

***TITLE Conventional antibiotics – revitalized by new agents.******In: Novel Antimicrobial Agents and Strategies.*****Authors: Anthony Coates and Yanmin Hu****SUMMARY**

Beta-lactamase inhibitors are clinically proven to revitalise old beta-lactam antibiotics by neutralising bacterial beta-lactamases. We call these compounds antibiotic resistance breakers. Unfortunately, bacteria express more than 1000 beta-lactamases, of which the metallo-beta-lactamases are proving difficult to neutralise. Here we describe other antibiotic resistant breakers, which are not yet in the clinic, but which potentially revitalise other classes of antibiotics. These include aminoglycoside modifying enzyme inhibitors, efflux pump inhibitors and compounds which are associated with increased permeability of the bacterial cell membrane. If it were possible to develop new antibiotic resistant breakers for many different classes of antibiotics, this approach could be a viable alternative to the more expensive single novel compound route which has been pursued for pyogenic bacterial infections.

**INTRODUCTION**

Antibiotic resistance develops to all antibiotics [1, 2, 3]. Over several decades, this leads to the need to replace old antibiotics with new ones. Unfortunately, the world has not produced antibiotics fast enough to cope with the emergence of antibiotic resistance, particularly for Gram-negative bacteria [4]. Between the 1940s and 1970s, the “Golden era”, about 20 new classes of antibiotics were produced, which led to more than 200 analogues. Since then, there have only been three new classes marketed, none of which are for Gram-negatives [5]. Can we recreate the Golden era? In other words, can we make 20 new classes of antibiotics which are active against highly resistant bacteria? There is much debate about this. Whilst new antibiotics against Gram-positive bacteria have been marketed in recent years, the main problem is that resistant Gram-negative bacteria are poorly served, with no new class being marketed for 40 years [5]. Furthermore, new antibiotics which are effective against the carbapenem resistant bacteria [6], which express, for example NDM-1 [7] are not being introduced into the market in good time, and we are playing ‘Catch-up’.

Is there a way forward? On the one hand, if enough money was provided by governments, perhaps in a similar way to the Marshall plan [8,9], or the Public Health Emergency Medical Countermeasures Enterprise [10] which is a public-private partnership of multiple agencies of the US Federal Government, many more antibiotics might reach the market.

This would need to be accompanied by global efforts by non-profit organisations such as the Bill & Melinda Gates Foundation, the Drugs for Neglected Diseases Initiative (a research and development organization which develops new treatments for neglected diseases) and Medicines for Malaria Venture, which is a public-private partnership with aim of providing

affordable antimalarial drug discovery and development. In addition, there would need to be changes in regulation, and encouragements for industry, for example the Generating Antibiotics Incentives Now (GAIN) Act in USA, and the proposed Antibiotic Development to Advance Patient Treatment Act in USA (“ADAPT Act”) for a Limited Population Antimicrobial Drug Pathway [8, 11].

On the other hand, in the long term, it may not be possible to market enough antibiotics to keep up with the relentless emergence of antibiotic resistance [12,13]. Whilst prevention will clearly play a greater role, this will not substitute for new antibiotics. The existing strategy is to discover and develop novel single antibiotic therapy[14]. Do we need to rethink this strategy? Considering costs alone, if we intend to discover and develop 200 new antibiotics, the cost will be somewhere in excess of \$1 billion per compound [15]. So this route would be very expensive. Is it scientifically feasible to endlessly produce more and more antibiotics? The past 40 years have shown that it is becoming more difficult to bring new antibiotics to the market. The absence of new classes of antibiotics for Gram-negative infections during this period is an important example[5]. Now we have virtually untreatable carbapenem resistant Gram-negative infections, exemplified by those bacteria which express metallo-beta-lactamase (MBL) [7], for which we are using, as a last resort, colistin [16], itself an old, relatively toxic antibiotic. Colistin resistance is now emerging in MBL Enterobacteriaceae [17]. Furthermore, the development of novel antibiotics against MBL resistant bacteria is still in early clinical development [14,18]. In addition, we know that antibiotic resistance arises to all antibiotics within a few years after entry into the marketplace [19]. Therefore a continuous flow new antibiotics into the market is needed. It seems unlikely that the world will be able to produce a limitless number of antibiotics far into the future. If the supply of effective antibiotics dries up, modern medicine is likely to suffer a devastating set back [13, 20].

We propose a new strategy. The world should revitalise conventional antibiotics by combining them with antibiotic resistance breakers (ARBs). This approach would mean that we could, potentially, continue to use conventional antibiotics. This has the advantage of being a cheaper option than developing hundreds of new antibiotics. For example, if each class of antibiotics could be resuscitated by a single antibiotic resistance breaker, theoretically, most of the 200 existing antibiotics could become useful again. Potentially, this could be achieved with many fewer new compounds than would be required for the replacement of the existing 200 compounds. There would be substantial financial savings and this would transform the feasibility of prolonging the Antibiotic Era. This chapter looks at the origins of combination antibiotic therapy and examines whether it is possible to extend this concept, namely the combination of conventional antibiotics (see Table 1) with resistance breakers, thereby revitalising a wide range of different classes of antibiotics.

