Combination therapy for carbapenem-resistant Gram-negative bacteria

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The emergence of resistant to carbapenems Gram-negative bacteria (CR GNB) has severely challenged antimicrobial therapy. Many CR GNB isolates are only susceptible to polymyxins; however, therapy with polymyxins and other potentially active antibiotics presents some drawbacks, which have discouraged their use in monotherapy. In this context, along with strong pre-clinical evidence of benefit in combining antimicrobials against CR GNB, the clinical use of combination therapy has been raised as an interesting strategy to overcome these potential limitations of a single agent. Polymyxins, tigecycline and even carbapenems are usually the cornerstone agents in combination schemes. Optimization of the probability to attain the pharmacokinetic/pharmacodynamic targets by both cornerstone drug and adjuvant drug is of paramount importance to achieve better clinical and microbiological outcomes. Clinical evidence of the major drugs utilized in combination schemes and how they should be prescribed considering pharmacokinetic/pharmacodynamic characteristics against CR GNB will be reviewed in this article.

Carbapenems are potent broad spectrum β-lactam antibiotics that have been used as the last resort treatment for many Gram-negative bacteria (GNB) causing serious nosocomial infections [1]. Prior to 2000, only relatively few clinical isolates were carbapenem resistant, mostly *Pseudomonas aeruginosa* and some *Acinetobacter baumannii*, due to the combination of high-level β-lactamase expression coupled with decreased permeability of the outer membrane and/or hyperexpression of efflux pumps [2]. Therefore, carbapenem resistance was not a major clinical problem before 2000 [3], but has since then become a major, global health concern. With imipenem having been US FDA-approved in 1985 and meropenem in 1996, this means that carbapenem resistance became a major clinical challenge within 15–20 years after approval of the first carbapenem. A similar relationship has been observed for other antibiotic classes [4].

The emergence of acquired carbapenem-hydrolyzing β-lactamases (carbapenemases) at the end of the past century and their worldwide dissemination is a major global threat to the antibiotic era and to all the clinical procedures that rely on effective antibiotic therapy [5–11]. The carbapenemases were initially described in a few organisms and restricted to specific geographic areas, but they have become a global concern by the middle of the past decade [5–11]. Some enzymes determining broad-spectrum β-lactam resistance in major nosocomial bacteria such as *P. aeruginosa, A. baumannii* and Enterobacteriaceae isolates have disseminated through the continents and completely changed the scenario of antibiotic resistance in GNB.

Unequivocally, the emergence of metallo-β-lactamases VIM, IMP and NDM (molecular class B), OXA-48 and its derivatives (molecular class D), and *Klebsiella pneumoniae* carbapenemases (KPCs, molecular class A) has rapidly caused several paradigm shifts in antibiotic therapy against GNB. This would not be a major concern if the discovery and development of new antimicrobials had evolved as quickly and effectively as the ability of these GNB to become resistant to antibiotics. However, the discovery and development pipeline

of new antibiotics against GNB has dried out since several years [12]. Consequently, physicians are now compelled to restore 'old' antibiotics as the last resort therapy against infections they had been used to successfully treat with broad spectrum β-lactam antibiotics, especially the carbapenems [13–17].

The global epidemics of carbapenem-resistant (CR) GNB and carbapenemases have been very dynamic and a detailed revision of this issue is beyond the scope of this article. Interested readers are invited to read thoughtful reviews published elsewhere [5–11,18]. Similarly, the reasons for the paucity of new antibiotics against GNB have also been extensively discussed previously [19,20] and will not be described here. Finally, GNB isolates with intrinsic resistance to carbapenems, such as *Stenotrophomonas maltophilia* [21], will also not be reviewed here. This review focuses on clinically available antibiotics and does not cover antibiotics currently under development.

In the past few years, antibiotic combinations against CR GNB have been proposed as the best practice in the management of infections by these organisms. In this report, we will review recent pharmacokinetic (PK) and PK/pharmacodynamic (PD) findings for the most often used antibiotics against major GNB with acquired resistance to carbapenems. Additionally, relevant pre-clinical and clinical data that may contribute to the choice of optimal combination regimens against these pathogens are summarized.

Carbapenem-resistance, multidrug-resistance, extended drug-resistance & pan-drug-resistance

Carbapenem resistance in *P. aeruginosa, A. baumannii* and Enterobacteriaceae is almost always associated with resistance to several other classes of antibiotics, because carbapenemase-encoding genes are located on mobile genetic elements that usually carry genes responsible for resistance to other antibiotics [2,8]. Recently, a group of experts proposed a consensus on the definitions for multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacteria [22]. Briefly, a MDR GNB is an isolate that is non-susceptible to at least one agent in at least three antimicrobial categories, which are potentially active against the respective GNB. An isolate is XDR, if it is non-susceptible to at least one agent in all but two or fewer antimicrobial categories, which are potentially active against the respective GNB. Finally, PDR is defined as non-susceptibility to all agents in all antimicrobial categories for this isolate [22]. Although the definitions for MDR and XDR do not require resistance to carbapenems, the CR phenotype is very common for MDR and particularly for XDR isolates. These and PDR GNB are the major clinical challenges for antimicrobial therapy. In the remaining sections of this review, we refer to CR GNB as isolates with XDR and, eventually, PDR phenotype.

Conceptual basis of combination therapy against CR GNB
Cornerstone therapy & adjuvant agents

Combination therapy for CR GNB is usually based on a cornerstone antibiotic for which the organism presents *in vitro* susceptibility, although this is likely not possible for PDR isolates. The main antibiotic is associated with an adjuvant drug to which the organism may be susceptible *in vitro* or not. It needs to be emphasized that the concept of susceptibility test refers to antibiotic monotherapy. An adjuvant drug, which may cause no bacterial killing in monotherapy, can still be highly beneficial to maximize bacterial killing or prevent resistance.

By far, polymyxins are the antibiotic class for which most CR GNB present *in vitro* susceptibility, and polymyxin-only-susceptible (POS) isolates account for a significant proportion of CR GNB with XDR profile [5–11]. Therefore, polymyxins (i. e., either colistin or polymyxin B) are the most common cornerstone agents in combination schemes. However, other agents such as tigecycline have also been the main antibiotic in some combination schemes for *A. baumannii* and Enterobacteriaceae infections. Finally, in some situations even carbapenems have been used as the main agent for the treatment of CR GNB infections.

The most frequently used adjuvant therapies for CR GNB infections are carbapenems, tigecycline, fosfomycin, aminoglycosides and rifampicin. Other agents are discussed later.

Why combination therapy?

There are no data from randomized clinical trials (RCTs) with an adequate sample size indicating that combination therapy is the standard of care for patients infected by CR GNB. The first RCTs assessing combination therapy against CR GNB have been only recently published, both assessing the combination of colistin with rifampicin against CR *A. baumannii* [23,24]. Neither of these two studies has shown a significant benefit of the combination compared with colistin monotherapy [23,24].

Indeed, there is clinical evidence indicating that combination therapy may not be superior to monotherapy in the treatment of GNB, including *P. aeruginosa,* when there is susceptibility to a β-lactam, and this drug is used as the cornerstone antibiotic in combination with another drug [25–28]. However, these meta-analyses include data over several decades, which have been subject to a dramatic increase in bacterial resistance over the years. Findings from these meta-analyses cannot be directly extrapolated to the treatment of MDR, XDR or PDR where a β-lactam is rarely used as the cornerstone therapy, and where often there is only a single agent with *in vitro* susceptibility available. While monotherapy may be appropriate for patients with less severe infections by susceptible isolates, patients with severe infections and critically ill patients would likely benefit most from rationally optimized combination therapy.

The vast majority of combination therapies were chosen empirically without being rationally optimized based on systematic *in vitro* and animal studies with subsequent translation to humans that is supported by translational modeling. Latest *in vitro* infection models and animal studies clearly showed that rationally optimized combination therapies are highly promising. The main microbiological reasons for using antibiotic combinations against CR isolates are to maximize the rate and extent of bacterial killing, prevent re-growth and minimize

bacterial resistance. These mechanistic reasons support intermediary clinical benefits and ultimately the final clinical and microbiological outcomes summarized in Figure 1.

