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**Antibiotic combination therapy against resistant bacterial infections: synergy, rejuvenation and resistance reduction**

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**Abstract**

**Introduction:** Anti-Microbial Resistance (AMR) is a pandemic which threatens modern medicine. There is a lack of effective drug treatment due to the slow pace, high cost and low achievable sales prices of new antibiotic monotherapies. A new hope comes in the shape of antibiotic combination therapy, which although used by mother nature, is under-explored and could provide the solution to AMR.

**Areas covered:** We performed a search of Pubmed and Medline using the keywords “combination therapy”, “antimicrobial resistance” for articles between 1930 and 2019, as supplemented with other relevant references to our knowledge. We will review the theoretical considerations for combination development and examine the existing and future clinical indications of combination therapies. We will discuss the potential of antibiotic combinations to provide therapeutic synergy, rejuvenating the effectiveness of old antibiotics to which the bacteria had developed resistance previously. We will examine the current thinking and evidence on resistance reduction using combination therapies, with a review on toxicity and drug-drug antagonism.

**Expert opinion:** Antibiotic combination therapy, exploiting synergies, old-drug rejuvenation and resistance reduction could provide the solution to AMR. The number of pharmaceutical companies in this area is likely to expand, bringing promising combinations to the bedside, to save millions of lives worldwide.

**Keywords:** Anti-Microbial Resistance; Antibiotic Combinations; Synergy; Superbug;

**Article highlights**

* Antimicrobial resistance (AMR) is a leading cause of mortality worldwide.
* Bacteria mutate faster than we can develop new antibiotics, combining antibiotics in novel regimens holds the key for controlling AMR.
* When certain carefully selected drugs are combined in the correct formulation, the bactericidal effects are synergistic, with potential to kill the highly resistant bacteria.
* Combination therapy is already used clinically for treating infections such as tuberculosis, the human immunodeficiency virus and non-infective diseases such as for cancer chemotherapy.
* Synergistic combination treatments can be developed cheaply, used in patients safely since the constituent drugs are well known, and thus could potentially be widely distributed.
* As new combination antimicrobial regimens emerge from the laboratories, these need urgent funding to be brought to the bedside, potentiating a new golden-age for combination therapy.

**1. Introduction**

In February 1941, an Oxford policeman, severely septic from a rose-thorn prick, became the first patient to receive a then revolutionary drug – penicillin [1]. His remarkable initial recovery was marred by rapid deterioration and death when this “wonder drug” ran out [1]. Indeed, since the discovery of penicillin in 1928, antibiotics have become our main armamentarium against bacterial infections [2]. However, even a year before penicillin was first used clinically, resistance to it had already emerged [3]. Much like how penicillin ran out for the first patient in 1941 [1] today, we are running out of antibiotics capable of killing the Anti-Microbial Resistant (AMR) “*Superbugs*” [4].

AMR kills around 700,000 people annually, and that figure could rise to 10 million annually by 2050, more than cancer and diabetes combined [5-8]. It threatens the achievements of medicine and brings a chilling notion that a minor scratch, prick or injury could kill [5].

Why do we find ourselves in this position? Mother Nature provides the answer. Bacteria themselves make antimicrobials, and some produce up to ten or more [9]. A single bacterium can become resistant to over twenty different antibiotics, suggesting that they are used to combatting attacks by multiple antibiotics [9]. When bacteria become resistant to an antibiotic, we simply develop another and use this new drug (sometimes un-sparingly) until, inevitably, the bacteria become resistant to it as well – this cycle simply repeats [5-8]. Bacteria develop resistance much faster than we can develop new antibiotics. In fact, new classes of antibiotics have not reached the market for Gram-negative bacteria for 30 years. We are fighting a losing battle, using a slow, expensive and incremental drug discovery strategy.

**1.1 Literature search methodology**

This review is based on Pubmed and Medline searches using the keywords “combination therapy”, “antimicrobial resistance” of articles between 1930 and 2019, as supplemented with other relevant references to our knowledge.