## CONVENTIONAL ANTIBIOTICS

The main classes of antibiotics which have been marketed, and many of their analogues, are listed in Table 1. Resistance has occurred to all of them. The  $\beta$ -Lactams are degraded by bacterial  $\beta$ -Lactamases which can be neutralised by combining the old antibiotic with a  $\beta$ -Lactamase inhibitor such as clavulanic acid [21]. This will be discussed in more detail in the next section, as will other combinations. Potential combinations only exist for a minority of classes.

Table 1 | **Main classes of antibiotics**

**Class Examples**

***Aminoglycosides*** Streptomycin, neomycin, kanamycin, paromycin, gentamicin, tobramycin, amikacin, netilmicin, spectinomycin, sisomicin, dibekalin, isepamicin

***β-Lactams***

**Penicillins** Penicillin G, penicillin V, methicillin, oxacillin, cloxacillin, dicloxacillin, nafcillin, ampicillin, amoxicillin, carbenicillin, ticarcillin, mezlocillin, piperacillin, azlocillin, temocillin

**Cephalosporins**

*First generation* Cephalothin, cephapirin, cephradine, cephaloridine, cefazolin

*Second generation* Cefamandole, cefuroxime, cephalexin, cefprozil, cefaclor, loracarbef, cefoxitin, cefmetazole

*Third generation* Cefotaxime, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime, cefixime, cefpodoxime, ceftibuten, cefdinir

*Fourth generation* Cefpirome, cefepime

**Carbapenems** Imipenem, meropenem

**Monobactams** Astreonam

***β-Lactamase Inhibitors*** Clavulanate, sulbactam, tazobactam

***Glycopeptides*** Vancomycin, teicoplanin

***Macrolides*** Erythromycin, azithromycin, clarithromycin

***Metronidazole***

***Lincosamides*** Lincomycin, clindamycin

***Lipopeptides*** Daptomycin

***Oxazolidinones*** Linezolid

***Polymyxin*** Polymyxin B, Polymyxin E (colistin)

***Quinolines*** Bedaquiline

***Quinolones*** Nalidixic acid, oxolinic acid, norfloxacin, pefloxacin, enoxacin, ofloxacin/levofloxacin, ciprofloxacin, temafloxacin, lomefloxacin, fleroxacin, grepafloxacin, sparfloxacin, trovafloxacin, clinafloxacin, gatifloxacin, moxifloxacin, sitafloxacin

***Rifamycins*** Rifampicin (also called rifampin), rifapentine, rifabutin, bezoxazinorifamycin, rifaximin

***Streptogramins*** Quinupristin, daflopristin

**Sulphonamides** Sulphanilamide, *para*-aminobenzoic acid, sulfadiazine, sulfisoxazole, sulfamethoxazole, sulfathalidine

**Tetracyclines** Tetracycline, chlortetracycline, demeclocycline, minocycline, oxytetracycline, methacycline, doxycycline, tigecycline

**Trimethoprim**

## THE PRINCIPLES OF COMBINATION ANTIBIOTIC THERAPY

In the clinic, combinations of antibiotics are often used. The main reasons for such combinations are:

1. Combinations which break resistance and rejuvenate old antibiotics. The best example of this approach is the combination of clavulanic acid and amoxicillin [21]. Clavulanic acid inhibits bacterial beta-lactamase which neutralises amoxicillin, thus allowing the latter to kill beta-lactamase producing bacteria. Clavulanic acid alone has no anti-bacterial activity. This chapter primarily deals with breaking resistance.
2. To prevent the emergence of resistance during chemotherapy. It is important to appreciate the limitations of this approach. Whilst combinations of antibiotics do prevent the emergence of resistance during tuberculosis chemotherapy [22], it is unlikely that this will be effective in multi-species environments such as the large intestine. In the case of *Mycobacterium tuberculosis*, combinations are effective because mutations only arise in the chromosome, and do not occur due to plasmid transfer from other species of bacteria[23]. *M. tuberculosis* lives on its own in a relatively sterile environment, for example inside macrophages in the lung. So there is little opportunity for plasmid transfer. Resistance due to transfer of plasmids does not occur in *M. tuberculosis*. In contrast, other bacteria, such as *Escherichia coli*, live in the large intestine in a multi-species environment where resistance is often transferred via plasmids [24]. Combinations such as sulphonamide and

trimethoprim(co-trimoxazole) already have high levels of resistance- for example, over 95% of Gram-negative bacteria from babies in rural India [25] in spite of early hopes that such a combination would prevent the emergence of resistance [26]. A meta-analysis (including data from eight randomised controlled trials) that compared aminoglycoside/beta-lactam combination therapy with beta-lactam mono-therapy to observe the emergence of antimicrobial resistance found that aminoglycoside/beta-lactam combination therapy was not associated with a reduced development of resistance when compared with beta-lactam therapy alone [27]. Nevertheless, for certain infections, where chromosomal resistance is thought to be important, combinations of different antibiotics may have the potential to prevent the emergence of resistance.

3. Combinations in which one antibiotic boosts the effect of a second antibiotic and visa-versa. This is called synergy. For instance, penicillin and gentamicin are synergistic [28], and are used to treat bacterial endocarditis.
4. A combination of antibiotics is used by clinicians to broaden the number of species of bacteria which are targeted. For example, if a seriously ill patient has suspected intra-abdominal infection with an unknown bacterium, an aminoglycoside and anti-anaerobe agents can be used [29].
5. Sometimes, the clinician may be faced with an infection which harbours dormant bacteria as well as fast multiplying ones. Tuberculosis is well known as an infection which persists due to the presence of dormant bacteria which are relatively tolerant to antibiotics. Combinations of antibiotics, typically containing four separate compounds (Rifampicin, pyrazinamide, isoniazid and ethambutol), are used in the initial stages of tuberculosis therapy. Rifampicin and pyrazinamide kill dormant

bacteria and so are responsible for the shortening of the duration of chemotherapy from 12 to 6 months [22].

