-analysis a difference of almost 40% in favor of the time-kill method was observed whenever polymyxin/carbapenem combinations were analyzed.

Despite the herein reviewed promising synergistic results with a variety of colistin combinations, there is no doubt that carefully performed RCT are required to evaluate in humans the reported *in vitro* interactions.

4.2. Evidence from animal studies

Since 2004 in eight studies, colistin monotherapy was compared to colistin combinations in XDR AB animal model infections [68–75]. From the reported results, the following were observed:

(i) Colistin with rifampicin irrespective of the presence of immunosuppression was synergistic against carbapenem resistant AB mouse and rabbit pneumonia models significantly decreasing bacteremia rates as well [68–70]. Also in the mouse pneumonia model, the combination of colistin with minocycline against minocycline-resistant AB strains improved survival rates [71] as was also observed with the combination of colistin plus tigecycline [72].
(ii) Colistin plus rifampicin decreased significantly mortality rates as well as tissue bacterial load in the neutropenic wistar rat thigh soft-tissue infection model caused by an XDR AB strain [73]. In the same model,, combinations of tigecycline, fosfomycin, or sulbactam with colistin, were indifferent [74]. On the other hand, combinations with meropenem were synergistic only whenever meropenem MIC was ≤32 μg/ml.
(iii) In a sepsis murine model, combination with daptomycin or teicoplanin against an XDR AB strain enhanced therapeutic efficacy [75].
(iv) In the Galleria mellonella model, colistin plus glycopeptides or daptomycin showed improvement in survival [76].

Since the reported studies in the animal models lack homogeneity, they are only indicative of effectiveness pointing out the necessity of prospective randomized clinical trials in humans.

4.3. Evidence from clinical studies

After excluding systemic reviews and meta-analysis, all retrospective, prospective, or randomized control trials comparing intravenous colistin or polymyxin B as monotherapy versus any polymyxin combinations in adult patients with documented infections caused by polymyxin susceptible, and mostly carbapenem-resistant XDR-AB strains were included after searching PubMed from 2000 until June 2017. In cases that <7 patients were admitted per treatment arm, or inhaled colistin was administered, patients were excluded. If more than one combination with other class(es) of antibiotics in the same trial was prescribed, if fulfilling the inclusion criteria, it was included. Primary outcome was considered mortality at 28–30 days with secondary outcomes clinical and microbiological efficacy. Fifty studies were considered as potentially eligible for full-text review, but only 17 fulfilled the inclusion criteria [77–93] and were included in the present review. Demographic characteristics as well as distribution of therapeutic groups are described in Table 2. In the included studies AB isolates were reported as

Table 2. Demographic characteristics of patients included in 17 studies fulfilling the inclusion criteria.

Total number of patients	1.674
Monotherapy arm	785
Combination arm	889
Number of randomized control studies	3 [83,85,86]
Number of prospective observational studies	1 [80]
Number of retrospective observational studies	13 [77–79,81,82,84,87–93]
Age (mean/median)	**Years**
Monotherapy arm	51.7/70.6
Combination arm	61/73
Duration of therapy	**Range-days**
Monotherapy arm	6.3–14
Combination arm	9.8–18
Type of predominant infection[a]	**Number of patients**
Monotherapy arm	645
VAP	450
BSI	195
Combination arm	884
VAP	550
BSI	334
Severe sepsis [81,82]	**Number of patients**
Monotherapy	70
Combination	39
Septic shock [81,82]	**Number of patients**
Monotherapy arm	26
Combination arm	26
Severe sepsis/septic shock[80,93]	**Number of patients**
Monotherapy arm	38
Combination arm	15

Distribution of patients in monotherapy and combination therapy arms	Number of patients	
Combination of polymyxin with:	Monotherapy	combination
Carbapenems – 6 studies: [77,79,84,88,90,92]	170	292
Tigecycline – 4 studies: [80,84,87,91]	181	95
Sulbactam – 4 studies: [82,84,88,90]	128	137
Rifampicin – 3 studies: [83,85,89]	147	237
Fosfomycin – 1 study: [86]	47	47
Aminoglycosides – 1 study: [93]	23	10
Glycopeptides – 2 studies: [78,81]	89	71
Total number of patients	**785**	**889**

BSI: blood stream infection; VAP: ventilator-associated pneumonia.
[a]In one study, only total numbers were referred [80] and in another total number in the two arms were not clarified [78]. In two studies, mixed VAP and other respiratory tract infections were included [79,92]. In 140 and 5 patients in monotherapy and combination arms, respectively, other type of infections were included.