The combination of polymyxins with another antibiotic has been first proposed for the treatment of POS GNB to overcome some shortcomings of polymyxins in monotherapy, including the potential for therapeutic failure due to the amplification or emergence of hetero-resistant subpopulations [29–32]. Hetero-resistance describes the scenario where resistant bacterial subpopulation(s) are present at initiation of therapy and eventually cause therapeutic failure, as they are not killed, for example, by polymyxin monotherapy. Hetero-resistance needs to be distinguished from adaptive resistance (also called 'tolerance'), which refers to a transient change of bacterial resistance in response to antibiotic therapy. Adaptive resistance has been found both for polymyxins and aminoglycosides in P. aeruginosa, for example. To minimize the impact of adaptive resistance, longer dosing intervals (i.e., 24 h) were suggested for aminoglycosides [33,34]. However, it is not clear whether once daily dosing of polymyxins minimizes emergence of resistance and thus more research is needed.

Also, polymyxins may only achieve limited bacterial killing against isolates with high minimal inhibitory concentrations (MICs) considering the unbound polymyxin concentrations that are achievable in patients [35,36]. Finally, recent studies suggested that polymyxin monotherapy may be inferior to other drugs in the treatment of GNB and have corroborated the idea that combination therapy is necessary [37–40].

Concomitantly, tigecycline was used as an alternative agent against polymyxin-resistant CR A. baumannii or Enterobacteriaceae isolates. As this drug has not been recommended in monotherapy for severe infections (see below), a second agent was also commonly added. Finally, many CR Enterobacteriaceae presented MICs for carbapenems within the previous susceptibility range, that is, ≤4 mg/l [41] and susceptibility to aminoglycosides. Therefore, carbapenems were prescribed as the cornerstone antibiotic against these organisms in combination with an aminoglycoside. Thus, combination regimens were first prescribed before unequivocal clinical evidence of superiority of this approach, over monotherapy, was available.

In fact, there is still no clinical evidence clearly demonstrating that combination therapy against CR GNB is superior to monotherapy; not even for infections in particularly difficult pathogens such as CR P. aeruginosa and A. baumannii. Indeed, apart from the recent RCTs with colistin and rifampicin against CR A. baumannii, no other study has primarily assessed the effect of combination therapy on clinical outcomes, neither against P. aeruginosa nor A. baumannii, with the exception of the study by Falagas et al. [41], that evaluated the role of meropenem in combination with colistin methanesulphonate sodium (CMS)/colistin against MDR GNB (predominantly P. aeruginosa and A. baumannii) infections. Nonetheless, no benefit was demonstrated by adding meropenem to the scheme [41]. A further evaluation of these patients [41] together with additional ones has also not found a statistically significant difference between combination therapy and monotherapy with colistin [42].

It was only for the treatment of CR K. pneumonia bacteremia that some evidence from observational studies has pointed toward a clearer advantage of combination schemes over monotherapy [43–45]. The lower mortality rates observed in patients receiving combination therapy compared with those treated with monotherapy have encouraged physicians to adopt this practice as the standard of care in the treatment of CR Enterobacteriaceae. Additionally, several authors have compiled data from case series and cohort studies and also concluded that combinations were superior to single drug schemes against CR Enterobacteriaceae, particularly those containing a carbapenem [8,46–48]. The promising results with combination therapy against CR Enterobacteriaceae have been extrapolated to the treatment of CR P. aeruginosa and A. baumannii, although, as stated earlier, no clinical benefit against these organisms has been clearly demonstrated so far.

Pre-clinical studies

Ultimately, a number of pre-clinical studies strongly support the use of rationally optimized combination dosage regimens against CR GNB. In vitro and animal infection models suggest that antibiotic combination regimens are superior to monotherapy for maximizing bacterial killing and minimizing the emergence of resistance (Figure 1). For the interpretation of in vitro studies, the presence of synergy is not important unless the combination also leads to adequate bacterial killing, minimizes the emergence of resistance or ideally achieves both of these goals.

P. aeruginosa

Most studies in contemporary P. aeruginosa isolates with different resistance phenotypes have been focusing on polymyxin-based combinations. Extensive and synergistic bacterial killing of P. aeruginosa by colistin combined with carbapenems (doripenem and imipenem) was most commonly found in static and dynamic in vitro models [49,50] and in murine infection models [51]. This synergy occurred at clinically relevant polymyxin and carbapenem concentrations. Latest dynamic infection models provided strong evidence for colistin plus doripenem preventing emergence of resistance and achieving substantial killing against a very high inoculum of a colistin-resistant and other isolates [52]. The triple drug combination of polymyxin B, doripenem and rifampicin achieved bactericidal activity against five of five CR P. aeruginosa isolates in static time-kill studies at a normal (i.e., low) inoculum [53]. Polymyxin B combined with supra-physiological concentrations of meropenem or amikacin and the associated triple combination achieve strain-dependent synergy against XDR P. aeruginosa [54]. Latest dynamic in vitro infection models showed that combination therapies of meropenem with tobramycin or levofloxacin achieved rapid and substantial killing and minimized resistance against P. aeruginosa with an overexpressed MexAB-OprM efflux pump [55,56]. This pump is clinically highly important as it effluxes almost all β-lactam antibiotics,
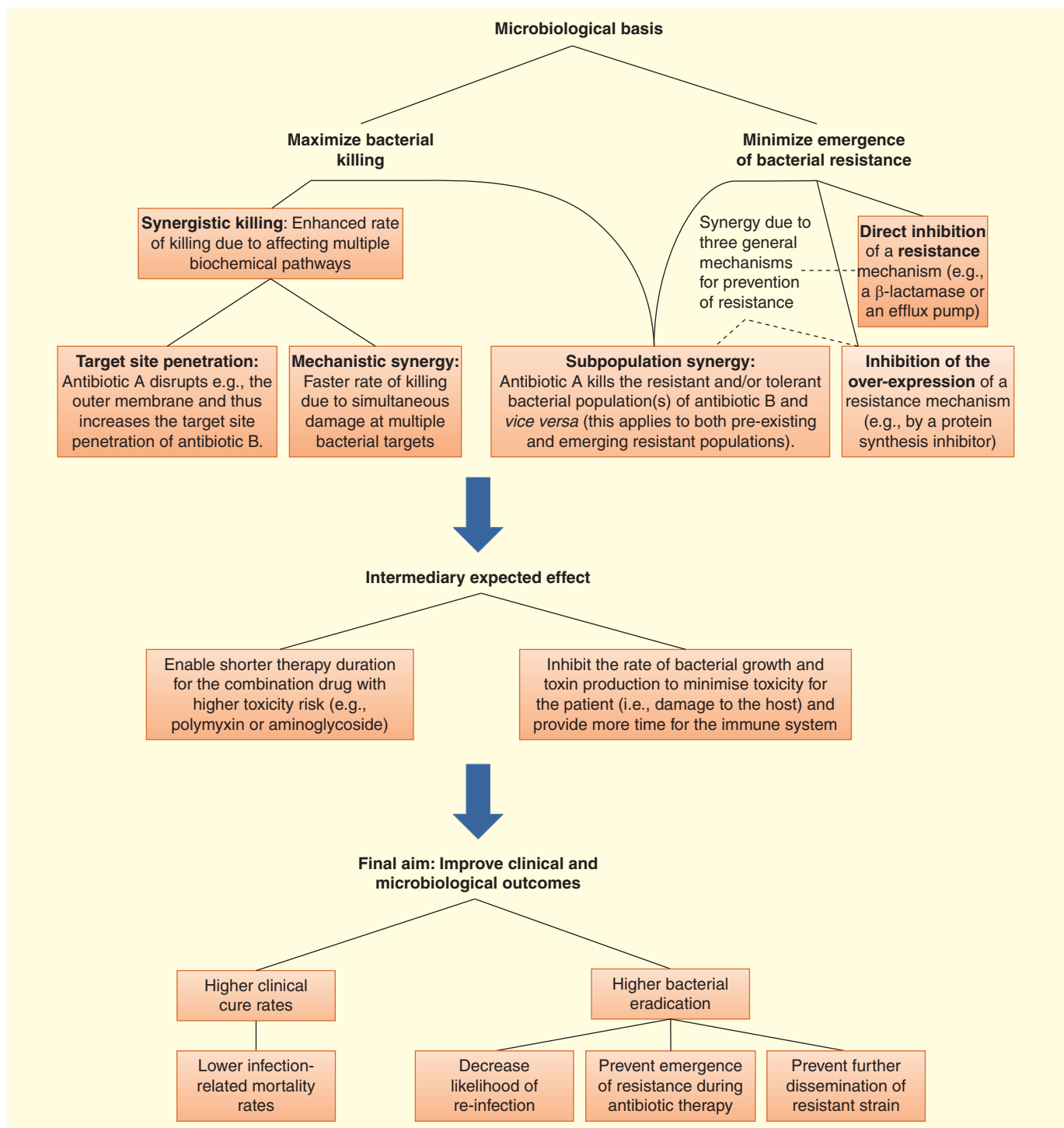
Figure 1. Conceptual basis of combination therapy against carbapenem-resistant Gram-negative bacteria.