## ANTIBIOTIC RESISTANCE BREAKERS

### REVITALISE CONVENTIONAL ANTIBIOTICS

The main threat to the effectiveness of a marketed antibiotic is the emergence of widespread resistance amongst its bacterial targets. Whilst prevention of resistance is clearly the ultimate answer to this problem, the world is a long way from reversing this trend. Since resistance to an antibiotic is an inevitable consequence of entry into the market, the main subject of this chapter is to examine the feasibility of revitalising conventional antibiotics by the addition of an antibiotic resistance breaker. The combination is active against resistant bacteria. In the large pyogenic bacterial field, combination therapy has not been developed to the extent that it has in tuberculosis, although, HIV and cancer therapy do use well characterised combinations of drugs.

There are a number of ways that conventional antibiotics can be revitalised by combining them with another agent.

#### 1. **Beta-Lactamase Inhibitors.**

Bacteria can produce beta-lactamases, which are enzymes that destroy the beta-lactam ring of beta-lactam antibiotics, thereby reducing their effectiveness [30]. There are over 1300 known beta-lactamases. The concept of combining a beta-lactam antibiotic with a beta-lactamase inhibitor in order to revitalise the antibiotic and to render it active against beta-lactamase expressing bacteria, was first introduced into the market by the combination of the beta-lactamase inhibitor clavulanic acid, derived from *Streptomyces calvuligerus*, with amoxicillin [31]. This combination is called Augmentin (GlaxoSmithKline, Brentford, UK). In a clinical trial [32] patients with non-bullous impetigo were treated with either amoxicillin alone or Augmentin. The causative organism of impetigo, *Staphylococcus aureus* was shown to be

present in lesions from all the patients. When tested for sensitivity to amoxicillin, all the bacterial isolates were resistant, but were sensitive to Augmentin. Clinically, the Augmentin group of patients responded better than the amoxicillin group. These data indicated that neutralisation of bacterial beta-lactamase can revitalise amoxicillin.

Unfortunately, bacteria produce many beta-lactamases which are not inhibited by clavulanic acid. There has been a 100 fold increase in the number of known beta-lactamase inhibitors in the past 40 years [30]. The classification of bacterial beta-lactamases is complicated. We have used the Bush (2013) system in this paper, bearing in mind that Extended Spectrum Beta-Lactamases (ESBLs which include TEM and SHV) and carbapenemases (such as NDM and KPC) in Gram-negatives are thought to be of the greatest clinical importance because they are difficult to treat and are relatively common in many countries [33,34]. Beta-lactamases can be divided into Ser- and Metallo-beta-lactamases, by their active sites. They are sub-divided into Molecular Classes A-D, which have Functional groups and Major functional subgroups. For example, the Serine beta-lactamases Molecular class C 1(1,1e) which degrade early cephalosporins and expanded spectrum cephalosporins in the case of 1e, Class A 2 (2a,2b,2be,2br,2f) which degrade penicillins and others, and in the case of 2f, penicillins, early and expanded spectrum cephalosporins, carbapenems and monobactams, and Class D 2d(2de, 2df) which destroy penicillins and in the case of 2df, carbapenems. The Metallo-beta-lactamases B 3 (3a and 3b) target carbapenems, and in the case of 3a, penicillins and early and expanded spectrum cephalosporins. Enzymes which are expressed are C 1(AmpC, CMY) and 1e(GC1), A 2a(PC1), 2b(TEM-1, SHV-1), 2be(CTX-M, ESBLs(TEM, SHV)), 2br(IRT, SHV-10), 2f(KPC,SME), 2de(OXA-11, OXA-15), 2df(OXA-23, OXA-48), and B 3a(IMP, VIM, NDM), 3b (CphA).

Clavulanic acid only neutralises the Serine beta-lactamases A(2a, 2b and 2be) and has a partial effect on A(2f), and D(2d). Clavulanic acid has also been combined with ticarcillin (Timentin;

GlaxoSmithKline, Brentford, UK). Other inhibitor combinations include tazobactam with piperacillin (Zosyn; Pfizer, Philadelphia, PA, USA), and sulbactam with ampicillin (Unasyn; Pfizer, Philadelphia, PA, USA). Unfortunately, the current beta-lactamase inhibitor combinations are not active against bacteria which express AmpC or ESBLs. Even worse [35], is that, so far, it is proving difficult to develop Metallo-beta-lactamase inhibitors which are effective against NDM.

Since the current marketed inhibitors are only active against class A enzymes but lack effectiveness against class A KPC carbapenemases, new inhibitors are under development which broaden the beta-lactamases which can be neutralised. For example, avibactam which is a bridged 1,6-diazabicyclo[3.2.1]octan-7-one (DBO) is in clinical development. This compound is active against a wide range of Class A and C serine b-lactamases [36], including ESBLs and class A carbapenemases. Although it neutralises Class D OXA-48, it is inactive against other D carbapenemases. This molecule also inhibits selected class D b-lactamases including OXA-48, but not other class D carbapenemases or B metallo-beta-lactamases. Avibactam combinations with ceftaroline (Cereza-Forest) and cefdzidime (AstraZeneca and Forest) are in clinical trials [35].