XDR in thirteen, as XDR/MDR in three, and as MDR in one. All strains were susceptible to colistin (≤2 μg/ml) without clarifying the applied methodology.

Length of hospital stay as well as superinfections and resistance development were not reported in the included studies, whereas underlying resistance mechanisms of AB to carbapenems were investigated only in one study in which isolates produced OXA-51-, OXA-23-, and OXA-24-type enzymes [93].

In 11 studies, colistin was given at a dose of 2–3 MIU/8 hourly [77–81,83–86,88,91], in three studies 1.5–2 MIU/8 or 12 hourly [82,89,90] whereas in one study no dosage schedule was reported [87]. In two studies, polymyxin-B was given at a dose of 150 mg/daily [92,93]. A loading dose was mentioned only in three trials [78,79,91].

Mortality at 28–30 days was evaluated in 14 studies, ranging from 23.5 to 67.6% and from 24.2 to 73%, respectively, when monotherapy and combination therapy were administered [77–83,85,86,88,89,91–93]. Three studies had to be excluded from evaluation because (i) 14-day mortality was described [84], (ii) in hospital death due to other reasons than infection was reported [87], and (iii) the monotherapy arm was not precisely determined [90]. With the exception of one study in which 92 patients with cancer (33 with hematological malignancies and 59 with solid tumors) were admitted [93], in the remaining 13 studies, no statistically significant differences were reported in mortality rates. In the latter study [93] overall 7-day and 30-day mortality rates were 71.7 and 83.7% respectively. However, the 7-day and 30-day mortality rates were 0.0 and 55.0% among patients receiving combination therapy compared with 47.8 and 60.9% among those on monotherapy ($p = 0.03$ and $p = 0.73$, respectively). Even more, in a multicenter trial where a heterogeneous group of infections caused by a variety of gram negatives were studied, therapy with combination of colistin plus a glycopeptide for ≥5 days had a protective effect on 30-day mortality [78]. Regarding cure and microbiological eradication rates, data were available only in 11 studies [79,81–90] in which cure and eradication rates ranged between 29.8–87% and 22–72.3%, respectively, in monotherapy, whereas relevant rates in combination therapy were 40–81.2% and 35–100%, respectively. Differences were statistically significant only in two studies [83,86]. Regarding the type of underlying infection, i.e. VAP (ventilator-associated pneumonia) and/or bacteremia as well as the presence of severe sepsis/septic shock, no superiority of combination therapy over monotherapy was observed. However, the number of recruited patients with

severe sepsis/septic shock is low, reported only in six studies [78,80–82,91,93] with distinction between severe sepsis and septic shock reported only in two studies [81,82]. In the remaining four studies, in two of them the number of included patients in each group was not precisely defined [78,93] and in the other two studies [80,91], severe sepsis and septic shock were presented as a mixed entity.

Finally, screening of nephrotoxicity was reported only in six studies [78,81,83,84,86,88]. With the exception of one [78], in which vancomycin was coadministered, no statistically significant differences were noticed in the remaining five studies.