including meropenem and doripenem but not imipenem [57]. Similar results were obtained for imipenem plus levofloxacin against a *P. aeruginosa* ioslate with overexpressed efflux or loss of the OprD outer membrane porin, which confers decreased susceptibility to carbapenems [58,59]. These results are in agreement with the synergistic and considerable killing by β-lactam plus aminoglycoside combinations in static time-kill studies,

which was observed at least for a considerable fraction of the tested CR *P. aeruginosa* isolates in older studies [60–67].

A. baumannii

In vitro and animal infection models against CR *A. baumannii* (including MDR, XDR and PDR isolates) suggested promising synergy with substantial killing for a polymyxin combined with

rifampicin or a carbapenem [53,68–72]. The colistin plus rifampicin combination provided substantial killing and minimized emergence of resistance in the dynamic hollow fiber *in vitro* infection model over 10 days [73–75] and in the murine thigh infection model [51]. Other studies suggested synergistic and extensive killing for carbapenem plus sulbactam combinations [70,76–78] and for rifampicin combined with imipenem or sulbactam [72,79]. Only a few studies are available on other combinations against CR *A. baumannii* such as colistin plus tigecycline [80,81] or minocycline [82]. The latter two studies showed promising activity, but further studies are needed on these combinations. Overall, polymyxin plus rifampicin or a carbapenem as well as two or three drug combinations containing a carbapenem, rifampicin or sulbactam are promising and should be further evaluated *in vitro* and *in vivo* against CR *A. baumannii*. More data on the mechanisms of synergy would be highly valuable to more thoroughly elucidate the mechanistic basis for these combinations.

Enterobacteriaceae

Most *in vitro* and animal data on antibiotic combinations against CR Enterobacteriaceae applies to CR *K. pneumoniae* and fewer systematic studies are available for *Escherichia coli*. Combinations of a polymyxin plus a carbapenem have shown the most consistently beneficial activity against *K. pneumoniae in vitro* [83–87] and in murine infection models [51]. The combination of colistin plus imipenem yielded synergistic killing against colistin-susceptible, metallo-β-lactamase-producing *K. pneumoniae* isolates; however, this combination was less promising against colistin-resistant *K. pneumoniae* [87]. A study in CR *K. pneumoniae* and CR *E. coli* showed >3.5 $\log_{10}$ killing at 24 h for polymyxin B plus doripenem against 4 of 5 tested *E. coli* strains. However, to achieve at least 2.7 $\log_{10}$ killing at 24 h in 5 of 5 *K. pneumoniae* strains, the triple drug combination of polymyxin B, doripenem and rifampicin was required [53]. Fosfomycin combined with meropenem achieved synergistic killing in 65% of 17 tested KPC-2 producing *K. pneumoniae* strains, but more studies are needed on this combination [88]. *In vitro* checkerboard data suggest synergistic killing for colistin plus rifampicin against KPC-producing *K. pneumoniae* [89], and further *in vitro* time-course studies are warranted. Overall, polymyxin plus carbapenem combinations seem most promising based on the available preclinical data. However, much more antibiotic combination studies using static and dynamic *in vitro* and animal infection models with CR Enterobacteriaceae are clearly needed to rationally optimize the associated combination therapies.

Optimizing antimicrobial prescription in combination therapy

In combination regimens, it is of paramount importance that the dosage regimens for both the cornerstone and the adjuvant drug are optimized to achieve relevant PK/PD targets and to maximize efficacy, decrease the potential for resistance emergence and decrease toxicity (Figure 1). Significant advances have

been made on the knowledge of PK and PK/PD of 'old' drugs used for the treatment of CR GNB, as increasing clinical experience has been published. The knowledge gained in these studies should be utilized as the basis for rational selection of the antibiotics and dosing regimens to successfully treat CR GNB infections and, therefore, is reviewed later. However, most of the PK/PD approaches developed to date only apply to antibiotic monotherapy and rational approaches to optimize combination regimens are scarce [55,56,90,91].

Polymyxins

Polymyxins are the most common class of antibiotics used to treat CR GNB as the cornerstone therapy. Although resistance rates have been increasing in some countries, particularly among Enterobacteriaceae [92–96], polymyxins are still considered the most active agents against CR GNB [97]. Polymyxin B and colistin are the two polymyxins available for clinical use [14,98,99]. Fortunately, significant advances have been made in the past decade in characterizing the PK and PK/PD of these drugs [36,100–110]. The first recommendations for dosing and dose adjustments in renal impairment have been made empirically without consistent PK data supporting them [14,99]. Additionally, no formal recommendations were available for patients on renal replacement therapy (RRT). Consequently, many patients have likely received suboptimal therapy, particularly those with renal dysfunction and those on RRT.

Both polymyxins differ by a single amino acid [98,111]. However, the PK characteristics of polymyxin B and colistin differ noticeably primarily due to the different pharmaceutical forms in which they are administered. While polymyxin B is administered as its active form (polymyxin B sulfate), colistin is administered as an inactive pro-drug, CMS (also called colistimethate), which leads to different PK behaviors.

The PK/PD index that best correlates with bactericidal activity of polymyxins in monotherapy is the free area under the curve (*f*AUC)/MIC. This was initially suggested by results from a hollow fiber *in vitro* PD model study in two strains [112], and more recently confirmed in more extensive dynamic *in vitro* infection model studies [113] and two animal models [114,115]. However, although *f*AUC/MIC has been demonstrated to be the best predictor of bactericidal activity of polymyxins, the *f*AUC/MIC target value has not yet been defined, as considerable between strain variability in the PK/PD target value exists. A broad range of *f*AUC/MIC values were associated with stasis (1.57–17.3), or 1-log (5.04–42.1), 2-log (6.61–95.0) and 3-log (53.3–141) bacterial killing at 24 h, in the *in vitro* infection model and in both murine thigh and lung infection models either with *P. aeruginosa* or with *A. baumannii* [113–115]. A 'target' average concentration at steady-state (Css,avg) of 2.5 mg/l colistin in patients was proposed by Garonzik *et al.* [102], which was similar to the median Css,avg in the 105 patients receiving physician-selected maintenance doses in the study. The Css,avg of 2.5 mg/l also corresponds to an AUC/MIC of 60, which generally led to a magnitude of effect between stasis and 1-log kill in the murine infection models

described earlier. This assumes that the average free fraction (*f*) is similar in infected mice and patients [114,115].

There are also two retrospective studies supporting that increasing the AUC may improve clinical outcomes [42,116]. Since the AUC value represents the total exposure to the drug, clinically the AUC can be increased by increasing the daily dose. Consequently, it can be expected that higher daily doses would be associated with improved clinical outcomes. Both retrospective studies assessing dosage regime and outcomes of patients treated with polymyxins have demonstrated such results [42,116]. The first study with CMS, assessing 258 intensive care unit patients, has shown that the overall mortality of patients treated with 3, 6 and 9 million IU/day was 38.6, 27.8 and 21.7%, respectively (p = 0.0011) [42]. Higher CMS doses were independently associated with lower mortality in the multivariate analysis [42]. The other study evaluated 276 patients treated with polymyxin B and found that hospital mortality was significantly lower in patients receiving daily doses equal to or higher than 200 mg (2 million IU) of polymyxin B. The hospital mortality rates were 66.4, 66.2 and 47.9% in patients receiving <150 mg/day, ≥150 and <200 mg/day and ≥200 mg/day, respectively (p = 0.03) [116]. High doses (≥200 mg/day) were independently associated with lower mortality in multivariate analysis, both in the subgroup of patients with microbiologically documented infections (n = 212) and in patients with bloodstream infections (n = 53) [116].

