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REVIEW

Novel approaches to developing new antibiotics for bacterial infections

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Antibiotics are an essential part of modern medicine. The emergence of antibiotic-resistant mutants among bacteria is seemingly inevitable, and results, within a few decades, in decreased efficacy and withdrawal of the antibiotic from widespread usage. The traditional answer to this problem has been to introduce new antibiotics that kill the resistant mutants. Unfortunately, after more than 50 years of success, the pharmaceutical industry is now producing too few antibiotics, particularly against Gram-negative organisms, to replace antibiotics that are no longer effective for many types of infection. This paper reviews possible new ways to discover novel antibiotics. The genomics route has proven to be target rich, but has not led to the introduction of a marketed antibiotic as yet. Non-culturable bacteria may be an alternative source of new antibiotics. Bacteriophages have been shown to be antibacterial in animals, and may find use in specific infectious diseases. Developing new antibiotics that target non-multiplying bacteria is another approach that may lead to drugs that reduce the emergence of antibiotic resistance and increase patient compliance by shortening the duration of antibiotic therapy. These new discovery routes have given rise to compounds that are in preclinical development, but, with one exception, have not yet entered clinical trials. For the time being, the majority of new antibiotics that reach the marketplace are likely to be structural analogues of existing families of antibiotics or new compounds, both natural and non-natural which are screened in a conventional way against live multiplying bacteria.
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Abbreviation: SPP, species

Antibiotics are one of the pillars of modern medicine (Ball *et al*., 2004). However, bacterial resistance emerges in a very small proportion of patients when an antimicrobial agent is introduced into the market or just after its introduction (Bax *et al*., 1998). There is a huge variation in the time for emergence of resistance, which varies among organisms and antibiotics. For example, penicillin resistance in *Staphylococcus* spp emerged rapidly, whereas penicillin resistance among *Streptococcus pneumoniae* took several decades. Eventually, resistance rises to such a high level that it reduces the efficacy of the drug in a human population. At this point, a new antibiotic is required, which is active against resistant bacteria. In response to bacterial resistance, the pharmaceutical industry has produced a remarkable range of antibiotics (Bax and Mullan, 1999).

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Current methods of antibiotic development

This section focuses on current well-tested strategies of selection of compounds from natural and non-natural sources and modifying existing classes. The current methods have concentrated on compounds that target logarithmic multiplying bacteria (Coates *et al*., 2002). For example, natural compounds, such as penicillin, have been discovered by scientific observation (Fleming, 1929; Abraham, 1987), or by searching for such compounds (Pelaez, 2006). These natural compounds have provided basic structures such as 6-aminopenicillanic acid, which chemists have used to produce analogues, such as amoxicillin (Rolinson and Geddes, 2007). The analogue route has been very successful for the development of new antibiotics, and continues to be so (Zhanel *et al*., 2004; Zuckerman, 2004). Novel compounds were also developed from the non-natural chemical route, for instance, prontosil (the precursor of sulpha drugs), metronidazole, isoniazid (Fernandez, 2006) and oxazolidinones. Arguably, quinolones may have been created through the non-natural chemical route, although they are originally derived from quinine. Screening of compound collections with enzymes or whole cells, such as target downregulation

by antisense RNA is also used (Wang *et al*., 2006), but have not resulted, as yet, in a marketed antibiotic (see the next section entitled The Genomics Revolution).

In retrospect, it seems as though virtually all of the easily discovered novel antibiotics were found between 1929 and 1962, because only two novel classes of antibiotics, the oxazolidinones (Barbachyn and Ford, 2003) and the cyclic lipopeptides (Finch, 2006; Kern, 2006), have entered the market in the past 40 years, both since 2000. In the intervening period, analogues of old compounds were developed. However, the number of chemical modifications that can be made to an old compound, such as penicillin, is finite, and new clinically important analogues that are active against resistant bacteria become more difficult to make as the options decrease. In addition to chemically modifying an antibiotic to address the emergence of bacterial resistance, a second compound can be added that counters a key mechanism of resistance, so that the patient takes a combination of two drugs. For instance, clavulanic acid that inhibits bacterial β-lactamase has been added to amoxicillin, and is called co-amoxiclav (Rolinson, 1982).

Unfortunately, during the past 20 years, the number of new drugs that reaches the marketplace has fallen to less than half the previous level (Spellberg *et al*., 2004; Coates and Hu, 2006). Concomitantly, resistance is rising to high levels among bacterial pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Reynolds *et al*., 2004), penicillin-resistant *S. pneumoniae* (Karchmer, 2004) and *Pseudomonas aeruginosa* (Paterson, 2006). This means that the rate of loss of efficacy of old antibiotics is outstripping their replacement with new ones for many species of pathogenic bacteria (Hancock, 2007), and this situation is particularly unsatisfactory for Gram-negative antimicrobials (Coates and Hu, 2006).