From the reported data, it is evident that there are no convincing studies to recommend combinations in targeted therapy with carbapenems, colistin or sulbactam. Probably the heterogeneity of the reported studies, attributed to the retrospective design in most of them, the variability in the severity of the underlying infections, the different dosage schedules and duration of therapy as well as the lack of the MIC determination without definition of the underlying resistance mechanisms, should be incriminated for the inconclusive results. However, statistically significant differences were not observed between monotherapy and combination therapy regarding mortality and clinical efficacy either in the 13 herein included observational studies [77–79,81,82,84,87–93] or in the three RCTs that showed no mortality benefit for rifampicin or fosfomycin in combination with colistin for MDR/XDR AB infections [83,85,86]. Therefore, it seems that at least for stable patients, combination therapy does not affect mortality. However, in septic shock patients and particularly the immunocompromised host as well as in case of infections caused by isolates with borderline MICs, combination therapy, until more prospective data are available, should be recommended [93,94]. As also pointed out in a recent systematic review and meta-analysis, which ended up with similar conclusions as herein described, it is evident that well organized RCT are urgently required in the severely ill patients, particularly in the effort to avoid unnecessary combinations, which fuel the epidemics, and subsequently the endemicity of XDR AB strains [10].

5. Pseudomonas aeruginosa

Pseudomonas aeruginosa exerts resistance to carbapenems through a variety of mechanisms [including production of carbapenemases such as metallo-beta lactamases (MBLs), overexpression of AmpC β-lactamases, porin loss due to mutations in *oprD* gene or overexpression of efflux pumps] which may coexist. Treatment options for carbapenems-resistant *Pseudomonas aeruginosa* (CRPA) are usually restricted to colistin and rarely aminoglycosides, fosfomycin and aztreonam [1]. Pseudomonas is known for the high mortality, which exceeds 30% [95] and may be doubled in nosocomial infections due to multi-resistant Pseudomonas [96]. The need for effective treatment and limited evidence in the past that combination therapy may reduce mortality in pseudomonal bacteremia [97] motivate the efforts to evaluate colistin combination therapy in comparison to monotherapy.

5.1. In vitro *studies*

Zusman et al. [14] published a systematic review and meta-analysis of studies exploring the *in vitro* synergism of polymyxins and carbapenems against gram negatives in 2013. In the latter study, a broad search without restrictions was conducted (including 39 published studies and 15 conference proceedings) and results were presented separately according to the *in vitro* method used (time-kill, checkerboard microdilution or E-test) and the pathogen tested. For *Pseudomonas aeruginosa*, the following information is noted: a) in 40 time–kill studies 136 Pseudomonas isolates were tested with 50% synergy of the colistin-carbapenem combination. The most active carbapenem in combination was doripenem (62% synergy) followed by imipenem (60%) and meropenem (24%). The results were similar if the combination included an isolate resistant to carbapenems. Bactericidality (>3 logs reduction) increased from 10 to 49% with the combination and this result persisted even when the isolate was both resistant to colistin and the carbapenem; (b) In 9 studies using the checkerboard microdilution method, 100 Pseudomonas isolates were tested with only 11% exhibiting synergy (and 18% additive results). (c) Among 240 isolates tested by the E-test methods synergy of the colistin-carbapenem combination was evident in only 2.5% (and 6% additive results). Discordance in synergy between *in vitro* methods used is not surprising since time-kill methods use serial viable counting to provide a picture of antimicrobial activity over time while checkerboard and E-test provide only inhibitory data at a single time point. Probably time – kill studies are more precise defining the nature of the *in vitro* interaction; and (d) Comparison of resistance development between monotherapy and combination therapy were found for 14 Pseudomonas isolates tested in four studies. Combination with doripenem suppress or delays the emergence of resistance to colistin which appears early after colistin monotherapy.

Lora-Tamayo et al. [98] explored colistin-doripenem combination therapy in biofilm-associated infections using a dynamic biofilm model. A standard reference strain sensitive and two carbapenems-resistant clinical Pseudomonas isolates were tested against constant colistin concentrations of 1.2 and 3.5 mg/L mimicking human levels. Combination was synergistic, killing planktonic and biofilm embedded bacteria, preventing also emergence of colistin resistance. Colistin monotherapy was ineffective against planktonic bacteria and effective in biofilm embedded bacteria only in the high-level regimen. Recent *in vitro* studies have also shown a synergistic effect of the colistin-fosfomycin combination (49.3% synergistic and partially synergistic) [99] and of the levofloxacin-colistin combination (90% the first 4 h and 85% at 24 h) [100] using time–kill experiments. Tängdén et al. [101] assessed recently in a dynamic time–kill experiment the early effect of the colistin-meropenem combination against four Pseudomonas isolates (two of them carbapenem resistant) and compared the results with the checkerboard method. While the dynamic time–kill study showed an enhanced bactericidal effect of the combination, the checkerboard showed no synergy.