**2. AMR**

The World Health Organisation (WHO) identified three Critical Priority bacteria, *Acinetobacter baumannii* (CRAB), *Pseudomonas aeruginosa* (CRPA) and Enterobacteriaceae (CRE), that are resistant to carbapenems, and most other penicillins [10]. Resistance is rising against the “*last resort*” antibiotic, Colistin [10,11]. Only six companies have drugs in clinical trials that can potentially kill all three pathogens [10,11], and five such therapies have only reached Phase I or II trials. Further, less than 20% of all antibiotics undergoing Phase I trials will ultimately be approved, leading to huge research costs for drugs that do not make it to market [12,13]. When a New Chemical Entity (NCE) does reaches the market, as much as $500 million may have been spent on its development, leading to high initial prices to reap a return for investors [13]. This is economical evidence that an alternative model to NCE development is needed, that is low cost and results in low priced antibiotics.

We need a paradigm shift, a new strategy, a new therapeutic approach [10,12].Rather than failing to keep pace with AMR using NCE development, would it not be better to revive the potency of the existing drugs to which the bacteria have become resistant? What if we could switch ineffective drugs that we already know are cheap, safe and widely-available back to the lethal bactericidal agents that they once were? Would it not be revolutionary if we could combine these drugs together in a formulation that is effective against AMR?

**3. Antibiotic Combinations**

*“A single twig breaks, but the bundle of twigs is strong.”* Tecumseh 1768-1813.

The fact that penicillin-resistant *Staphylococci* had already emerged a year before penicillin was first used clinically [3] shows that bacteria are always one-step ahead. For over seventy years, the development and subsequent clinical use of antibiotics for the treatment of the bacterial infections has mostly been driven by monotherapy discovery [4,8,12]. Notable exceptions to the monotherapy “rule” are antibiotic combination therapy of bacterial diseases such as *Mycobacterium tuberculosis* [14] and *Helicobacter pylori* [15-17] infections. Prolonged combination antibiotic therapies are often employed for the treatment of bacterial infective endocarditis [18,19]. Successful application of combination therapy is also used in the treatment of the Human Immunodeficiency Virus (HIV) [20,21]. In non-infectious diseases, combination chemotherapy is used to treat solid tumours and haematological malignancies [22-24].

Combination therapies have several key advantageous features:

**3.1 Synergy**

When certain antibiotics are combined in vitro, the therapeutic effect is greater than the sum of each drug [25-27]. An example is the potentiation of vancomycin against *E. coli* when combined with trimethoprim or nitrofurantoin [28]. Owing to the large number of antibiotics in the market and almost limitless permutations, there exists a vast potential for therapeutic gains from this phenomenon.

**3.2 Rejuvenation**

Perhaps the most powerful effect of synergistic combinations is the potential to rejuvenate the effectiveness of old antibiotics against bacteria which have previously developed resistance against them [29,30]. This “Antibiotic Recycling” means that instead of the current practice of discarding old antibiotics when resistance arises, they can be reused [29,30].

**3.3 Resistance**

Antibiotic combinations can reduce the emergence of resistance. For example, for tuberculosis (TB), while monotherapy leads to resistance and disease relapse, combination therapy provides clinical improvement with reduced emergence of resistance [14,31].

**3.4 Spectrum**

For clinically suspected bacterial infections of unknown cause and before sensitivities can be obtained from culture results, empirical combination therapy widens the coverage of bacterial species. An example of this is the treatment of community-acquired pneumonia [32].

**3.5 Toxicity**

Lower doses of each drug in combinations can be used to avoid toxicity while exploiting the greater therapeutic effect [26,28]. For example, low-dose gentamicin with amoxicillin for bacterial endocarditis [19,33].

**3.6 Duration**

Some combinations can shorten the duration of therapy. An example of this is TB therapy [34].