Another combination under development (Cubist) is tazobactam and ceftolozane [37]. Tazobactam increases the activity of the combination against ESBL-producing Enterobacteriaceae, and can partially neutralise AmpC and KPC carbapenemases.

A new DBO (MK-7655 Merck) has been combined with imipenem, and is in clinical trials [38]. This combination is active against KPC-2-producing *K. pneumoniae* and AmpC-overexpressing isolates of *P. aeruginosa* but not those which express metallo-carbapenemases [39].



2. **Aminoglycoside-modifying enzyme inhibitors.** Whilst these type of inhibitors have not yet reached the clinical trials phase of development, some interesting in vitro experience has been achieved. In general, inhibitors of aminoglycoside-modifying enzymes [40,41] have struggled with numerous different targets because bacteria may express multiple enzymes. However, inhibition of aminoglycoside phosphotransferases and acetyl transferases has been shown by cationic antimicrobial peptides [40]. Indolicidin is a bovine antimicrobial peptide. This peptide and its synthetic analogues inhibited both aminoglycoside phosphotransferase and aminoglycoside acetyltransferase classes. This is the first description of broad-spectrum inhibitors of aminoglycoside resistance enzymes. Crystallography studies have shed light on the molecular structure of aminoglycosidephosphotransferases or kinases (APHs). A review of APH structures and inhibitors is covered by Shi and colleagues [42]. These data suggest that the commercial development of a universal APH inhibitor may not be feasible.

3. **Antibiotic efflux pump inhibitors** Although there are numerous examples of antibiotic efflux pump inhibitors, none are in clinical trials as yet.

The main families of bacterial efflux pumps which are chromosomally expressed and which are associated with multi-drug resistance [43], are the resistance nodulation division (RND) family (encodes AcrA/B-TolC), the major facilitator superfamily (MFS) (encodes QacA), and the staphylococcal multiresistance (SMR) (encodes QacC),

the multidrug and toxic compound extrusion (MATE) family(NorM) and the ABC (ATP binding cassette)(LmrA). Efflux pump inhibitors include reserpine [44] which is too neurotoxic to be used at effective concentrations in humans [45], berberine and palmatine [46], and other compounds (reviewed in [43]) including plant extracts, synthetic molecules, thioxanthenes, phenothiazines, and arylpiperazines. Whilst some inhibitors perform well in vitro, problems with toxicity have not resulted in extensive clinical trials. In addition, particularly in some Gram-negative bacteria, treatment with an inhibitor may lead to compensatory upregulation of other efflux pumps. For example [47], RamA expression is induced by inhibition of efflux or inactivation of *acrAB* in *Salmonella typhimurium*.

#### **4. Synergy associated with bacterial membrane permeators**

Synergy between non-antibiotics and antibiotics, and between antibiotics themselves is well-known. In some cases this synergy is associated with one of the pair in the combination being a bacterial membrane permeabiliser. Whether this is responsible for the synergy is unknown in many cases, but it has been suggested [48] that permeabilisation of the membrane may increase the intracellular concentration of the antibiotic in the combination, and this, in turn may increase the anti-bacterial potency of the antibiotic. Some of these associations are described here.

Gram-negative bacteria have two membranes. In the case of fluoroquinolones, outer membrane proteins play a key part in helping these molecules to cross the membrane [49,50]. In contrast, passive diffusion is thought to be important for translocation of the inner membrane of Gram-negatives and the single membrane of Gram-positives [51,52,53,54]. In the 1960s [55,56] improved penetration of fluoroquinolones was achieved by the addition of a 7-piperazine side-chain and this is thought initiate

translocation across the membrane [57]. This suggests that adding side-groups such as piperazine or membrane permeabilisation compounds in combinations could be a way of increasing the activity of current antibiotics.

One of the most serious problems in clinical practice in the world, is the emergence of carbapenem resistant Gram-negative bacteria. Carbapenems are often used as the antibiotics of last resort. Combinations of antibiotics are used to treat patients with carbapenem resistant metallo-beta-lactamase producing Gram-negative infections such as *Klebsiella pneumoniae* [58], and these combination often contain colistin. This antibiotic, which is a polypeptide of the polymyxin group, increases the permeability of Gram-negative membranes [59]. The polycationic regions of colistin displace the bacterial counter ions in the lipopolysaccharide of the outer membrane. The inner membrane is solubilised by the hydrophobic/hydrophilic regions of colistin. Whilst clinical data regarding the efficacy of different antibiotic combinations is sparse, in vitro data [58] suggests that a combination of colistin, rifampicin and meropenem is effective against metallo-beta-lactamase producing *K. pneumoniae*(VIM; NDM-1).

Antimicrobial peptides can also increase the permeability of bacterial membranes, and can synergise with conventional antibiotics. For example [60] antimicrobial peptides have been created which synergise with conventional antibiotics such as cefotaxime, ciprofloxacin or erythromycin against highly resistant strains of the Gram-negative bacterium *Acinetobacter baumannii*. There are three models of AMP membrane interaction(Reviewed in [61]): Barrel-stave pores, toroidal pores and carpet mechanism in which peptides form a layer on the surface and dissolve the membrane [62]. AMPs have numerous other effects on bacterial cells, and so synergy may not necessarily be the most important as far as a bactericidal effect is concerned.