In conclusion, *in vitro* evidence by time–kill studies indicate a probable synergistic effect between colistin and carbapenems (which is the combination mostly studied).

5.2. Evidence from animal studies

There are three animal studies exploring the effectiveness of combination treatment versus monotherapy against carbapenem resistant pseudomonas. Aoki et al. [102] used a hyperoxic mice pneumonia model, mimicking the conditions of VAP and evaluated the effectiveness of colistin in combination with imipenem or rifampin, given either parenterally or intranasally. Combinations were first tested *in vitro* with colistin plus rifampin exhibiting 100% synergy and colistin plus imipenem 100% synergy or additive results. Control animals and those treated with monotherapy with colistin, imipenem, or rifampin had a 100% lethality rate during the first 48 h. Treatment with intranasal colistin plus rifampin and intranasal colistin plus imipenem increased survival of the animals to 75% and 62.5%, respectively. The same combinations with colistin given subcutaneously resulted in only 14% survival, supporting the superiority of the direct administration of colistin, which leads to higher concentrations in the lung with a longer half-life. Combination treatment led also to a reduction in inflammatory cytokines in the lung.

Cirioni et al. [103] used an experimental rat model of sepsis, comparing colistin monotherapy to colistin-rifampin combination therapy against a sensitive and an XDR isolate of *Pseudomonas aeruginosa*. Colistin was given intravenously and the pathogen was inoculated intraperitoneally. Monotherapy with colistin in the experiment with the sensitive isolate led to a 73.3% survival of the animals, which increased to 93.3% when colistin was combined with rifampin. Colistin was less effective as monotherapy against the XDR isolate with survival rate of 46.6%, which increased to 73.3% with combination therapy. The differences in survival in both experiments and the rate of bacteremia were not statistically significant. Significant was the antibacterial effect expressed in the cultures of peritoneal fluid and the reduction noted in inflammatory cytokines. The same study group showed similar results when they evaluated the combination of colistin plus imipenem in a mouse sepsis model using a sensitive and a multi-resistant Pseudomonas strain [104].

The restricted data from animal studies indicate a promising synergistic effect of the combination of colistin with a carbapenem (imipenem) or rifampin in serious pseudomonal infections that needs to be proved in clinical studies.

5.3. Evidence from clinical studies

There are no randomized clinical trials to compare monotherapy to combination therapy in infections due to CRPA. The scarce evidence retrieved from literature is derived from a small number of studies including low numbers of patients with infections from CRPA (Table 3).

During the early days of gram-negative multi-resistance, when polymyxins were reported as salvage treatment in serious infections, Linden et al. [105] reported a prospective study with 23 patients in a solid organ transplant unit who presented with serious infections from MDR Pseudomonas and were treated with colistin. In 13 of them colistin was given in combination with amikacin or an antipseudomonal b-lactam with the same rate of favorable outcome (60%). A few years later Furtado et al [106] treated 74 critically ill patients with nosocomial or ventilator associated pneumonia (VAP) due to MDR Pseudomonas with polymyxin B. In 28 patients polymyxin was combined with a b-lactam, mostly imipenem (24/28). Favorable response appeared in 47% overall and it was not affected by combination therapy.

In recent years, Peña et al. [108] in a *post hoc* analysis of their prospective cohort of 593 Pseudomonal bacteremias evaluated the impact of adequate single drug versus combination therapy, without evidence of mortality benefit from the combination therapy. This cohort included a group of 131 MDR Pseudomonas bacteremias among which 68 cases were due to XDR Pseudomonas with the same results. Petrosillo et al. [78] presented retrospective data from 184 critically ill patients treated for VAP or BSI with colistin either as monotherapy or combined with a glycopeptide (given either

Table 3. Clinical evidence comparing polymyxin/colistin monotherapy to combination therapy in infections due to MDR/XDR *Pseudomonas aeruginosa*.