**3.7 Fast and low-cost development**

The commercial development of a combination containing known marketed drugs is faster and lower cost than for NCE. With added perks of lower resistance emergence, this strategy could enable us to keep pace with AMR emergence. Compared to the very expensive traditional NCE monotherapy approach, combination therapy development is a better method already used by Mother Nature. This is being developed in clinical trials by Helperby Therapeutics, namely antibiotic combinations for common bacterial diseases. This radically different approach, compared to other companies (Figure 1), could provide the low-cost solution against AMR.

The interplay between research academia and pharmaceutical industries is the kernel of successful antibiotic development not only in bringing novel therapies from the bench to the bedside, but also in allowing the successful marketing of new drugs and combinations. Combination therapies often contain off-patent antibiotics, many of which are already used in clinical practice for a different clinical purpose [35]. The key novelty resides much more in the combination itself, rather than the individual drugs. For the purpose of Intellectual Property (IP) protection, one would expect challenges with the use of off-patent antibiotics. Each combination and formulation are patentable, as well as any novel steps in the manufacturing process. The key challenge for IP protection rests with picking the correct combination to take forward, which is where experience and refined drug selection methods become important. As the range of combination therapy widens, it is expected that IP protection will become commonplace.

**4. Synergy**

“*The whole is greater than the sum of its parts.*” Aristotle, 384-322 BC

Conceptually, we can differentiate between *net* synergy and *emergent* synergy when examining drug combinations. Traditionally, net synergy is the total amount of synergy of a group of antibiotics compared to all the individual effects of each drug within the combination. Recently, *emergent* synergy was introduced to indicate that some synergies can be considered emergent properties – that is, the synergy only emerges because all *x* drugs are in the combination, and not as a result of a lower-order interaction [36,37]. For example, in a five-drug combination, the emergent interaction is the total interaction after subtracting all lower-order interactions. In this case, all lower-order interactions would include all the interactions from all unique four-drug combinations, all unique three-drug combinations, all unique pairwise combinations, and all five single drug effects.

For clinical applications, it is advantageous for a combination to express both net and emergent synergy. This potential is under-exploited. A recent study of higher-order antibiotic combinations against *Escherichia coli* showed that net synergy is predominant over emergent synergy [38]. As seen in Figure 2, net synergy was observed in less than 5% of 251 pairwise, approximate 12% in 1,512 three-drug, 30% in 5,670 four-drug and more than 45% in 13,608 five-drug combinations [38]. These data suggest that the larger the combination, the greater the net synergy, at least up to 5-drugs. Antagonism also increased with larger combinations.

Synergy is measured *in vitro* using the Fractional Inhibitory Concentration Index (FICI) [39,40], which is the standard for assessing synergy, indifference or antagonism between two antibiotics [35,39]. FICI is the measured using the checkerboard method which determines the growth of microbes in the presence different concentrations of two antibiotics in 96 combinations. It is defined as the sum of the minimal inhibitory concentrations (MIC) of each antibiotic in combination divided by the MIC of the antibiotic when tested alone. FICI of ≤0.5 is synergistic; >0.5 to 4.0 is “no interaction”; and >4.0 is antagonistic [41].

Synergy can also be observed in time-kill experiments [42,43]. The dynamics of synergy can be demonstrated using hollow fibre experiments in which bacteria are grown in special tubes and are subjected to continuous flow of medium and antibiotics over time [44]. Lenhard and colleagues showed that while the polymyxin B + meropenem combination was unable to kill *Acinetobacter baumanii* resistant to both antibiotics (Figure 3) [44], addition of Ampicillin/Sulbactam achieved complete kill, despite the bacterium being resistant to Ampicillin/Sulbactam [44]. Bulman and Colleagues showed that colistin resistant *E. coli* could not be eradicated using polymixin B, aztreonam or amikacin individually, while complete kill was achieved using the combination of these drugs (Figure 4) [45].