A recent development has been the observation of enhancement or synergy between a compound which was developed against dormant *Staphylococcus aureus* [63] and three different classes of antimicrobials [64]. The compound (HT61; Helperby Therapeutics Ltd, London) depolarises the bacterial cell membrane and is in clinical trials.

Another example is Loperamide (Immodium; McNeil Consumer Healthcare, Fort Washington, PA, USA) [65] is an opioid receptor agonist which enhances the activity of minocycline against *Escherichia coli*, *S aureus* and *Pseudomonas aeruginosa*. Loperamide interferes with the electrical component of the proton motive force of the bacterial membrane. This leads to an increase in the pH gradient which enhances the entry of tetracycline into the cell.

## DISCUSSION

Revitalising old antibiotics by combination with a second compound means that resistance to the old antibiotic is broken by the second compound, either directly or indirectly.

There is only one clear, clinically proven example of rejuvenation of old antibiotics in this way, namely the addition of beta-lactamase inhibitors to beta-lactams. Arguably, the addition of the 7-piperazine ring to quinolones in order to enhance the initiation of translocation, could be regarded as another example. Antibiotic-antibiotic combinations which are frequently used in clinical practice, for example in tuberculosis chemotherapy, do not break resistance as such. Such antibiotic-antibiotic combinations (with the exception of those which include colistin, and perhaps other membrane permeators) have other functions such as preventing the emergence of resistance (tuberculosis chemotherapy), or synergy (increasing efficacy). If resistance exists to the primary antibiotic, a second antibiotic is added to which the organism is sensitive and this renders the combination effective. Combinations can also broaden the spectrum of species which are targeted. For example, in abdominal sepsis patients, two antibiotics such as an aminoglycoside and anti-anaerobe agents are used together to cover as many aerobic and anaerobic species of bacteria as possible before the results of microbiological tests are available. Some combinations contain drugs which kill dormant organisms (for instance pyrazinamide and rifampicin in tuberculosis chemotherapy), thus shortening the duration of therapy.

The advantages of revitalising old antibiotics, such as beta-lactams with a beta-lactamase inhibitor, is that the existing antibiotic can be used once again to effectively treat a resistant bacterial infection which was previously untreatable by that antibiotic. Further advantages of

this approach is that it is relatively low cost because one antibiotic resistance breaker can be used to rejuvenate several old antibiotics. In addition the risk which is associated with this approach is lower than developing a novel antimicrobial because once the ARB has been shown to be safe in clinical trials in combination with one compound, it can be used to rejuvenate other old antibiotics. Furthermore, instead of reproducing the Golden era of antibiotic discovery by creating 200 novel antibiotics, the world could, potentially, rejuvenate existing antibiotics with 20 or less ARBs in combination with 200 existing antibiotics.

Could ARBs prevent the emergence of resistance? Whilst combinations of drugs are used in tuberculosis, HIV and cancer chemotherapy to reduce the emergence of resistance, there are certain fundamental differences between these combinations and ARBs for the treatment of pyogenic bacterial infections such as urinary tract disease due to Gram-negative bacteria. The first difference is that *Mycobacterium tuberculosis* resistance is not transmitted by plasmids. It is chromosomally mediated. This contrasts with resistance in pyogenic bacteria which is transmitted by plasmids in some cases, is chromosomally mediated in others and both mechanisms in some. It is unlikely that ARBs could reduce the emergence of plasmid mediated resistance, but they might be able to impact upon chromosomal resistance. A second important difference is that some ARBs, such as some beta-lactamase inhibitors have no anti-bacterial activity by themselves. These ARBs are unlikely to be able to prevent even chromosomal resistance because resistance emergence is effectively appearing to the one old antibiotic alone. If, however, the ARB has some antibacterial activity in its own right, such as HT61 (Hu et al 2010), mutants which arise to the old antibiotic can be killed by the ARB and thus the combination may be able to prevent the emergence of chromosomally mediated resistance.

Could ARBs be used to reduce the dose of old antibiotics against sensitive bacterial strains, and so decrease the incidence of toxic side-effects? If the ARB can boost the effect of the old antibiotic against sensitive strains, it may be possible to use a lower dose of the old antibiotic to achieve cure.

Would ARBs enhance activity against dormant bacteria? This depends upon the ARB. Beta-lactamase inhibitors have no action against dormant bacteria and so would not increase a beta-lactam's activity against dormant bacteria. In contrast, other ARBs such as HT61 which was selected for anti-dormancy activity [63,64] boost the activity of the combinations against dormant bacteria.

Historically, resistance has eventually emerged to every antibiotic after entry into the market. Clearly, resistance will appear to ARB combinations. Experience with beta-lactamase inhibitors suggests that mutant bacteria emerge over time which express beta-lactamases, such as the B3a metallo-beta-lactamase NDM that are resistant to, for example, clavulanic acid [7]. Since bacteria produce over 1000 beta-lactamases, it seems likely that, when challenged with a new beta-lactamase inhibitor, mutants will emerge which can neutralise the inhibitor with a novel beta-lactamase. Ways need to be found which slow the emergence of resistance. One possible route could be to use ARBs which target the cell membrane, on the grounds that it may take bacteria longer to develop resistance against combinations which act on the bacterial membrane [66].