Colistin is administered as CMS, an inactive pro-drug that needs to be converted *in vivo* to the active drug colistin [35,117]. However, only a small fraction of CMS is converted to colistin *in vivo* and this conversion is quite slow [102,107]. Therefore, without loading doses, therapeutic concentrations of colistin are only reached after 48 h of CMS administration [101,102,107]. Thus, loading doses of CMS are required to reach therapeutic concentrations of colistin in the first 12–24 h [102,107]. Even with a CMS loading dose, the required conversion from CMS to colistin means that it likely takes several hours until effective colistin concentrations can be achieved.

In contrast, higher plasma concentrations in relation to steady-state (i.e., ~65% of steady-state) are attained after the first polymyxin B dose [36]. If a loading dose of 20.000–25.000 IU is given on day 1 of therapy, 85–87% of the steady-state concentration are reached after the first administration of polymyxin B [36]. So, loading doses are also recommended for polymyxin B, although not mandatory as for CMS, particularly in severely ill patients or in infections by organisms with MICs ≥1.0 mg/l.

Although colistin clearance is mainly by the non-renal route, CMS is predominantly cleared by the kidneys. CMS concentrations increase as creatinine clearance decreases, which results in higher concentrations of CMS to be converted to colistin. Therefore patients with impaired renal function require dose adjustment of CMS [102]. In contrast, patients with normal, but especially those with increased creatinine clearances, such as those in initial phases of sepsis and septic shock, will likely present low concentrations of colistin in plasma with usually recommended doses. This is caused by low concentrations of CMS, which is eliminated by the kidneys, and the consequently low fraction of CMS converted to colistin [102]. This is very problematic, particularly for patients with creatinine clearances above 60–70 ml/min. Therefore, it was proposed that colistin be best used as part of a highly active combination, particularly for patients with good renal function and infections by isolates with MICs >0.5 mg/l [102].

In contrast, the clearance of polymyxin B is not related to creatinine clearance; therefore dose adjustments are not required in renal dysfunction [36,103]. Although one may consider decreasing the daily dose in cases of renal dysfunction, it will ultimately result in low plasma concentrations with potential negative consequences for clinical and microbiological outcomes [36]. It should be noted that in a retrospective cohort study the benefit of high doses of polymyxin B was maintained regardless of the presence of renal dysfunction during therapy [116].

In patients under RRT (both continuous and intermittent), both CMS and colistin are partially removed [102,109,118,119], requiring adjustment of dosage regimens as has been proposed by Garonzik *et al.* [102]. There are less data on the PK of polymyxin B in patients under RRT. Data from two patients showed that only 5–12% of polymyxin B are removed in continuous venovenous hemodialysis, indicating that only minimal, if any, increase in the dose would be necessary [106].

Considering currently available data on the PK of polymyxins, it can be concluded that there are some PK advantages of polymyxin B over CMS/colistin. With currently recommended dosages, polymyxin B reaches higher serum concentrations than colistin, and these polymyxin B concentrations are reached much more quickly, even without a loading dose, which is recommended but does not seem to be as essential as for CMS. Finally, different brands of CMS have similar elemental compositions, but they lead to different exposures to the microbiologically active formed colistin; this is another complication for adjusting dosage regimens since it seems to be unpredictable [120].

A potential advantage for CMS lies in the treatment of urinary tract infections. As there is substantial tubular reabsorption of polymyxin B (and also colistin), very low concentrations of polymyxin B or colistin are found in urine [36,103]. In contrast, CMS is highly eliminated by the kidneys without tubular reabsorption, and a large amount of CMS is converted to colistin in urine leading to high urinary concentrations of the latter [35]. Thus, although polymyxin B may be successfully used for the treatment of lower urinary tract infections [121], CMS might potentially have a higher capacity of sterilization of the urine owing to the higher colistin concentration reached at this site. TABLE 1 summarizes the major differences between the two polymyxins.

Carbapenems

Although it may seem paradoxical at first sight, carbapenems have been commonly prescribed against CR GNB, particularly for KPC-producing Enterobacteriaceae infections, either

Table 1. Key differences and similarities in pharmacokinetic and dosing of colistin and polymyxin B.

	Colistin	Polymyxin B
Form in which it is administered	CMS (inactive pro-drug), slow and incomplete conversion to colistin (active moiety)	Polymyxin B sulfate (active moiety)
Dose units	CBA (mg) or IU, 1 million IU approximately 30 mg CBA [99]	International units (10,000 IU/ mg)
Need for a loading dose	Loading dose clearly required [101,102,107]	Loading dose recommended [36]
Renal handling of the active moiety	Minimal renal clearance of colistin (high extent of tubular reabsorption) [99], high accumulation in renal tissues [216]	Minimal renal clearance (high extent of tubular reabsorption) [36], high accumulation in renal tissues
Elimination of pro-drug	Mainly by renal clearance (tubular secretion) [99]	Active drug is administered
Dose adjustment for renal function and dialysis	CMS doses need to be adjusted [102]	Not recommended at this time [36,106]
Urinary concentrations	High (for CMS and colistin)	Low
Most predictive PK/PD index for anti-bacterial effect	fAUC/MIC	fAUC/MIC

CBA: Colistin base activity; CMS: Colistin methanesulphonate sodium; fAUC/MIC: Free area under the curve/minimal inhibitory concentration; IU: International units; PK/PD: Pharmacokinetic/pharmacodynamic.

as an adjuvant or even as the cornerstone drug [8,46–48]. This is mainly due to the fact that many carbapenemase-producing Enterobacteriaceae isolates present carbapenem MICs near to or even at the current susceptibility breakpoints, that is, 1–4 mg/l; this occurs especially for meropenem and doripenem. Numerous studies have shown that higher doses and optimal modes of administration, either by extended or continuous infusion of the drugs [122–125], can lead to an acceptable probability of attaining the PK/PD target (i.e., time of free drug during the dose interval above the MIC >40%) for pathogens with carbapenem MICs between 1 and 8 mg/l, even in critically ill patients [126]. Combination therapy may provide further benefits (**Figure 1**) for the use of carbapenems in these CR GNB isolates with borderline susceptibility. These PK/PD data have been corroborated by the analysis of many case series and some cohort studies demonstrating lower mortality rates among patients treated with a carbapenem-containing regimen for infections caused by CR *K. pneumoniae* with MICs below 8 mg/l, and particularly below 4 mg/l [8,46–48,127].

In contrast to Enterobacteriaceae, carbapenem MICs in CR non-fermentative organisms are often very high (>32 mg/l), either because more potent carbapenemases are involved or other resistance mechanisms are additionally present [2]. This fact along with the lack of clinical data supporting the use a carbapenem in combination therapy against CR *P. aeruginosa* or *A. baumannii* may discourage the use of carbapenem-containing regimens against such pathogens at this time. However, as described earlier, many preclinical studies have demonstrated potential benefits, particularly for combinations of carbapenems with a polymyxin or an aminoglycoside. This is caused by synergistic killing and resistance

prevention, as carbapenems are not subject to the same resistance mechanisms as polymyxins and aminoglycosides.

Another recently proposed approach for treating KPC-producing Enterobacteriaceae is the double-carbapenem combination therapy [128,129]. Specifically, the rationale is using a carbapenem with increased affinity for KPC, that is, ertapenem, to act as a 'suicidal' drug in order to improve the action of another carbapenem, especially doripenem, with increased stability against the hydrolyzing activity of KPC [128,129]. Indeed, experimental data simulating high dose doripenem regimens (2 g every 8 h infused over 3 or 4 h) plus ertapenem (1 g daily) have shown enhanced microbiologic efficacy of this combination over doripenem in monotherapy and this effect has been attributed to the interaction between ertapenem and the carbapenemase enzyme [128,129]. Anecdotal case reports have shown clinical success with combinations of high dose doripenem or meropenem plus ertapenem in the treatment of PDR Enterobacteriaceae [130,131], and double-carbapenem therapy may be a promising alternative against pathogens with such a resistance profile, particularly in combination with a third drug. However, non-carbapenemase-mediated resistance to carbapenems also occurs among CR GNB and combining carbapenems is expected to be ineffective against such isolates. Thus, such a strategy may be potentially useful against carbapenemase-producing strains, but more clinical data are needed to routinely recommend such practice.

