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REVIEW

Combination therapy for carbapenem-resistant Enterobacteriaceae: increasing evidence, unanswered questions, potential solutions

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Abstract Carbapenem-resistant Enterobacteriaceae (CRE) are associated with a high mortality rate and are an increasing problem worldwide. In this mini-review, we consider the growing number of observational studies in favour of combination therapy but highlight the absence of randomised control trials. We discuss the importance of data on minimum inhibitory concentrations (MICs), both for surveillance and for individual patient management. We examine the issues surrounding the use of carbapenems, polymyxins and tigecycline in the treatment of CRE. When and how should we be using carbapenems? Which polymyxin is best? Is tigecycline much maligned? Further studies are urgently needed to validate drug combinations, doses and ratios to maximise efficacy whilst reducing drug exposure and adverse effects.

Introduction

Antimicrobial resistance is the biggest infection challenge of our generation. Carbapenem-resistant Enterobacteriaceae (CRE) are now found worldwide and there is evidence of dissemination outside healthcare institutions in some regions [1]. Infections with CRE are associated with increased mortality [2, 3].

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Combination therapy: growing evidence?

There is increasing evidence to support the use of combination therapy in severe infections caused by CRE. In vitro data suggest high rates of synergy between polymyxins and carbapenems [4]. Recent retrospective observational studies of patients with isolates of *Klebsiella pneumoniae* producing *Klebsiella pneumoniae* carbapenemase (KPC-KP) have shown improved survival in patients on combination therapy with two or more antibiotics [5–7]. A review of 20 descriptive and observational studies by Tzouvelekis et al. suggests that combination therapy is superior to monotherapy in the treatment of CRE and that mortality in those patients who received monotherapy did not differ significantly from those receiving no active therapy at all. The authors acknowledge, however, the difficulties in collating data and that the vast heterogeneity of study design included in this review made it impossible to perform a methodologically rigorous meta-analysis [8]. Two randomised controlled trials (RCTs), NCT01732250 and NCT01597973, are currently investigating colistin versus carbapenem–colistin combination therapy for multidrug-resistant Gram-negative organisms. These are due to complete in 2016 and 2017, respectively.

In addition, combination therapy may reduce the development of drug resistance by suppressing hetero-resistant subpopulations [4, 9]. This has both clinical and ecological benefit.

Opponents of combination therapy complain of the additional cost, the increased risk of adverse effects and the greater use of antibiotics creating further resistance pressure in the hospital environment. They point to previous meta-analyses that have failed to show a mortality benefit in combination therapy [10, 11]. These meta-analyses are fundamentally misleading. They include a large number of heterogeneous studies performed over a long period of time. The 2014 Cochrane

Review, for instance, searched for studies performed between 1966 and 2013 but the majority of included studies were performed between 1980 and 2000. This was long before the emergence of CRE [11]. They do not analyse sub-groups with multidrug-resistant organisms and, thus, do not address the question of combination therapy in this group.

Minimum inhibitory concentration: ignore at your peril

There are still many unanswered questions. It is clear that the incidence of resistant Gram-negative infections globally is rising. However, our understanding of the level of resistance is poor. In both academic and clinical medicine, we refer to bacterial isolates as either susceptible or resistant to antibiotics depending upon their breakpoints but this oversimplifies the problem. Few clinical studies have reported minimum inhibitory concentrations (MICs) among Enterobacteriaceae, and this is important because an isolate with, for instance, a meropenem MIC of 8 mg/ml is very different to one with an MIC of 32 mg/ml or above. This is highlighted in Tumbarello et al.'s retrospective study of KPC-KP; on sub-group analysis, higher survival rates were seen where meropenem was used in combination with another antibiotic if the isolate had a meropenem MIC of ≤8 mg/l [7]. Ultimately, we will need to establish treatment guidelines based upon the MICs of CRE to ensure that we adequately treat, but do not over-treat, these organisms. Under-treatment may result in the emergence of resistance within the patient. Over-treatment may result in the emergence of resistance due to antibiotic pressure in the hospital environment.