Ref.	Year	Study	Setting	Number of patients with MDR PA or CRPA	Type of infection	Monotherapy (no)	Combination therapy (no)	Result/outcome
[105]	2003	Prospective cohort	ICU (SOT)	23	Pneumonia Intra-abdominal	Colistin (10)	Amikacin (4) Antipseudomonal b-lactam (9)	No difference in favorable outcome (resolution of symptoms by the end of therapy without death)
[106]	2007	Retrospective	ICU	74	VAP	Polymyxin B (46)	Imipenem (24) b-lactam (2) Ciprofloxacin (2)	No difference in favorable outcome (resolution of symptoms by the end of therapy)
[78]	2014	Retrospective	ICU	31	VAP, BSI	Colistin (14)	Glycopeptide (5) or antipseudomonal drug (7) or both (5)	No difference in 30-day mortality Colistin-Glycopeptide combination for ≥5 days a protective factor for mortality in multivariate analysis
[107]	2014	Retrospective	Hematology-Oncology	15	Bacteremia	Colistin (8)	Not mentioned (7)	No difference in mortality during the episode (37.5% for monotherapy and 57.1% for combination therapy, $p = 0.8$)
[92]	2015	Retrospective	ICU	18	VAP, BSI	Polymyxin B (15)	Carbapenem (3)	Significant difference in 30-day mortality between monotherapy (93.3%) and combination therapy (0%) for Pseudomonal infections and the overall cohort

BSI: blood stream infection; CRPA: carbapenem-resistant *Pseudomonas aeruginosa*; ICU: intensive care unit; MDR: multi-drug resistant; MDR PA: multi-drug-resistant *Pseudomonas aeruginosa*; no: number; SOT: solid organ transplant; VAP: ventilator-associated pneumonia; XDR: extensively drug resistant.

empirically or for a gram-positive coinfection). In the cohort, 31 patients (18.7%) had infections due to MDR Pseudomonas and 17 received combined therapy. Combination with a glycopeptide if given for ≥5 days was a protective factor for 30-day mortality. Moreover, Rigatto et al. [92] in a retrospective cohort study analyzed 101 patients treated with polymyxin B alone or in combination (73% with a carbapenem) for infections (mostly pneumonia and BSI) due to XDR Gram negatives among which 18 were XDR *Pseudomonas aeruginosa* and 15 received combination therapy. Despite low numbers, there was a survival benefit for combination therapy (30-day mortality 42.4 vs. 67.6%) overall and for patients with pseudomonal infections. Additionally, Ribera et al. [109] also presented a case series of 34 osteoarticular infections due to MDR Pseudomonas, among which 23 were XDR. They compared monotherapy (19 cases) to combination therapy (15 cases) to show a significantly better cure rate with combination therapy overall (31.6 vs. 73.3%). Combination therapy in 13 cases included colistin plus a b-lactam, but in the monotherapy group only 4/19 cases received colistin and no comparison was made between colistin monotherapy and combination therapy. Last, Samonis et al. [107] in a retrospective cohort study explored risk factors and outcome in 89 cancer patients with 97 *P. aeruginosa* infections. Bacteremia was the most prevalent infection (54%), and neutropenia was present in 15% of episodes. Among them, 22 cases (3 with neutropenia) were due to CRPA sensitive only to colistin. Due to early mortality, only 15 cases were evaluable for treatment outcome, eight given colistin monotherapy and seven colistin combination therapy. No difference in mortality between combination therapy and monotherapy was observed (37.5 and 57.1% for monotherapy and combination therapy, respectively, $p = 0.8$).

The available limited and of low-quality clinical evidence does not support the use of combination therapy for the treatment of CRPA.