Finally, there is some pre-clinical evidence that carbapenems may present higher microbiological efficacy against non-carbapenemase-producing CR *K. pneumoniae* in comparison with KPC-producing isolates [132]. Additionally, one study reported higher microbiological activity of carbapenems against
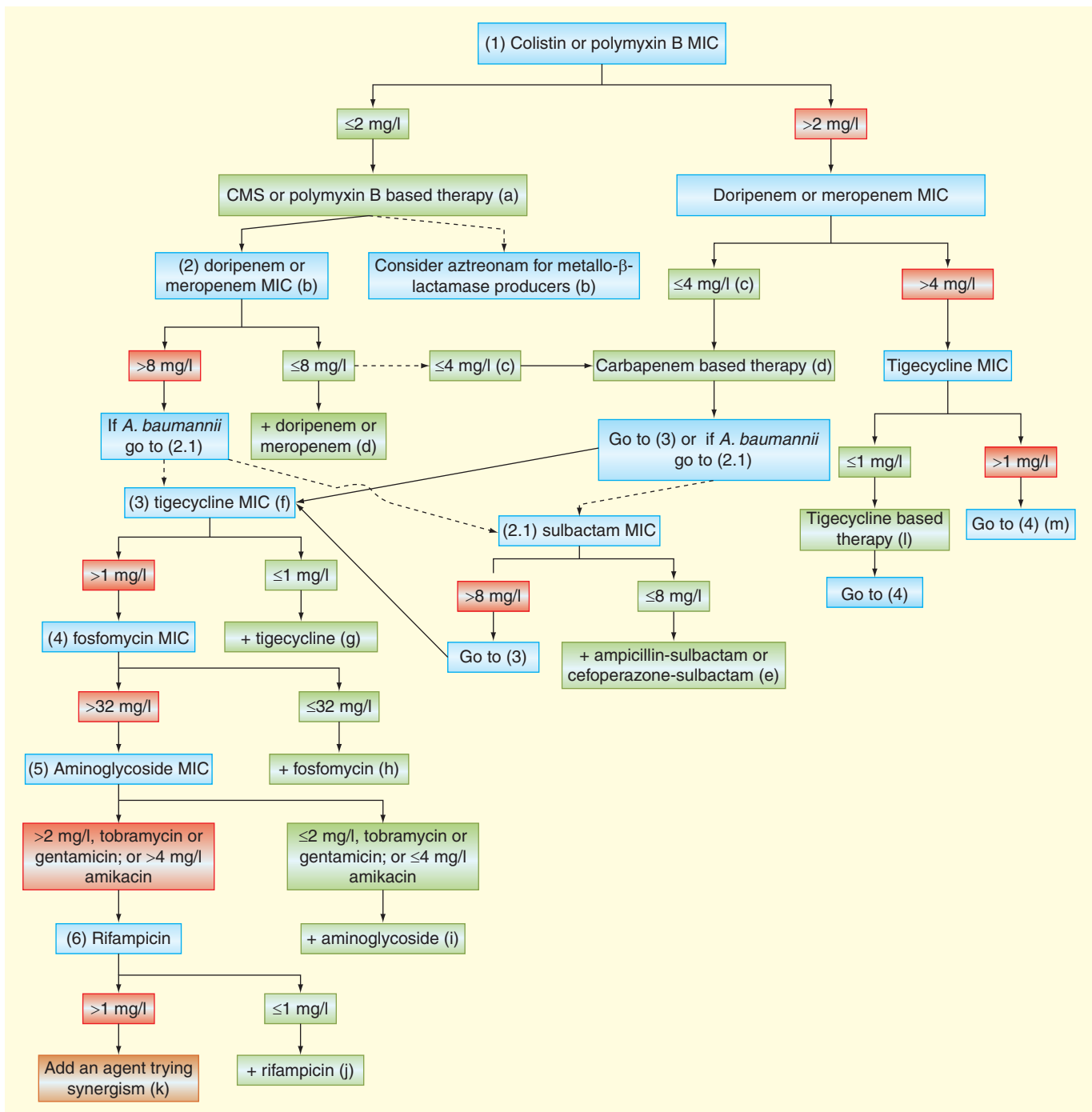
Figure 2. Flowchart for selecting mainstream and adjuvant therapy against Gram-negative bacteria. (a) Colistin methanesulfonate sodium (CMS) – Loading dose: 150,000 IU (corresponding to ~5 mg colistin base activity) × weight in kg; caution should be taken in using any dose above the current maximum approved daily dose of 10 million IU (~300 mg of colistin base activity); maintenance dose started 12 or 24 h later: 9–12 million IU/day split into 2 or 3 doses (every 8 or 12 h) for patients with creatinine clearance ≥60 ml/min. Adjust for renal dysfunction [102]. Polymyxin B – Loading dose recommended: 20,000–25,000 IU/kg (~2–2.5 mg/kg) followed 12 h later by 25,000 IU/kg/day (MIC<1 mg/l) to 30,000 IU/kg/day (MIC = 1 or 2 mg/l) split into two daily doses (every 12 h). For polymyxin B, no need for dose adjustment in renal dysfunction or continuous venous–venous hemodialysis [36]. CMS may be preferred for urinary tract infections owing to high urinary concentrations. **(b)** If the pathogen is suspected to be a metallo-β-lactamase-producing GNB, the aztreonam MIC may be evaluated at the same time as the MIC of the carbapenems. For aztreonam MICs ≤8 mg/l, consider aztreonam as the preferred combination drug at a dose of 6–8 g/day split into 3–4 doses that are given as 3–4 h infusion. **(c)** Some authors suggest that if the MIC ≤4 mg/l for carbapenems (maybe ≤2 mg/l for doripenem), a carbapenem should be the cornerstone drug in the combination scheme. **(d)** Doripenem: 2 g every 8 h infused over 3–4 h. Meropenem: 2 g every 8 h over 3–4 h. Imipenem may be used 1 g every 6 h, but there is few data concerning its stability in extended infusion and it poses a higher risk of convulsion at higher doses. Many carbapenemases possess a higher hydrolytic activity against imipenem. **(f)** Go to step **(4)** if the organism is a *Pseudomonas aeruginosa*.

(e) 9–12 g/day of the sulbactam component every 6–8 h infused over 3–4 h. High-dose extended infusion sulbactam may also be considered against organisms with MIC = 16 mg/l. **(g)** 200 mg as loading dose followed by 100 mg every 12 h for MIC = 0.5 or 1 mg/l, or 100 mg as loading dose followed by 50 mg every 12 h may be appropriate for MIC ≤0.25 mg. Higher doses may be considered for severe urinary tract infections. **(h)** Since the pharmacokinetic/pharmacodynamic (PK/PD) parameters of fosfomycin are not well defined, high doses (20–24 g/day divided in 3 or 4 doses) are recommended for fosfomycin MIC = 16–32 mg/l. Lower doses (12–16 g/day) may be appropriate for MIC<16 mg/l. Further studies are required to confirm these suggestions. **(i)** Gentamicin and tobramycin should be chosen on the basis of the lower MIC; MIC ≤0.5: 5 mg/kg once daily. MIC 1 or 2 mg/l: 7 mg/kg once daily (for MIC = 4 mg/l even higher doses may be more appropriate); a loading dose must be administered in critically ill patients. Amikacin: 15 mg/kg once daily is more likely appropriate for MIC ≤4; for MIC = 8 or 16 mg/l higher doses may be necessary; a loading dose ≥25 mg/kg must be administered in critically ill patients. Increased doses and shorter duration of therapy (preferably <7 days) may be necessary, since toxicity depends on aminoglycoside therapy duration and dose. If drug level monitoring is available, consider to apply target concentration intervention software or to follow a nomogram, for example, the Hartford algorithm [217]. **(j)** Few PK/PD data for GNB; IV is preferable if available; 10 mg/kg every 12 h. Higher doses have been used but toxicity must be further evaluated [196]. **(k)** Doripenem or meropenem at doses indicated above + ertapenem 1 g daily. Addition of a third non-carbapenem drug should also be considered. **(l)** If tigecycline is the cornerstone drug, high doses should always be considered regardless of the MIC. **(m)** There are very few or no data for fosfomycin, aminoglycosides and rifampicin as the mainstream drug. If the use of any of these drugs is supported by the MIC, the use of two other drugs is strongly recommended. Emergence of resistance to fosfomycin, rifampicin and aminoglycoside monotherapy is very common, both *in vitro* and in patients.