**4.1 Synergy in the Clinical Setting**

Despite several clinical indications for antibiotic combination therapy for common bacterial infections, the data from Randomised Controlled Trials (RCT) remain limited. TB, however, is a notable exception with both pre-clinical and Phase III trials demonstrating efficacy of combination therapy [14,34]. This evidence is further boosted recently with the Federal Drugs Agency approval of the novel anti-TB agent Bedaquiline [46], which is being incorporated into novel anti-TB combination regimens [46]. Synergies between different anti-TB antibiotics have not been robustly demonstrated.

Colistin combinations are often used for highly resistant Gram-negative bacteria including the three Critical Priority Pathogens [47]. International consensus guidelines (Figure 5) [47] for the use of colistin (and other polymyxins) combinations acknowledged the lack of clear clinical evidence. The expert Panel voted for colistin combinations rather than monotherapy [47], recognising the lack of adequately powered RCTs that assessed colistin combination versus colistin alone. Against severe CRE infections, although the recommendation was strong, the evidence, mainly based on observational data, was limited [47]. Concerns were expressed over the risk of colistin resistance with monotherapy [47].

A novel combination between colistin and azidothymidine (AZT) is currently being developed [35]. This is distinctive since AZT is an antiretroviral drug which is not routinely used to treat bacterial infections; it constitutes a new class of antibacterial agent [35]. AZT blocks the incorporation of thymidine into replicating DNA [35]. *Ex vivo* studies showed synergistic activities against Carbapenem Resistant and Colistin Resistant Enterobacteriaceae (CRE) in patients treated with the combination therapy at approved therapeutic doses [35].

For invasive CRAB infections, the Panel voted for colistin combinations and this recommendation was based on randomized open-label studies [30,47-49]. In the case of CRPA infections, the Panel also recommended combination therapy [47,50-55].

For Methicillin resistant staphylococcus aureus (MRSA), whether patients should be treated with vancomycin alone (current standard) vs combination therapy is an area of controversy [56]. Promising in vitro results of combination therapy against MRSA have often failed to translate to clinical benefit in later trials [56-58]. The Combination of Vancomycin and β-Lactam Therapy for Methicillin-Resistant Staphylococcus aureus Bacteremia (CAMERA 1) [57] randomised 60 MRSA patients to either vancomycin monotherapy vs vancomycin-plus-flucloxacillin [57]. The combination therapy group had shorter mean time to bacteraemia resolution, without mortality benefit compared to the vancomycin monotherapy group [57]. The results of the follow up multi-centre study (CAMERA 2) [58] was recently presented. The investigators randomised 344 patients to either standard therapy (either vancomycin or daptomycin) vs standard therapy plus a β-lactam (flucloxacillin, cloxacillin or cefazolin) [59]. The investigators found no significant difference in primary clinical endpoints between the two groups, but a greater prevalence of complications such as acute kidney injury in the combination group.

While colistin based combination therapies have been shown to be effective against drug resistant gram-negative bacteria in vitro, these results have not always translated to improved clinical outcomes in randomised controlled trials (RCT) [30,48]. This disconnect may be multifactorial. The presentation of AMR infections is invariably different in patients compared to in-laboratory test tubes. Factors such as volume of distribution, pharmacodynamics and pharmacokinetics play a major role in the efficacy of antibiotics in the human body [60]. The measurement of clinical outcomes in RCT are often variable. For instance, while haemodynamic instability is a well-known sign of sepsis, this marker is variable between different patients, with variable links of mortality [61]. The body’s response to sepsis secondary to AMR infections and its relation to mortality may therefore be variable between different patients. RCTs powered to detect univariate differences in clinical outcomes may be underpowered in multivariate analysis, taking into these factors (and more) into account. Demonstrating the true efficacy of novel combination therapies in clinical RCTs is an important challenge to overcome. Pharmacokinetic and pharmacodynamic studies on smaller patient cohorts may provide a more feasible alternative, which needs further investigation.

Antibiotics such as penicillins and gentamicin form the cornerstone of treatment for bacterial infective endocarditis [19]. In uncomplicated infections, 6 weeks’ therapy is indicated for left sided valves and a minimum of 4 weeks is required for right sided disease [19]. In stable patients with left sided endocarditis who have received at least 2 weeks of intravenous antibiotics, recent data indicated similar clinical outcomes between those switched to oral, versus those continued on intravenous regimens, for up to a total of 6 weeks [62]. With infected pacemakers and implantable cardiac defibrillators, after device removal, a course of combination antibiotics is often administered [19].