ARBs which can rescue old antibiotics from a wide range of resistance challenges are needed, and those which can counteract metallo-beta-lactamases are urgently needed.

## ACKNOWLEDGEMENTS

We would like to acknowledge financial support from the Burton Trustees and Helperby Therapeutics Ltd.

## REFERENCES

- [1] Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings AS, Jones DL, Lee NM, Otten W, Thomas CM, Williams AP(2013). The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. *Lancet Infect Dis*, 13(2), 155-65
- [2] Arias, C. A. and Murray, B. E. (2009). Antibiotic-resistant bugs in the 21st century--a clinical super-challenge. *New England Journal of Medicine*, 360, 439-43.
- [3] Livermore, D.M. (2009). Has the era of untreatable infections arrived? *Journal of Antimicrobial Chemotherapy*, 64, 29-36.
- [4] Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen Ø, Seifert H, Woodford N, Nordmann P; European Network on Carbapenemases (2012). Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect*, 18(5):413-31.
- [5] Coates AR, Halls G, Hu Y(2011). Novel classes of antibiotics or more of the same? *Br J Pharmacol*, 163(1):184-94
- [6] Nordmann P (2013). Carbapenemase-producing Enterobacteriaceae: Overview of a major public health challenge. *Med Mal Infect*, pii: S0399-077X(13)00336-3. doi: 10.1016/j.medmal.2013.11.007
- [7] Bushnell G, Mitrani-Gold F, Mundy LM(2013). Emergence of New Delhi metallo- $\beta$ -lactamase type 1-producing enterobacteriaceae and non-enterobacteriaceae: global case detection and bacterial surveillance. *Int J Infect Dis*, 17(5):e325-33.
- [8] Laxminarayan R, Duse A, Watal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H, Greko C, So AD, Bigdeli M, Tomson G, Woodhouse W, Ombaka E, Peralta AQ, Qamar FN, Mir F, Kariuki S, Bhutta ZA, Coates A, Bergstrom R, Wright GD, Brown ED, Cars O.(2013) Antibiotic resistance-the need for global solutions. *Lancet Infect Dis*, 13(12):1057-98.
- [9] The Marshall Plan 1948. <http://www.marshallfoundation.org/TheMarshallPlan.htm>. Accessed by AC 2nd March 2014.
- [10] The Public Health Emergency Medical Countermeasures Enterprise 2010. <https://www.medicalcountermeasures.gov/media/1138/mcmreviewfinalcover-508.pdf>. Accessed by AC 2nd March 2014.

- [11]Antibiotic Development to Advance Patient Treatment Act. <http://usamobiledrugtesting.com/adapt-act-would-create-a-new-limited-population-approval-pathway-for-antibiotics-and-antifungals/>. Accessed by AC 3rd March 2014.
- [12]Grossi PA, Tebini A, Dalla Gasperina D(2014). Novel multi-drug resistant microorganisms in critically ill: a potential threat. *Minerva Anesthesiol*. Mar 5. [Epub ahead of print]
- [13]Davies S (2013). *The Drugs Don't Work*. Penguin Special. March.
- [14]Coates AR, Halls G. Antibiotics in phase II and III clinical trials(2012). *Handb Exp Pharmacol*,211,:167-83.
- [15]Huttner A, Harbarth S, Carlet J, Cosgrove S, Goossens H, Holmes A, Jarlier V, Voss A, Pittet D (2013). Antimicrobial resistance: a global view from the 2013 World Healthcare-Associated Infections Forum. *Antimicrob Resist Infect Control*,2(1):31. [Epub ahead of print]
- [16]Tzouveleki LS, Markogiannakis A, Psychogiou M, Tassios PT, Daikos GL (2012). Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clin Microbiol Rev*, 25(4):682-707
- [17]Tascini C, Tagliaferri E, Giani T, Leonildi A, Flammini S, Casini B, Lewis R, Ferranti S, Rossolini GM, Menichetti F (2013). Synergistic activity of colistin plus rifampin against colistin-resistant KPC-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*, 57(8):3990-3
- [18]Chen J, Shang X, Hu F, Lao X, Gao X, Zheng H, Yao W (2013).  $\beta$ -Lactamase inhibitors: an update. *Mini Rev Med Chem*,13(13):1846-61.
- [19]House of Lords inquiry into antimicrobial resistance (1998). *Commun Dis Rep CDR Wkly*. 8(17):147, 150.
- [20] So AD, Gupta N, Cars O (2010). Tackling antibiotic resistance. *BMJ Editorial* 340: c2071.
- [21]Watkins RR, Papp-Wallace KM, Drawz SM, Bonomo RA (2013). Novel  $\beta$ -lactamase inhibitors: a therapeutic hope against the scourge of multidrug resistance. *Front Microbiol*. 4:392.
- [22]Mitchison D, Davies G (2012). The chemotherapy of tuberculosis: past, present and future. *Int J Tuberc Lung Dis*, 16(6),724-32.
- [23]Mitchison DA (2012). Prevention of drug resistance by combined drug treatment of tuberculosis. *Handb Exp Pharmacol*, 211, 87-98.
- [24]Dalhoff A (2012). Resistance surveillance studies: a multifaceted problem--the fluoroquinolone example. *Infection*, 40(3):239-62.
- [25]Viswanathan R1, Singh AK, Ghosh C, Dasgupta S, Mukherjee S, Basu S (2012). Profile of neonatal septicaemia at a district-level sick newborn care unit. *J Health Popul Nutr*, 30(1):41-8.