NDM-1-producing compared with KPC-2-producing isolates [133]. Thus, although clinical support is still lacking, carbapenems may be especially attractive as the mainstream agents against non-KPC-producing *K. pneumoniae* isolates with MIC ≤ 4 mg/l and offer an excellent safety profile.

Tigecycline

Tigecycline is a minocycline derivative belonging to the new class of antimicrobials known as glycylcyclines [134]. It is a broad-spectrum antimicrobial with activity against many Gram-positive, Gram-negative and anaerobic pathogens and has been frequently prescribed as a part of combination schemes against CR Enterobacteriaceae and also CR *A. baumannii* [48,135,136]. Unfortunately, tigecycline is not active against *P. aeruginosa* [134]. Despite some differences in the reported susceptibility breakpoints of this drug (1 or 2 mg/l), it has been shown in many surveillance studies that tigecycline presents good *in vitro* activity against many MDR and XDR Enterobacteriaceae and *A. baumannii* isolates [137,138].

Several meta-analyses of RCTs involving tigecycline versus comparators have shown that tigecycline therapy was associated with lower cure and higher mortality rates than comparators in patients treated with tigecycline [139–142]. Additionally, a RCT compared tigecycline (using the approved dose in the product label: 100 mg loading dose followed by 50 mg every 12 h) plus ceftazidime with imipenem-cilastatin plus vancomycin for the treatment of hospital-acquired pneumonia (HAP) [143]. A significantly lower cure rate was found in the tigecycline group in the subset of patients with ventilator-associated pneumonia [143]. These studies discouraged the use of tigecycline alone for the treatment of severe infections, especially pneumonia. However, considering its *in vitro* activity against many CR Enterobacteriaceae and *A. baumannii*, tigecycline has been used as a part of combination schemes, usually as the adjuvant agent but also as the cornerstone treatment [8,46,144].

The fAUC/MIC is the PK/PD index that best correlates with *in vitro* activity of tigecycline [145]. The tigecycline PK is characterized by low serum concentrations, frequently below the MIC of many GNB [146]. This fact has led many physicians to prescribe higher tigecycline doses in order to increase serum concentrations and optimize the AUC [147,148]. Considering the linearity of tigecycline PK, the AUC increases in proportion with increasing tigecycline doses [149]. Thus, a second RCT comparing two higher dosage regimens of tigecycline (150 mg followed by 75 mg every 12 h and 200 mg followed by 100 mg every 12 h) with imipenem/cilastatin in subjects with HAP was performed. It demonstrated that clinical response rates with the 100-mg dosage regimen were higher than with the 75 mg tigecycline dose and the imipenem/cilastatin control [150], supporting the benefit of higher doses to improve clinical outcomes. Importantly, the safety profile of the higher doses was similar to the approved dose of tigecycline [150].

To assess the PK/PD and patient-specific factors affecting clinical and microbiological outcomes, PK and clinical data retrieved from patients enrolled in the first RCT of tigecycline for the treatment of HAP were further analyzed [145]. Assuming an unbound fraction of tigecycline of 0.20, the authors found that a fAUC/MIC ≥0.90 and ≥0.35 were associated with higher clinical and microbiological response rates, respectively, suggesting that these values should be targeted when prescribing tigecycline, at least for pulmonary infections [145]. Thus, considering a protein binding of 80% and the mean AUC_{0-24h} reached after a 100-mg dose [151], a fAUC/MIC ≥0.90 against pathogens with an MIC for tigecycline of 0.5 and 1 mg/l will be more easily reached with the high dosage regimen. For MICs below 0.5 mg/l, the usual dose of 50 mg every 12 h may be appropriate for achieving an fAUC/MIC ≥0.90. In contrast, if the MIC is 2 mg/l (susceptibility breakpoint for Enterobacteriaceae according to the FDA), this target is unlikely to be reached.

Fosfomycin

Fosfomycin, a phosphonic acid derivative, is another old broad-spectrum antibiotic that has become attractive as an alternative agent against CR GNB, particularly against Enterobacteriaceae [17,152–155]. Fosfomycin demonstrates considerable

in vitro activity against many of these organisms [156–160]. It is an extremely low molecular weight antibiotic, which is chemically unrelated to any other anti-bacterial agent [152,153]. It also has a unique mechanism of action through inhibition of a specific component of peptidoglycan synthesis by blocking the formation of N-acetylmuramic acid [152,153]. This drug is available for oral use as fosfomycin tromethamine and as fosfomycin disodium for parenteral use. The latter form is available only in a few countries, mostly in Europe, where it has been increasingly used as an adjuvant treatment against CR GNB [17].

Fosfomycin tromethamine is only used for the treatment of non-complicated urinary tract infections, because it rapidly reaches high urinary concentrations (~1000–4000 mg/l), far above the MIC of most Enterobacteriaceae (≤64 mg/l), after the administration of the usual 3 g single dose [161]. However, most attention has been given to the intravenous use of fosfomycin, considering its favorable PK and safety profile, and its use as an adjuvant treatment against many CR Enterobacteriaceae has been increasingly reported [162]. It is less frequently used against *P. aeruginosa*, especially because of higher MICs and a higher potential for development of resistance during therapy in this organism [88,163–169].

Fosfomycin has a negligible protein binding and high serum concentrations are reached following intravenous administration, with Cmax ranging from approximately 200 to 600 mg/l, depending on the dose and duration of infusion (bolus to 60 min), according to most recent PK studies [161]. Good distribution into many tissues and body fluids, including cerebral spinal fluid and lung, has also been reported [161]. However, despite these attractive PK characteristics, there are still many gaps in the knowledge of PK and PK/PD of fosfomycin that must be overcome to recommend this drug based on more complete scientific data. Its use, either as the main or adjuvant treatment against CR GNB, mostly relies on empirical experience.

The PK/PD index associated with microbiological activity of fosfomycin is still not elucidated and both concentration- and time-dependent activity have been suggested [161]. Thus, although well tolerated even in 'high' dosage regimens (20–24 g/day, divided in 3 or 4 doses), these doses are still empiric and no further recommendation can be made at this time. Dosage regimens have not been optimized based on clinical data comparing distinct dosage regimes or on PK/PD, as discussed earlier.

Finally, rapid emergence of resistance during therapy has been described for fosfomycin, especially in *P. aeruginosa* [166–168]. It has been suggested that the combination of fosfomycin with other active drugs might protect against this effect [88,169]. However, development of resistance to fosfomycin in three cases of KPC-producing *K. pneumoniae* bacteremia, where this drug was used as a part of combination schemes, raised concerns on the ability of such a combination to prevent the emergence of fosfomycin resistance, at least in severe infections by KPC-producing *K. pneumoniae* [169]. Further studies on prevention of resistance strategies for fosfomycin are warranted.

Aminoglycosides

Aminoglycosides have been used for more than 50 years against a large variety of infections. They act at the 30S subunit of the ribosome, interfering with bacterial protein synthesis [170]. This effect likely contributes to prevention of emergence of resistance in combination regimens, as inhibition of protein synthesis will prevent the over-expression of resistance mechanisms that depend on protein synthesis (Figure 1). The main aminoglycosides prescribed for GNB infections are gentamicin, tobramycin and amikacin, and the PK properties of these drugs are quite similar [170,171]. Aminoglycosides cause concentration-dependent bacterial killing and have a prolonged post-antibiotic effect [170,171]. Whereas a Cmax/MIC ratio of 8–10 for aminoglycosides has been associated with maximal bacterial killing and clinical efficacy [170–172], it has also been suggested that the AUC/MIC may be more closely associated with bactericidal activity, especially in Enterobacteriaceae [173].