Increased antibiotic use in agriculture and in humans leads to an increase in resistance in the whole environment [63,64]. For example, a recent study found that over 50% of bacteria identified in the Diptera insects in UK hospitals are antibiotic resistant [65]. At the same time, bacteria living in the guts of insects could also provide a novel source of antibiotics [66], and potentially important determinants of antibiotic resistance [67]. The existence of antibiotic resistance in the ecosystem is important, although concerning, could provide novel methods to detect and manage antibiotic resistance. Treatment of volunteers with common antibiotic monotherapy leads to antibiotic resistant bacteria in the stool within one week after commencement of therapy [68]. The resistant bacteria are detectable in the faeces for up to one year. The introduction of faeces into inefficient sewage systems or without toilet facilities may also have an impact on the ecosystem and the global transmission of AMR.

**4.2 Resistance reduction**

“*Excellence consists in breaking the enemy’s resistance without fighting.*” Sun Tzu, 545-496 BC.

Not only is combination therapy able to treat resistant bacterial infections, it can also reduce the probability of resistance emergence [69]. The evolution of antibiotic resistance to two different drugs can be independent [69]. In such cases, the probability of spontaneous resistance evolving is the product of the two probabilities for the individual drugs [69].

Michael Fischbach: “*Imagine that the probabilities of spontaneous resistance to drugs A and B are 1 × 10−6 and 1 × 10−7, respectively. If spontaneous resistance to drugs A and B are independent events, then the probability of spontaneous resistance to the A+B combination will be the product of the two rates, or 1 × 10−13*.” [69]

By combining antibiotics, the probability of spontaneous development of resistance is dramatically decreased [70,71]. If this phenomenon serves, the greater number of antibiotics used, the lesser the chance of resistance emergence (Figure 6). However, there are some cases where evolution of resistance to two different drugs are not independent. These are cases where cross-resistance occurs—that is, a single mutation confers resistance to multiple antibiotics. This can occur in cases such as mutations within the efflux pump, which can pump multiple antibiotics out of a bacterial cell.

Suzuki and colleagues conducted laboratory evolution of *E. coli* in the presence of three pairwise combinations of the antibiotics: amikacin, chloramphenicol, and enoxacin [72]. These were compared to samples evolved in the presence of single antibiotics, as control experiments. They found that resistance acquisition was significantly suppressed by the combination of amikacin and chloramphenicol [72]. These exhibit collateral sensitivity to each other, which is the process where the mutation causing resistance to one drug simultaneously enhances sensitivity to another unrelated drug. Development of combinations which capitalise on this phenomenon would be a valuable avenue of research.

Zheng and colleagues exposed Vancomycin Sensitive *Staphylococcus aureus* (VSSA) to vancomycin in pairwise combination with cefazolin, fosfomycin, gentamicin, meropenem, rifampin, piperacillin-tazobactam or trimethoprim-sulfamethoxazole to identify combinations that prevented the development of vancomycin intermediate *S. aureus* (VISA) [73]. They found that vancomycin plus the beta lactams: piperacillin-tazobactam, cefazolin, and meropenem were effective at preventing VISA emergence whilst fosfomycin, gentamicin, rifampin, and trimethoprim-sulfamethoxazole were not [73]. There was significant variation between repeats of the same strains, especially for fosfomycin and gentamicin combinations [73]. This study is promising but larger studies with more variables and repeats are necessary to fully explore the options for future combinations [73].

Zapata and colleagues exposed *Streptococcus mitis oralis* samples to combinations of daptomycin plus one of: gentamicin, rifampin, trimethoprim–sulfamethoxazole, imipenem, ceftaroline, tedizolid, or linezolid [74]. They found that ceftaroline, gentamicin, and trimethoprim–sulfamethoxazole reduced the MIC of daptomycin from 256 µg/ml to a range of 16–32 µg/ml [74]. These combinations need further testing on a larger clinical scale.