Revised PK/PD and clinical outcome data, presumably reflecting the rise in the MIC90 of clinical isolates, resulted in the Clinical and Laboratory Standards Institute (CLSI) reducing the breakpoints for Enterobacteriaceae in 2011. However, there has been little investigation into the trends in the MIC90 of Gram-negative organisms over time. These data are important, as they help to identify the development of resistant strains within the population. Data from Italy published as part of the Tigecycline Evaluation and Surveillance Trial showed the MIC90 of *K. pneumoniae* to meropenem increased from just 0.12 mg/l in 2006 to 0.2 mg/l in 2008 to ≥32 mg/l in 2010. During this time, the percentage of isolates resistant to meropenem increased from 1.4 % (2006) to 2.7 % (2008) to 16.4 % (2010) [12]. This mirrors our own unpublished data, which suggest that the MIC90 of Enterobacteriaceae to meropenem, colistin and tigecycline is rising year on year. This lack of data may be partly because the common methods performed to establish drug resistance are unable to evaluate the end point of resistance for organisms with high MICs. The meropenem Etest, for example, ranges from a concentration of 0.002 μg/ml to 32 μg/ml (CLSI breakpoint ≤1 mg/ml, European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint ≤2 mg/ml). Of the large antimicrobial surveillance studies, only the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) programme performs an MIC dilution to 256 mg/l for meropenem [13]. The other studies finish dilution at 16 mg/l and report any MICs above this as ≥16 mg/l. As Gram-negative organisms become increasingly resistant and CRE more common, it is more relevant to know how resistant a bacterial strain is (the factor by which the MIC exceeds the breakpoint) than how susceptible such a bacterial strain is (the factor by which an organism's MIC is less than the breakpoint), as this will direct antimicrobial therapy.

Carbapenems: when and how?

Carbapenems probably have a role in the treatment of some carbapenem-resistant organisms when used in combination therapy. The important question is when and how they should be used. As already mentioned, a mortality benefit may be seen if patients with meropenem MIC ≤8 mg/l are treated with combination therapy including meropenem [7]. With β-lactams, time over the MIC is the most important pharmacodynamic parameter. This can be increased by raising the dose or prolonging the infusion time. To achieve maximal bactericidal activity, the free drug concentration should exceed a pathogen's MIC by four-fold for 40–60 % of the dosing interval [14]. A number of clinical trials have evaluated prolonged infusion time in severely ill patients but the results have been inconsistent [15]. Some groups are now advocating this for CRE based on data from Monte Carlo simulation studies [15, 16]. Although this may be an effective strategy for isolates with borderline MICs [15, 17], it is impossible to achieve adequate free drug concentrations without significant drug toxicities for those with high MICs; for instance, a meropenem MIC >16 μg/ml. Furthermore, by exposing organisms with intermediate or borderline resistance to sub-optimal antimicrobials and doses, we amplify the resistant population, resulting in hetero-resistance and, eventually, frank drug resistance [18]. The strategy of prolonged β-lactam infusion as part of combination therapy for CRE has not yet been evaluated in clinical trials.

Isolates with meropenem MICs ≥16 mg/l are often only susceptible to polymyxins and tigecycline. Unfortunately, these drugs are both far from ideal and many questions surround their usage.

Polymyxins: which is best?

Polymyxins were first marketed in the 1950s when novel antibiotics were subjected to little regulatory scrutiny. They were

rapidly sidelined due to renal toxicity and, thus, their clinical utility was never fully characterised. Colistin (polymyxin E) and polymyxin B contain mixtures of products produced by fermentation, resulting in significant product heterogeneity. However, colistin undergoes further chemical modification to produce colistimethate (CMS, the prodrug which is given intravenously to patients), resulting in an even more heterogeneous mixture of up to 30 sulphomethylated derivatives [19]. This results in product-to-product and batch-to-batch variation that can result in variable pharmacokinetics and clinical efficacy.

Both colistin and polymyxin B have similar structures and in vitro efficacy, but they behave very differently in vivo. Polymyxin B is given in the active sulphate form. It rapidly achieves therapeutic concentrations when a loading dose is given and there is little inter-patient variability in serum concentrations [20]. By contrast, colistin is given as the inactive prodrug CMS and only around 25 % is converted to colistin in vivo. There is significant delay in attaining therapeutic serum concentrations, even when loading doses are given and, when this is achieved, it is difficult to maintain effective plasma concentrations in patients with normal renal function. In patients with similar renal clearance given the same dose of CMS, there may be a ten-fold range in the plasma concentration [21].

Therapeutic drug monitoring should be used for both drugs due to the narrow therapeutic index and concentration-dependent killing. However, it is more important for colistin due to the variable plasma levels. Sub-optimal plasma levels may result in clinical failure but may also expose already multidrug-resistant organisms to sub-optimal drug concentrations. Colistin is known to promote hetero-resistance and this may evolve to frank resistance in the presence of low serum concentrations or the absence of a second combination drug [9].

Finally, dosing strategies for colistin are extremely confusing; whilst Europe and India dose in international units (IU), the rest of the world doses in the number of milligrams of colistin base activity (CBA), which is different from the dose in milligrams of CMS. 30 mg of CBA equates to 80 mg of CMS, which equals about one million IU. Dosing errors have resulted in death and are probably under-reported [22].