- [26]Grüneberg, R. N (1979). The microbiological rationale for the combination of sulphonamides with trimethoprim. *J. Antimicrob. Chemother*, 5 (Supplement B), 27-36
- [27]Bliziotis IA, Samonis G, Vardakas KZ, Chrysanthopoulou S, Falagas ME (2005). Effect of aminoglycoside and beta-lactam combination therapy versus beta-lactam monotherapy on the emergence of antimicrobial resistance: a meta-analysis of randomized, controlled trials. *Clin Infect Dis*, 41(2):149-58.
- [28]Indrelie JA, Wilson WR, Matsumoto JY, Geraci JE, Washington JA 2nd (1984). Synergy of imipenem or penicillin G and aminoglycosides against enterococci isolated from patients with infective endocarditis. *Antimicrob Agents Chemother*, 26(6):909-12.
- [29]Investigators of the Piperacillin/Tazobactam Intra-abdominal Infection Study Group (1994). Results of the North American trial of piperacillin/tazobactam compared with clindamycin and gentamicin in the treatment of severe intra-abdominal infections. *Eur J Surg Suppl*, 573, 61-6
- [30]Bush K. Proliferation and significance of clinically relevant  $\beta$ -lactamases (2013). *Ann N Y Acad Sci*, 1277:84-90
- [31]Brogden RN, Carmine A, Heel RC, Morley PA, Speight TM, Avery GS (1981). Amoxicillin/clavulanic acid: a review of its antibacterial activity, pharmacokinetics and therapeutic use. *Drugs*,22(5), 337-62. Review.
- [32]Dagan R1, Bar-David Y (1989). Comparison of amoxicillin and clavulanic acid (augmentin) for the treatment of nonbullous impetigo. *Am J Dis Child*, 143(8):916-8.
- [33]Savard, P. & T.M. Perl (2012). A call for action: managing the emergence of multidrug-resistant Enterobacteriaceae in the acute care settings. *Curr. Opin. Infect. Dis*, 25: 371–377.
- [34]Lascols C, Hackel M, Hujer AM, Marshall SH, Bouchillon SK, Hoban DJ, Hawser SP, Badal RE, Bonomo RA.(2012). Using nucleic acid microarraysto perform molecular epidemiology and detect novel beta-lactamases: a snapshot of extended-spectrum betalactamases throughout the world. *J. Clin. Microbiol.* 50: 1632–1639.
- [35]Buynak JD (2013). Beta-Lactamase inhibitors: a review of the patent literature. 2010 – 2013. *Expert Opin. Ther. Patents*(2013), 23(11), 1-13.
- [36]Drawz SM, Papp-Wallace KM, Bonomo RA (2013). New  $\beta$ -lactamase inhibitors: A therapeutic renaissance in an "MDR world". *Antimicrob Agents Chemother*, [Epub ahead of print]
- [37]Hong MC, Hsu DI, Bounthavong M (2013). Ceftolozane/tazobactam: a novel antipseudomonal cephalosporin and  $\beta$ -lactamase-inhibitor combination. *Infect Drug Resist*, 6, 215-223. eCollection 2013. Review.
- [38]Shlaes DM (2013). New  $\beta$ -lactam- $\beta$ -lactamase inhibitor combinations in clinical development. *Ann N Y Acad Sci*, 1277, 105-14



- [39]Livermore DM, Warner M, Mushtaq S (2013). Activity of MK-7655 combined with imipenem against Enterobacteriaceae and *Pseudomonas aeruginosa*. *J Antimicrob Chemother*, 68(10), 2286-90.
- [40]Boehr DD, Draker KA, Koteva K, Bains M, Hancock RE, Wright GD (2003). Broad spectrum peptide inhibitors of aminoglycoside antibiotic resistance enzymes. *Chem Biol*, 10(2), 189-96.
- [41]Singh H, Thangaraj P, Chakrabarti A (2013). *Acinetobacter baumannii*: A Brief Account of Mechanisms of Multidrug Resistance and Current and Future Therapeutic Management. *J Clin Diagn Res*, 7(11), 2602-5.
- [42]Shi K, Caldwell SJ, Fong DH, Berghuis AM (2013). Prospects for circumventing aminoglycoside kinase mediated antibiotic resistance. *Front Cell Infect Microbiol*, 3, 22.
- [43]Piddock LJ (2006). Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin Microbiol Rev*, 19(2), 382–402.
- [44]Neyfakh, A. A., V. E. Bidnenko, and L. Bo Chen (1991). Efflux-mediated multidrug resistance in *Bacillus subtilis*: similarities and dissimilarities with the mammalian system. *Proc. Natl. Acad. Sci. USA* 88,4781–4785.
- [45]Markham, P. N., and A. Neyfakh (1996). Inhibition of the multi-drug transporter norA prevents emergence of norfloxacin resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother*, 40, 2673–2674.
- [46]Hsieh, P.-C., S. A. Siegel, B. Rogers, D. Davis, and K. Lewis (1998). Bacteria lacking a multidrug pump: a sensitive tool for drug discovery. *Proc. Natl. Acad. Sci. USA*, 95, 6602–6606.
- [47]Lawler AJ1, Ricci V, Busby SJ, Piddock LJ (2013). Genetic inactivation of *acrAB* or inhibition of efflux induces expression of *ramA*. *J Antimicrob Chemother*, 68(7), 1551-7
- [48]Buyck JM, Plésiat P, Traore H, Vanderbist F, Tulkens PM, Van Bambeke F (2012). Increased susceptibility of *Pseudomonas aeruginosa* to macrolides and ketolides in eukaryotic cell culture media and biological fluids due to decreased expression of *oprM* and increased outer-membrane permeability. *Clin Infect Dis*, 55(4), 534-42.
- [49]Masi M, Pagès JM (2013). Structure, Function and Regulation of Outer Membrane Proteins Involved in Drug Transport in Enterobacteriaceae: the *OmpF/C* - *TolC* Case. *Open Microbiol J*, 7, 22-33
- [50]Tran QT, Williams S, Farid R, Erdemli G, Pearlstein R (2013). The translocation kinetics of antibiotics through porin *OmpC*: insights from structure-based solvation mapping using WaterMap. *Proteins*, 81(2), 291-9
- [51]Bayer AS, Schneider T, Sahl HG (2013). Mechanisms of daptomycin resistance in *Staphylococcus aureus*: role of the cell membrane and cell wall. *Ann N Y Acad Sci*, 1277, 139-58.