In some instances, combination therapy has been linked to the evolution of broad spectrum antibiotic resistance [75,76]. Vestergaard and colleagues investigated the effects of combinations of ceftazidime, ciprofloxacin and tobramycin on *Pseudomondas aeruginosa* *in vitro* on both the frequency and spectrum of resistance [75]. They found that some combinations (ceftazidime + ciprofloxacin) led to selection of mutants with broad spectrum antibiotic resistance, whilst other combinations (ceftazidime + tobramycin) prevented the evolution of resistant mutants [75]. In hollow fibre experiments, there are many reports of the prevention of resistance emergence by the use of combinations (Figure 7) [77].

Adjuvant molecules have long been used in combination with antibiotics to boost efficacy and prevent the emergence of AMR [78]. Adjuvant molecules such as β-lactamase inhibitors [79], permeabilsers [80,81] and efflux-pump inhibitors [82], have well known efficacy. The caveat of adjuvant combination is that if the adjuvant molecule has limited antibacterial action in its own right, it may not be able to prevent the emergence of resistance to its partner antimicrobial(s) [78]. The effect of synergy on the adjuvant molecule and its partner antibiotics deserves further investigation. Overall, new adjuvants will add to our anti-microbial armamentarium in the future.

**4.3 Resistance Reduction in the clinical setting**

**4.3.1 Tuberculosis**

Clinical reduction in resistance emergence using combination therapy is best exemplified by TB therapy [14]. In 1948, TB patients were treated with streptomycin alone [14,34]. Although most initially improved, many died later from streptomycin resistant TB. The British Medical Research Council introduced then the first documented use of antibiotic combination therapy to treat TB, namely streptomycin and para-amino salicylic acid [14]. This combination not only cured the patients but also did not induce resistance [14]. After decades of RCTs, a four-drug regimen was developed [14]. Today, the WHO recommends combination therapy for 6-9 months [10]. These drugs target different metabolic pathways. Isoniazid inhibits fatty acid synthase and produces damaging free radicals [83]; rifampicin inhibits RNA polymerase [84]; ethambutol inhibits arabinosyl transferase (involved in cell wall synthesis) [85]; and pyrazinamide targets persistent TB bacilli [86].

However, even with the extensive use of combination therapy, TB resistance has developed [87]. This is likely due to poor-adherence to the long treatment regimens, which are costly and difficult to implement [14,34,46,87]. To combat this, new drugs and drug combinations that reduce the duration of the 6-9month chemotherapy are needed for effective disease control [14,46,88].

**4.3.2 Helicobacter pylori**

Combination therapy is indicated for the treatment of *H. pylori* infections, a common infection that causes 95% of duodenal ulcers and predisposes to gastric cancer [15-17]. Standard treatment for *H. pylori* infection is the use of triple therapy including a proton pump inhibitor (PPI), clarithromycin and either amoxicillin or metronidazole [15-17]. This treatment is effective in eradicating the bacteria in over 80% of cases [15-17]. However, resistant strains have developed [15-17,89]. To combat this, further combinations including bismuth-based quadruple therapy are used [90]. This consists of a PPI and a trio of antimicrobials including tetracycline, bismuth salts, and metronidazole [15-17].

**4.3.3 Human Immunodeficiency Virus (HIV)**

The retrovirus HIV killed over one million people in 2016 [91]. It has an extremely high mutation rate, leading to rapid development of drug resistance [20,21]. Antiretroviral combination therapy is the mainstay treatment to maintain viral suppression, retard disease progression and prevent emergence of resistance [20,21]. The first line treatment for HIV is a combination of two nucleoside reverse transcriptase inhibitors along with either: a non-nucleoside reverse transcriptase inhibitor; a boosted protease inhibitor; or an integrase inhibitor [20,21]. Although the antiretroviral drugs and combinations have well known side effects, their availability have contributed to HIV becoming a “chronic illness”, rather than a terminal illness [92].