Clearly, polymyxin B has pharmacological and safety advantages over colistin. However, these drugs are often considered equivalent and clinicians use whichever drug is available [20]. Worldwide, colistin is more widely available than polymyxin B. This is probably the reason that most clinical trials are conducted using colistin rather than polymyxin B. A recent meta-analysis on the efficacy of polymyxins in CRE included five studies where polymyxin B was used and 21 where colistin was used. The analysis did not distinguish between the two drugs [23]. The research community needs to further investigate the role of polymyxin B in the treatment of CRE, particularly in combination with meropenem or tigecycline, and by head-to-head comparison with colistin.

Tigecycline: much maligned?

Tigecycline is the first in the class of glycylcycline antibiotics. It inhibits bacterial protein synthesis by binding the 30s ribosomal subunit with five times the affinity of tetracyclines. This novel mechanism of action means that there is no cross-resistance with pre-existing antimicrobial classes, nor does tigecycline stimulate cross-resistance in other antimicrobial classes [24]. In a world where multidrug-resistant organisms are rapidly increasing, this is a highly advantageous property.

Tigecycline is licensed for complicated skin and soft tissue infections and complicated intra-abdominal infections. However, its broad spectrum and in vitro activity against multidrug-resistant organisms has tempted clinicians to use it for other indications. This resulted in observational studies and clinical trials in which tigecycline was used for a variety of unlicensed indications, including bloodstream infections and ventilator-associated pneumonia. It was used alone or in combination, for empirical or directed therapy, in critical care and non-critical care settings. This generated a lot of confused data which is further marred by concerns that standard doses of tigecycline are inadequate in patients with increased volumes of distribution and clearance, such as may be seen in patients with severe infections [25]. Subsequent meta-analyses analysed this muddle of data and concluded that tigecycline was associated with increased overall mortality and clinical failure [26, 27] or with increased incidence of adverse effects [28, 29]. A U.S. Food and Drug Administration (FDA) black box warning was issued citing increased mortality for both approved and non-approved indications of tigecycline.

Although the meta-analysis data and black box warning are concerning, it is important to recognise that these meta-analyses do not specifically address tigecycline use in multidrug-resistant organisms where other treatment options are extremely limited. In vitro data suggest possible synergism between tigecycline and colistin against CRE [30]. There are no RCTs investigating this or the use of tigecycline in combination with carbapenems or polymyxins. These are urgently needed.

Which drugs at what dose?

Although these three drug classes are far from ideal, they are currently the best which we have available for treating most CRE. We need to establish which drugs should be combined to maximise efficacy, minimise toxicity and limit the emergence of further resistance. Combination therapy including a carbapenem is currently favoured by observational studies in isolates with a meropenem MIC ≤8 mg/l. However, there are far fewer studies investigating carbapenem-sparing strategies for CRE. Sbrana et al. achieved an enviable 92 % clinical response rate when treating carbapenem-producing

K. pneumoniae. They used individualised carbapenem-sparing regimens comprising one, two or three drug combinations of tigecycline, colistin, fosfomycin and gentamicin [31]. Other groups have suggested using double-carbapenem therapy with ertapenem and doripenem in KPC-KP. The postulated mechanism is that the antimicrobial effect of doripenem is protected from carbapenemases by ertapenem, which is preferentially hydrolysed and acts as a sacrificial agent. There are in vitro data to support this approach [32, 33] but scanty data in vivo. A small number of case reports have suggested some clinical improvement in patients treated with a doripenem–ertapenem combination with or without a third agent [33, 34]. It is unclear whether carbapenem pairing, carbapenem sparing or double carbapenem therapy is better at preventing the emergence of resistance within the patient and the hospital environment.

The dose and ratio between drugs in combination therapies is also important. For synergy to occur in vitro, it may not be necessary for both drugs to be at maximum dose. Our own, as yet unpublished, data investigating synergy between isolates of multidrug-resistant *Acinetobacter baumannii* with meropenem and sulbactam MIC of 32 mg/l show that synergism is achieved when meropenem is used at standard doses but sulbactam is used at 1/8th of the standard dose. However, if the meropenem dose is reduced, then the sulbactam dose must be increased several times to obtain synergy depending upon the MIC of the particular isolate. Further investigation is needed to validate doses and ratios of combination therapy in clinical studies to maximise clinical efficacy whilst minimising adverse effects.

Conclusion

In conclusion, there is increasing evidence to support the use of combination therapy in carbapenem-resistant organisms but there are many unanswered questions. Further studies are needed in order to investigate the trends of rising MIC90s and clinical studies should report the MIC of the involved isolates rather than whether organisms are resistant or sensitive. Further work on polymyxins and tigecycline is urgently needed. Drug combinations and ratios must be validated in clinical trials to maximise efficacy whilst reducing drug exposure and adverse effects.

Conflict of interest The authors declare that they have no conflict of interest.

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