6. Conclusions

The current state of evidence regarding combination treatment versus monotherapy against XDR gram-negative microorganisms is based on heterogeneous retrospective studies characterized as low quality with the exception of three randomized trials regarding *Acinetobacter baumannii*. The rationale for using two or more agents is supported by the high mortality associated with serious CRE infections and current evidence for carbapenems-resistant *K. pneumoniae* suggests that combination therapy is associated with reduced mortality in patients with septic shock and rapidly fatal underlying diseases. However, patients with less severe BSIs and in non-bacteremic intra-abdominal or UTIs, monotherapy is reported as an adequate therapeutic choice. Regarding *Acinetobacter baumannii*, combination treatment does not appear to provide therapeutic advantage over monotherapy. However, for immunosuppressed patients, patients with septic shock and infections with borderline MICs, combination therapy could be recommended. Last, there are minimal clinical data and inconsistent evidence in support of the use of combinations for treating *P. aeruginosa* infections and therefore treating XDR gram-negative infections should be done in consultation with an expert whenever possible.

7. Expert commentary

In the early literature of antimicrobial chemotherapy, combination of antibiotics was recommended for the following reasons: (a) to protect resistance development; (b) to obtain synergistic results; (c) to decrease the incidence of adverse effects because synergy permitted the lowering of dosages; and (d) to enhance the spectrum of antimicrobial activity in empirical therapy of septic shock patients. Traditionally in the past, combinations were given in the case of suspected or proven *Pseudomonas aeruginosa* bacteremia implicated mainly in the immunocompromised – neutropenic host. In this case, the administration of an anti-pseudomonal β-lactam plus gentamicin or amikacin was considered as possessing a synergistic result; however, recent meta-analysis has doubted the efficacy of similar combinations.

Nowadays, *P. aeruginosa* along with the predominance of *Acinetobacter baumannii* and *Klebsiella pneumoniae* strains producing carbapenemases, engaged physicians to rediscover and revive life-saving older antibiotics, mainly polymyxins. There is no doubt that colistin in particular possesses several drawbacks like peculiar PK/PD leading to a variety of dosage schedules, the necessity of MIC determination with broth microdilution in order to escape false sensitivities, nephrotoxicity, and neurotoxicity and *in vivo* selection of Proteaceae (by definition resistant to colistin) with subsequent superinfections. Therefore, the need of testing both *in vitro* and *in vivo* various combinations is currently reintroduced in clinical praxis. After applying checkerboard or the time–kill curve techniques in a large number of studies, it was concluded that at least against Cp-Kp and AB, the combination of colistin with meropenem was synergistic to a high percent irrespective of the sensitivity pattern of carbapenems, whereas combination suppressed the emergence of resistance to colistin.

On the other hand, fosfomycin, another revived antibiotic of the sixties, which is active *in vitro* against Cp-Kp, including also XDR *P. aeruginosa* strains, is confronted by definition *in vivo* with resistance development when given as monotherapy, with subsequent therapeutic failures and relapses. The necessity of a combination partner is imposed to escape resistance, which at least from the available *in vitro* data should be a carbapenem.

Tigecycline, an improved tetracycline that overcomes the active efflux and the ribosomal protein resistance mechanisms, is active *in vitro* against a high percent of XDR Cp-Kp and AB as well, however combination with an anti-pseudomonal agent is indispensable to overcome the lack of activity against *P. aeruginosa* strains. Another important problem imposing combination therapy is the disadvantageous PK/PDs of tigecycline connected mostly with therapeutic failures in VAP caused by XDR gram negatives.

Surprisingly, rifampicin combinations with colistin against AB were associated *in vitro* with almost 100% synergistic results, while colistin plus glycopeptide was found synergistic against colistin-R AB strains. Unfortunately, *in vivo* experience

of colistin and rifampicin combination is disappointing, whereas the role of glycopeptides *in vivo* is still uncertain.

Has any important lesson been obtained from the animal models? Colistin in various combinations, but mainly with carbapenems in a variety of animal models against Cp-Kp, XDR AB and CRPA was associated with a promising synergistic effect. However, lack of homogeneity in the applied animal model in a variety of other combinations, makes it difficult for constant conclusions to be derived.