A gentamicin or tobramycin dose of 7 mg/kg infused over 30 min leads to peak concentrations from approximately 15–30 mg/l [174,175]. After 15 mg/kg of amikacin over 30 min, the maximum concentration was on average 40.9 mg/l [176]. Considering the protein binding of aminoglycosides (usually <10%), these doses would be optimal against pathogens with MICs <2 mg/l for gentamicin and tobramycin, and ≤4 mg/l for amikacin. It should be noted that the susceptibility breakpoints for these drugs are 2 or 4 mg/l for gentamicin and tobramycin, and 8 or 16 mg/l for amikacin depending on the organism or if established by European Committee on Antimicrobial Susceptibility Testing or Clinical and Laboratory Standards Institute [301,302]. However, there are some data associating AUC/MIC with surrogate clinical outcomes, such as the probability of afebrility by day 7 of aminoglycoside therapy as well as nephrotoxicity [177–180]. For gentamicin and tobramycin, the highest probabilities of clinical success with lower probabilities of renal toxicities were seen when 5 mg/kg once daily was administered for organisms with MIC ≤0.5 mg/l [180]. When using 7 mg/kg once daily against organisms with MIC = 4 mg/l (CLSI susceptibility breakpoint), the probability of afebrility by day 7 drops to 58% and the probability of nephrotoxicity increases to 51% [180]. According to these latter studies, the current breakpoints may be too high if a high probability of successful treatment with acceptable toxicity is expected [177]. In addition, it is well demonstrated that critically ill patients have a larger volume of distribution and therefore require higher aminoglycoside loading doses to achieve therapeutic concentrations [181]. A first dose of ≥25 mg/kg amikacin should be administered to achieve therapeutic concentrations in these patients [181]. It is likely that these higher doses would be still required along the entire therapy, but clinical data on this are lacking [181].

The administration of a single daily dose of aminoglycosides has been widely used in order to achieve higher peak serum concentrations and decrease the risk for nephrotoxicity and ototoxicity [177]. However, it should be noted that this benefit on nephrotoxicity depends on the cumulative dose and there is

virtually no difference between once- or multiple-daily dosage regimens after 5 or 6 days of therapy using currently recommended doses [179]. Once daily dosing has also been associated with a lower propensity for adaptive resistance in *P. aeruginosa* owing to the adaptive over-expression of the MexY transporter component of the MexXY-OprM efflux pump [182]. Additionally, the post-antibiotic effect of aminoglycosides had a longer duration for higher peak concentrations [177].

Nonetheless, even potentiating activity and lowering toxicity with once-daily regimens, it is difficult to achieve optimal activity of aminoglycosides in monotherapy when the MICs of CR GNB for these organisms are above the susceptibility breakpoint, considering the narrow therapeutic window of aminoglycosides. Fortunately, there are some CR GNB isolates, especially some KPC-producing Enterobacteriaceae that still remain susceptible to at least one of these agents. It is also likely that aminoglycosides can achieve synergistic killing in combination with another antibiotic at sub-MIC concentrations [183]. However, determination of the MIC of the drug may be useful to adjust dosage regimens to maximize therapeutic effect and decrease toxicity. Except for synergistic combinations, aminoglycoside monotherapy has a limited role when the isolate presents with *in vitro* resistance.

Another point is that it may be useful to assess the susceptibility profiles of the different aminoglycosides, since they may present some differences in potency against distinct species and in resistance profiles, depending on the molecular mechanism implied in aminoglycoside resistance [184]. Some data indicated that tobramycin was the most active agent against *P. aeruginosa* and *A. baumannii*, with MICs that were 2- to 4-fold lower than those for gentamicin [171]. Thus, considering the similar PK of the latter drugs, tobramycin might be preferred against non-fermenters. Against Enterobacteriaceae, amikacin usually presents lower resistance rates than gentamicin and tobramycin [185].

Aminoglycosides are generally administered as once-daily doses. Due to a narrow therapeutic index, individualizing dosage regimens is important to attain PK/PD targets and decrease toxicity [170]. Various nomograms have been developed to guide dosing by therapeutic drug monitoring. Furthermore, advanced clinical software that incorporate the aminoglycoside concentrations observed in a patient with Bayesian population PK models and the effect of specific patient characteristics on the PK to provide optimized individualized dosage regimens are available and recommended [186,303]. It is beyond the scope of this review to discuss each nomogram or algorithm, but these can be found elsewhere [170,187]. Finally, plazomicin (formerly ACHN-490), a new aminoglycoside with increased resistance to some aminoglycoside-modifying enzymes, has been clinically evaluated and it may be potentially useful in the near future against isolates with resistance to other drugs in this class [184].

Rifampicin

Rifampicin is a derivative of rifamycin with intra-cellular antibacterial activity determined by the suppression of RNA synthesis initiation by inhibiting DNA-dependent RNA polymerase [188]. It has a broad spectrum of activity including Gram-positive and -negative pathogens, although it is not recommended as a single therapeutic agent because of rapid emergence of high-level resistance *in vitro* and *in vivo* [188]. Apart from its use against some *Staphylococcus aureus* infections, rifampicin has been used in combination with polymyxins against CR GNB, most notably against *A. baumannii* [189,190]. This use is based on pre-clinical studies indicating synergism of such combinations, but it has not been corroborated by clinical evidence of benefit according to two recent RCTs [23,24]. As described earlier, PK/PD approaches based on pre-clinical data show a strong benefit of colistin plus rifampicin combination therapy compared with monotherapy.

Indeed, neither CLSI nor EUCAST have defined breakpoints of rifampicin for Gram-negative organisms. The susceptibility breakpoint proposed for *S. aureus* and *Enterococcus* spp. is ≤1 mg/l [301,302]. The French Society for Microbiology has established a rifampicin breakpoint for *A. baumannii* based on MIC distributions (susceptible, ≤4 mg/l; intermediate, 8–16 mg/l and resistant, >16 mg/l) [191]. Nonetheless, the PK/PD index that best correlates with anti-bacterial activity has not been elucidated so far. It is known that it has a Cmax/MIC related activity with a potent post-antibiotic effect against *M. tuberculosis* [192], but the ratio of AUC/MIC, an exposure-dependent metric, has also been correlated with a reduction in bacterial counts [193]. Also the long post-antibiotic effect may not translate to other more rapidly replicating bacteria.

The administration of 600 mg rifampicin orally resulted in peak serum concentrations of 7–10 mg/l, and following intravenous administration of 300 or 600 mg over 30 min peak concentrations of 9 or 17.5 mg/l are reached [188]. However, considering an 80% protein binding [188,194], it is unlikely that unbound rifampicin concentrations will either reach peak concentrations able to achieve a Cmax/MIC, which has been associated with best anti-bacterial activity (~8–10) [195] or appropriate *f*AUC/MIC [194,195], considering the MICs of rifampicin against most CR *A. baumannii* [23,195]. Thus, any activity of this drug most likely relies on its potential synergistic proprieties rather than on an anti-bacterial activity *per se*. Finally, although doses as high as 600 mg every 8 h have been administered to some patients, the safety of these dosages should be further evaluated and therefore they cannot be routinely recommended [196].

Other agents
Sulbactam

Sulbactam is a β-lactamase inhibitor with a chemical structure similar to β-lactams. It is commercially available mainly in combination with ampicillin or cefoperazone. However, sulbactam clearly has intrinsic activity against *A. baumannii* isolates by binding to penicillin-binding proteins and contributes the major part of the activity in the combinations with ampicillin or cefoperazone [197]. There is reasonable clinical experience with sulbactam against *A. baumannii* [39,198,199], but few studies

have assessed the PK/PD of this agent, particularly against CR *A. baumannii*. Nonetheless, an experimental study demonstrated that $fT_{>MIC}$ is the PK/PD index that best correlates with sulbactam efficacy [200]. Although no specific target has been defined, sulbactam was as efficacious as imipenem against *A. baumannii*, when the sulbactam $fT_{>MIC}$ was similar to that of imipenem [200]. In a recent *in vitro* PD model of *A. baumannii* infection with human-simulated exposures of ampicillin/sulbactam, the $fT_{>MIC}$ of sulbactam against the three sulbactam non-susceptible isolates (MIC: 16–32 mg/l) was 50–65% with 3 g sulbactam every 8 h as 4 h infusion, compared with 5–30% with 1 g of sulbactam every 6 h over 30 min [201]. Extended infusion regimens achieved significantly more bacterial reduction against these non-susceptible isolates than standard 30 min infusions [201].