- [52] Weingart H1, Petrescu M, Winterhalter M (2008). Biophysical characterization of in- and efflux in Gram-negative bacteria. *Curr Drug Targets*, 9(9), 789-96.
- [53] H. Nikaido, D.G. Thanassi (1993). Penetration of lipophilic agents with multiple protonation sites into bacterial cells: tetracyclines and fluoroquinolones as examples. *Antimicrob. Agents Chemother*, 37, 1393–1399
- [54] J.L. Vázquez, S. Merino, O. Domenech, M. Berlanga, M. Viñas, M.T. Montero, J. Hernández-Borrell (2001). Determination of the partition coefficients of a homologous series of ciprofloxacin: influence of the n – 4 piperazinyl alkylation on the antimicrobial activity. *Int. J. Pharm*, 220, 53–62
- [55] Domagala JM (1994). Structure-activity and structure-side-effect relationships for the quinolone antibacterials. *J Antimicrob Chemother*, 33(4), 685-706.
- [56] Sanchez, J. P., Domagala, J. M., Hagen, S. E., Heifetz, C. L., Hutt, M. P., Nichols, J. B. et al. (1988). Quinolone antibacterial agents. Synthesis and structure-activity relationships of 8-substituted quinolone-3-carboxylic acids. *Journal of Medicinal Chemistry*, 31, 983-91.
- [57] Cramariuc O1, Rog T, Javanainen M, Monticelli L, Polishchuk AV, Vattulainen I (2012). Mechanism for translocation of fluoroquinolones across lipid membranes. *Biochim Biophys Acta*, 1818(11), 2563-71.
- [58] Tängdén T, Hickman RA, Forsberg P, Lagerbäck P, Giske CG, Cars O (2014). Evaluation of Double and Triple Antibiotic Combinations for VIM- and NDM-producing *Klebsiella pneumoniae* by in vitro time-kill experiments. *Antimicrob Agents Chemother*, [Epub ahead of print]
- [59] Dhariwal AK, Tullu MS (2013). Colistin: re-emergence of the 'forgotten' antimicrobial agent. *J Postgrad Med*, 59(3), 208-15.
- [60] Gopal R, Kim YG, Lee JH, Lee SK, Chae JD, Son BK, Seo CH, Park Y (2013). Synergistic effects and anti-biofilm properties of chimeric peptides against MDR *Acinetobacter baumannii* strains. *Antimicrob Agents Chemother*, [Epub ahead of print]
- [61] Tavares LS, Silva CS, de Souza VC, da Silva VL, Diniz CG, Santos MO (2013). Strategies and molecular tools to fight antimicrobial resistance: resistome, transcriptome, and antimicrobial peptides. *Front Microbiol*, 4, 412. eCollection 2013.
- [62] Pietiäinen M, François P, Hyyryläinen HL, Tangomo M, Sass V, Sahl HG, Schrenzel J, Kontinen VP (2009). Transcriptome analysis of the responses of *Staphylococcus aureus* to antimicrobial peptides and characterization of the roles of *vraDE* and *vraSR* in antimicrobial resistance. *BMC Genomics*, 10, 429.
- [63] Hu Y, Shamaei-Tousi A, Liu Y, Coates A (2010). A new approach for the discovery of antibiotics by targeting non-multiplying bacteria: a novel topical antibiotic for staphylococcal infections. *PLoS One*. 5(7), e11818.

[64]Hu Y, Coates AR(2013).Enhancement by novel anti-methicillin-resistant *Staphylococcus aureus* compound HT61 of the activity of neomycin, gentamicin, mupirocin and chlorhexidine: in vitro and in vivo studies. *J Antimicrob Chemother*, 68(2), 374-84.

[65]Ejim L, Farha MA, Falconer SB, Wildenhain J, Coombes BK, Tyers M, Brown ED, Wright GD (2011). Combinations of antibiotics and nonantibiotic drugs enhance antimicrobial efficacy. *Nat Chem Biol*, 7, 348–50.

[66]Hurdle JG, O'Neill AJ, Chopra I, Lee RE (2011). Targeting bacterial membrane function: an underexploited mechanism for treating persistent infections. *Nat Rev Microbiol*, 9(1), 62-75