Which is the relevant experience of current combinations in humans? Despite the fact that almost all studies are retrospective including small number of patients, evidence of synergistic results has been reported mostly from Italian and Greek studies in patients with infections by Cp-Kp, permitting their application in the clinical setting with confidence. The role of combination therapy and monotherapy for XDR gram negative has been analyzed in detail in the current review. It is however interesting to be pointed out that XDR *P. aeruginosa* remains the most inconsistent strain regarding evidence of superiority of combination therapy, probably attributed to the priority given by the investigators to the threatening Cp-Kp and XDR AB infections with seems to have replaced nowadays older pathogens like Pseudomonas. After all, there is no doubt that carefully conducted prospective randomized trials are required to clarify with certainty the role of combinations against XDR gram-negative pathogens.

Recently, the emergence of colistin-R Cp-Kp which is steeply raising particularly in South Eastern European countries, points out the urgent necessity of novel active compounds. We are looking forward to welcome the carbapenemase inhibitors like avibactam, relebactam, and vaborbactam, the newer promising aminoglycoside plazomicin as well as the siderophore cephalosporin, cefiderocol, which possesses the broadest spectrum of activity against XDR Enterobacteriaceae, AB and CRPA. However, not far from its introduction in the USA market, resistance development during ceftazidime-avibactam treatment reaching 30% was reported.

While awaiting the active compounds to be brought into the global market, which is the most plausible salvage therapy against XDR and even more PDR gram-negative pathogens? At least against Cp-Kp strains, the double carbapenem combination approach, based on the administration of ertapenem as a suicide KPC inhibitor with prolonged infusion of meropenem, is a revolutionary and provocative concept proven effective *in vitro*, in animal models as well in humans.

In the meantime, what else is left in the universal battle against XDR pathogens? Despite the pending availability in the future of the newer compounds and the reported herein salvage approach, the prevention of resistance development and the spread of the resistant clones in the nosocomial environment by the strict application antibiotic stewardship and infection control, including careful contact precautions are the two cornerstones. Otherwise, as reported by Jim O' Neill in the 'Review of Antimicrobial Resistance' in February 2015, by 2050 10 million people will die annually from infections caused by bacteria for whom no antibiotic will be available.

8. Five-year view

The use of combination therapy nowadays is motivated by the decreasing activity of currently available options, i.e. colistin, tigecycline, and fosfomycin, and their apparent selection of resistance. This approach in the next 5 years is to be reassessed after the adoption of novel antimicrobial agents with activity against XDR gram-negative pathogens like novel beta-lactam/beta-lactamase inhibitor combinations, such as those containing avibactam, vaborbactam and relebactam, plazomicin, and cefiderocol, and in light of highly anticipated randomized studies looking at the matter in specific patient populations. However, the prospective of administration of novel agents as monotherapy or in combination will be a debate in the coming years and the real potential of those new therapeutic agents may only be revealed with the adoption of rapid molecular diagnostics and the prudent use in the nosocomial setting.

Key issues

- This century has been marked by the emergence of XDR gram-negative pathogens that have been confronted as major threats with high mortality, represented mainly by *Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa*
- Combination therapy for targeted treatment of carbapenem resistant *K.pneumoniae* is indicated for severe infections, in particular in cases of septic shock, rapidly fatal underlying diseases and patients with high INCREMENT-CPE mortality score. On the other hand, monotherapy is preferred for non-bacteremic infections like intra-abdominal or urinary tract infections.
- High dose carbapenem based combination therapy may be a reasonable treatment option against carbapenem resistant *K.pneumoniae*, provided that carbapenem is given in a dose of 2 gr every 8 h and 3 – hours infusion, MIC of the infecting organism is ≤ 8mg/L, and carbapenem is given in combination with another active *in vitro* antimicrobial agent.
- There is moderate evidence in support of the use of monotherapy for treating *Acinetobacter baumannii* infections. However, for septic shock patients as well as in the immunocompromised and infections with borderline MICs, combination therapy could be recommended.
- There is lack of evidence regarding combination treatment for the treatment of Pseudomonal infections, even in cancer patients. Consultation with an infectious disease expert is warranted, pending randomized clinical trials addressing the issue of the benefit and drawbacks of combination in XDR *P.aeruginosa* infection

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