**4.3.4 Cancer**

Combination therapy is the mainstay treatment for cancer [22,23], targeting multiple metabolic pathways simultaneously and preventing the use of salvage pathways by cancer cells [22,23,52]. The cytotoxic chemotherapeutic agents have severe and sometimes fatal side effects, including cardiotoxicity and organ failure [23]. The use of synergistic drugs in combination allows a lower dose to be used whilst maintaining efficacy and reducing negative side effects [22,23,52]. The high level of heterogeneity within a tumour population and the presence of cancer stem cells mean that a single drug is often unable to target all cells within a tumour [22]. Cancer stem cells that survived chemotherapy can rapidly repopulate a tumour [93] and are linked to the development of drug resistance [22,23]. Combinations offer a broader spectrum of action against both the tumour core and the stem cells, allowing improved prognosis [22]. In a recent study, metastatic non-small cell lung cancer patients treated with platinum-based chemotherapy in combination with pembrolizumab had better prognosis than those treated with platinum-based chemotherapy alone [94].

**5. Toxicity and Antagonism**

Adverse effects in combinations are caused by both pharmacokinetic and pharmacodynamic interactions [95]. Pharmacodynamic interactions occur where drugs directly influence each other’s effects [95]. These interactions can be either synergistic or antagonistic. Pharmacodynamic effects can also occur outside the target bacteria in the body, leading to unwanted effects [95,96]. Pharmacokinetic interactions affect the absorption, distribution, metabolism, and elimination of drugs which leads to alterations in effective concentrations in blood and tissue. This is important because exposure to sub-inhibitory concentrations of antibiotics accelerates the development of resistance [96].

Recent data have linked the vancomycin and piperacillin–tazobactam combination (common empirical hospital treatments) to acute kidney injury (AKI) [97]. The combination had a greater probability of developing AKI compared with either vancomycin or piperacillin–tazobactam monotherapy (27.66% vs 6.98% or 7.92%, respectively) [97]. This highlights the importance of detecting and assessing for toxicity in combination therapy.

Using multiple drugs at once can also have either synergistic or antagonistic effects which, depending on requirements, can both be useful [27,34,38,71]. Antagonism occurs when drug interactions produce an overall effect less than the sum of the individual drugs, leading to an attenuated effect [71]. Synergistic antibiotic combinations lead to more effective killing of bacteria and can rejuvenate antibiotics to which resistance has developed, but select more for resistance [27,34,38,71]. Synergistic combinations can also lead to a reduction in the dose of a toxic antibiotic which means that patients will have less side-effects from treatment. Antagonistic combinations produce less selection pressure but are less bactericidal [98,99]. The adverse effects of combination therapy should be tested in both pre-clinical and clinical trials.

**6. Conclusion**

The AMR pandemic cannot be tackled by monotherapy antibiotic development. Antibiotic combination therapy, exploiting synergies, old-drug rejuvenation, resistance reduction, and greater permutational diversity could provide the solution. Combinations are already used for selected infectious and non-infectious diseases, recommendation for combination treatment should be supported by clinical studies, especially where there is good pre-clinical data, phase one pharmacokinetic/dynamic RCTs would be important. The small number of pharmaceutical companies in this field is likely to expand in the future, bringing promising combinations from the bench to the bedside, to save millions of lives worldwide.

**7. Expert opinion**

The development of new antibiotics for common bacterial infections is at a crossroads. On the one hand, the traditional method of making new chemical entities is stalling in the market because of the high costs relative to the low sales price which the market will tolerate. On the other hand, there is good evidence that combinations of antibiotics have much lower development costs and could be sold at a lower price which may be more acceptable in the present market conditions. Because of powerful synergy between members of the combination it may be possible to rejuvenate old antibiotics. This means that it may be possible to reuse many of our old antibiotics, and by making large combinations recycle our old antibiotics in large numbers of different combinations.