Only recently, a study has evaluated the PK/PD of sulbactam using Monte Carlo simulations [202]. This study predicted that doses of 2 g sulbactam given as 4 h infusions every 8 h had a probability of 97% to achieve concentrations above the MIC of 4 mg/l (CLSI susceptibility breakpoint) over 40% of the dosing interval [202]. With this extended infusion, administration of 4 g every 8 h achieved a probability of target attainment (PTA) for $fT_{>MIC} \geq 40\%$ of 97% for an MIC of 8 mg/l. Considering these recent data and that sulbactam presents a relatively good stability at 37°C for up to 24 h [203] it should be considered as an adjuvant drug when the MIC is ≤4 mg/l, and potentially when the MICs are 8 or 16 mg/l. Extended infusion is recommended.

Aztreonam

Aztreonam is a monobactam antibiotic with a similar mechanism of action compared with other β-lactams such as cephalosporins [204]. The monobactam class is 'unique' among the clinically available β-lactams in its capacity of not being hydrolyzed by metallo-β-lactamases [11]; thus, aztreonam is an important therapeutic option against metallo-β-lactamase-producing CR GNB [11,205]. The major cause for its limited use is that the vast majority of metallo-β-lactamase-producing isolates also produce extended spectrum β-lactamases and/or AmpC enzymes that can hydrolyze aztreonam [11]. In practice, it is only an option for a few isolates that produce metallo-β-lactamase and do not express other broad spectrum β-lactamases that inactivate aztreonam. However, considering its low potential for AmpC induction [206], it may be a good option even for Enterobacteriaceae isolates with inducible chromosomal AmpC β-lactamases, if there is susceptibility *in vitro*. Aztreonam may also be a valuable component of β-lactam plus aminoglycoside combination therapies [207,208].

There are few PK/PD studies available with aztreonam, but in a murine thigh infection model with human simulated doses, 2 g aztreonam every 6 h over 30 min was able to reach a $fT_{>MIC}$ of 100, 90, 65 and 38% against isolates with aztreonam MICs ≤4 (CLSI susceptibility breakpoint), 8, 16 and 32 mg/l, respectively [209]. Hence, aztreonam might be considered even for some non-susceptible isolates, in particular for combination therapies.

Alternative adjuvant therapies
Inhalatory therapy

Although therapy with inhaled antibiotics is not being considered as a part of combination schemes, it has been used as an adjuvant to systemic therapy in ventilator-associated pneumonia caused by CR GNB. The main drug used is CMS and the rationale is reaching higher drug concentrations at the site of the infection while avoiding or minimizing systemic toxicity. Although many case series have reported good response rates with inhalatory therapy, most have lacked a control group [210–212].

In the few comparative studies, including two RCTs, no benefit in mortality was found with adjuvant inhalatory therapy [213–215]. Nonetheless, most, but not all studies [213–215], have found higher rates of microbiological eradication with inhalatory therapy. Considering the current clinical evidence, inhalatory therapy cannot be routinely recommended in the treatment of ventilator-associated pneumonia caused by CR GNB. However, it might be considered in cases where systemic polymyxins are not tolerated and/or when microbiological eradication is an objective of the therapy. Indeed, it might have implications in the control of dissemination of these organisms, but it still requires further investigations. In the authors' opinion inhalatory therapy cannot substitute an adjuvant parenteral drug in combination schemes.

Expert commentary

To date combination therapy for infections by CR GNB is not supported by evidence from a series of adequately sized RCTs. However, pre-clinical data and emerging clinical evidence from observational studies have suggested that antibiotic combinations may be better than monotherapy against CR GNB. Although the preliminary clinical evidence is mainly based on studies involving CR *K. pneumoniae*, this practice has been extrapolated and applied to the management of other important CR GNB such as *P. aeruginosa*, *A. baumannii* and other Enterobacteriaceae.

There are some data available indicating that combination therapy containing a carbapenem drug is associated with improved outcomes in the treatment of CR *K. pneumoniae*. However, there is still no definitive evidence of which is the most appropriate combination scheme, and it is likely that the best antibiotic combination should be individualized, depending on the organism, its susceptibility profile, the site of the infection and the patient to be treated. In order to help clinicians decide which would be the best combination therapy, we propose an algorithm for the choice of the antimicrobial drugs (FIGURE 2). The primary aim of the therapy is to optimize the use of the cornerstone therapy. Second, physicians should look for an adjuvant agent that is active or most likely active against the pathogen and further optimize its PK/PD target attainment. The activity of the drug is not necessarily based on the breakpoints defined by CLSI or EUCAST, but on the probability of PK/PD target attainment when dosage regimens are optimized based on the known PK proprieties. The proposed flowchart for selecting the main and adjuvant drug considered the published data and the authors' clinical experience with antibiotics against CR GNB infections. Additionally, we considered the probability of achieving anti-bacterial activity by attaining PK/PD targets through

the optimization of dosage regimens, the toxicity profile and the potential for synergism based on preclinical studies. When using the fluxogram, it should be kept in mind that an adjuvant compound may be completely inactive in monotherapy and still be highly beneficial in a rationally designed combination regimen.

Five-year view

There is a need for systematic studies specifically designed to assess the efficacy of rationally optimized combination dosage regimens versus monotherapy in the treatment of CR GNB. From our perspective, it is critical to leverage latest *in vitro* and animal infection models to rationally optimize combination dosage regimens and consider the infection site specific PK profiles of each antibiotic before evaluating optimized combinations in patients. This translational process can be excellently supported by mechanism-based modeling. Future studies should address the following questions for each specific CR GNB species: Is rationally optimized combination therapy superior to monotherapy? If so, is it superior even when combining a non-susceptible drug? Which combination schemes may be more suitable for each pathogen and site of infection? Is high-dose initial combination therapy beneficial? What is the optimal duration of therapy and should therapy be de-escalated after, for example, 2 or 3 days?

Indeed, it is unlikely that these questions will be answered in the next five years. Nonetheless, it can be expected that a clearer definition on the role of combining drugs against CR GNB, particularly against CR Enterobacteriaceae, will be obtained during this period. Meanwhile, it seems that combination therapy will continue to be the standard of care in the treatment of severe infections by CR GNB. This approach and further studies assessing it should always take into account the PK/PD principles for optimizing the use of cornerstone agents (specifically polymyxins) as well as adjuvant antibiotics in combination treatments to maximize bacterial killing and minimize further emergence of resistance.

Key issues

- Resistance to carbapenems in *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Enterobacteriaceae, mainly determined by the production of carbapenem-hydrolyzing β-lactamases, has emerged worldwide and is severely challenging antimicrobial therapy against these pathogens.

- Most resistant to carbapenems Gram-negative bacteria isolates are only susceptible to polymyxins, which are commonly the main antibiotic class used against these isolates.

- Some shortcomings of polymyxins, as well as other potentially active agents, for use in monotherapy against infections by CR GNB have been raised, including emergence of resistance during monotherapy and possible lower clinical efficacy. Combined with strong preclinical evidence supporting the use of antibiotic combinations, this has led to the common practice of prescribing two or more agents, even without solid clinical evidence for this practice.

- Combination schemes usually rely on a cornerstone drug, most often polymyxin B or colistin, but also tigecycline and even a carbapenem, plus an adjuvant agent, which may or may not present *in vitro* susceptibility against the carbapenems Gram-negative bacteria (CR GNB) isolate considering the current clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing breakpoints.

- Optimization of pharmacokinetic/pharmacodynamic target attainment both with the cornerstone and the adjuvant antibiotic should be attempted to improve clinical and microbiologic outcomes. More systematic studies to rationally optimize combination therapies against resistant to carbapenems Gram-negative bacteria are urgently needed.

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