We could have a rebirth of the antibiotic era – the dawn of the “Antibiotic Combination Golden Age”.

In comparison to monotherapy, combinations may contain lower doses of certain antibiotics thus reducing side effects. The fundamental problem with monotherapy is that it induces resistance. Experimental data suggests that resistance evolution in common bacterial infections might be reduced if combinations were used instead of monotherapy. Further advantages of combination therapy are that it may reduce the duration of therapy and broaden the spectrum of activity.

Combination therapy is powerful. It is of no surprise that it is the established standard of care not only for many important infectious diseases, including HIV, TB and endocarditis, but also for cancer. The potential to enlist the power of synergy to develop novel and cheaper combinations is extremely attractive. In fact, for common bacterial infections, doctors often already use unapproved combinations to treat seriously ill patients, for example in the intensive care units. There is now a strong case for the regulators to help doctors with their choice of combinations by reducing the burden and hence the cost of development of old already marketed antimicrobials, simply by radically shortening the duration of development. By doing this, many new affordable combinations could continuously be brought into the market for many years into the future.

The major issues in the field is the global preference of grant bodies, industry and governments to develop new single-drug antimicrobials. Since every such antimicrobial ever developed has been (and will continue to be) rendered ineffective by antimicrobial resistance (AMR), this strategy is futile (bacteria mutate faster than drug development) for the control of AMR. This strategy is also financially detrimental, as reflected by recent bankruptcies of such companies trying to develop new single-agents and many big pharmaceutical companies leaving the field in search of more profitable alternatives. As a result, although governments worldwide publicise the importance of controlling AMR as a pandemic health problem, the reality is that when novel combination therapies come out of the laboratories, there is limited funding available from grant bodies and industry to scientists to carry the combinations towards the bedside. This can only be solved by the governments and funding bodies joining together to give combination therapy an equal emphasis. There needs to be a “paradigm shift” in our thinking that combination therapy is the way forward as treatment that is cheaper, easier to develop and with potentially high penetrance to patients worldwide.

The challenge facing novel combination therapies is generating sufficient clinical evidence for treating highly resistant infections and prevent the emergence of resistance, since regulators often insist on clinical trials powered for clinical outcomes including mortality. The huge expense of replacing the entire antibiotic arsenal very 30-40 years makes this approach impossible. If preclinical data and much more limited human pharmacokinetic and pharmacodynamic studies were to be the measures sufficient for approval, this would be feasible.

As far as fighting AMR is concerned, it is “in with combinations, out with single agents.” Antimicrobial combinations should become accepted mainstream strategy for combating AMR.

In summary, the AMR pandemic cannot be tackled by monotherapy antibiotic development. Antibiotic combination therapy, exploiting synergies, old-drug rejuvenation and resistance reduction could provide the solution to AMR. The ultimate goal is to start a program of harnessing the power of potentially thousands of synergistic antimicrobial combinations which could enter the market with a short low-cost development duration, at an affordable price. The number of pharmaceutical companies in this area needs to expand, bringing promising combinations to the bedside, to save millions of lives worldwide.

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**Declaration of interest**

A Coates is the CSO of Helperby Therapeutics Ltd, and a director and shareholder. Y Hu is the Scientific Director of Helperby Therapeutics Ltd. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

**Reviewer disclosures**

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**Figure Legends**

**Figure 1.** Combination therapy approach versus conventional monotherapy approach.

**Figure 2.** The larger the combination of antibiotics, the greater the net synergy.

**Figure 3.** An illustration of synergy: eradication of highly resistant bacteria by antibiotics which lack individual activity.

**Figure 4.** An illustration of synergy:combination therapy eradicates colistin resistant MCR-1, carbapenem resistant NDM-5 *E coli*.

**Figure 5.** International consensus guidelines for optimal use of the polymyxins.

**Figure 6.** A greater number of antibiotics in combination leads to reduced resistance emergence.

**Figure 7.** Resistance prevention of highly resistance bacteria by combinations.CFU: colony forming units.