What has gone wrong? The antibiotic industry is probably a victim of its own success. After penicillin and the other major antibiotic classes were discovered between 1929 and 1962, it was generally considered that infectious diseases had been conquered. In developed countries, life expectancy increased each year, and the number of deaths caused by infectious diseases fell. Grant funding agencies reduced financial support for fundamental antibiotic research, believing that large pharmaceutical companies would fund antibiotic research (Hancock, 2007). Many major pharmaceutical companies and large biotech companies left the antibiotic research and development arena. For example, since 1999, 10 of the 15 largest companies have significantly reduced or eliminated their antibiotic research programmes (Projan and Shlaes, 2004), although recently GlaxoSmith-Kline and Astra-Zeneca have announced renewed and expanded efforts in antibiotics. The main reason for this withdrawal is that antibiotics are less valuable (diminished net present value) than many other drugs. For example, antibiotics are short-course therapies that cure infections. In contrast, billion dollar blockbuster drugs are typically chronic treatments for incurable diseases, such as cholesterol-lowering compounds. Companies make much larger profits from selling drugs that patients take every day for 20 years than they do selling drugs that patients take for 7 days and then stop. In addition, the pharmaceutical industry has found it difficult to discover new classes of antimicrobial agents, and only two novel classes were introduced in the past 40 years, although most of the companies active in the field tried to identify fundamentally novel compounds (Zurenko *et al*., 1997; Ford *et al*., 2001; Fox, 2006). The research that took place delivered analogues of known classes, but no new ones. Why did this happen? In part, it was because of the reduction in research effort, in particular the decline in natural product research and in part the over-reliance on screening compound collections that were focused on subclasses of mammalian targets and combinatorial chemical libraries which are now known to be non-productive. Also, the current financial reward for large pharmaceutical companies to develop new antibiotics has become unfavourable. For example, it has been suggested (Metlay *et al*., 2006; Bradley *et al*., 2007) that increasingly severe market restrictions on the use of antibiotics, which have been introduced to control resistance, will, paradoxically, reduce the rate of development of new antibiotics because potential earnings will be reduced. In addition, the increase in regulatory requirements (Norrby *et al*., 2005) makes the market conditions for new antibiotics even less attractive.

Things could not get much worse. But unfortunately, they did. The research community and the pharmaceutical industry then undertook an expensive experiment that (to date) appears to have yielded little. They tried to develop new antibiotics using genomics (Payne *et al*., 2007). In retrospect, this was probably a mistake.

The genomics revolution

The complete sequencing of the genomes of many pathogenic bacteria has led to an explosion in knowledge about these organisms (Monaghan and Barrett, 2006). The first whole-bacterial genome to be sequenced was *Haemophilus influenzae* in 1995 (Fleischmann *et al*., 1995). Since then, hundreds of bacterial genomes have been completely sequenced, yielding a massive amount of new information (McDevitt and Rosenberg, 2001; Haney *et al*., 2002; Dougherty *et al*., 2003; Freiberg and Brotz-Oesterhelt, 2005; http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi or http://www.tigr.org/tdb). Genomics is used to select potential antibacterial targets (Sakharkar *et al*., 2004; Arcus *et al*., 2006) and can also be used to provide insights into, for example, pathogenesis (Field *et al*., 2004; Polissi and Soria, 2005) and antibiotic resistance (Black and Hodgson, 2005; Fournier *et al*., 2006). An antibacterial target may be the receptor of a ligand in a bacterial molecule, a specific function of a bacterial molecule such as an enzyme or a metabolic pathway. Target identification is achieved in a variety of ways (see below), but the main principle is that the target should not be shared with the human host, should be present in those bacteria that cause the disease for which a cure is needed, and if removed or inhibited leads to the death of the bacteria (essentiality). Libraries of compounds may be screened at this time to find a lead molecule that, for example, inhibits the enzymatic function of the target. Further development includes searching for antibacterial

activity of the molecule. Several lead compounds are chosen and lead optimization then proceeds to complete the preclinical programme, which is required before entry into clinical trials.

While many molecular targets have been tested, a recent analysis suggests that there may not be many new metabolic targets for broad-spectrum antibiotics, and that most possible metabolic pathways are already known (Becker *et al*., 2006; Schmid, 2006a). An example of the genomic approach, which covers many different routes, is one in which a target is identified by bioinformatics, expression profiling of all predicted open-reading frames using DNA microarrays and knockout mutants are used to pinpoint essential genes (Pucci, 2006). Essentiality may be shown in cultures of bacteria or *in vivo* in the infected host. Proteins are expressed at a high level and in almost all cases are then used for high-throughput screening in enzyme-inhibition assays. After potentially interesting inhibitors are identified for a target, NMR or X-ray crystallography is used to predict the molecular structure of key proteins such as enzymes (Saidijam *et al*., 2006). The current computational programs in use that can predict compounds that would bind to a given protein structure are presently unreliable, and work much better when used to suggest additional compounds that might bind using structures from initial inhibitors identified by high-throughput screening. Fine tuning of the structure of the inhibitory compound can be achieved by co-crystallization with the protein. Molecules that obey Lipinski's Rule of 5 (see below) can be selected if an oral drug is desired. In the case of antibiotic discovery, this approach and many other similar ones have been and are being attempted (Freiberg and Brotz-Oesterhelt, 2005). However, so far, no marketed antibiotics have reached the marketplace via the genomics route (Mills, 2006). The reason for this failure is not entirely clear. Certainly, an abundance of molecular targets has been discovered (Dougherty and Miller, 2006), and there are numerous compounds that inhibit the activity of these targets. However, it seems that inhibition of, for example, enzyme activity by a candidate compound does not always correlate closely with bactericidal activity. Sometimes, factors such as poor penetration into the cells and active efflux of the compound from the cell impede antibacterial activity. Identifying good leads has been slower than expected, and optimizing lead compounds into drug candidates that are suitable for entry into Phase 1 clinical trial has been a further obstacle to progress (Schmid, 2006b). Peptide deformylase is one target that has been identified with the assistance of genomic information (Yuan and White, 2006), and inhibitors of this enzyme have been developed, which have entered into human clinical trials (Clements *et al*., 2002). Unfortunately, these inhibitors seem to generate mutants at a high rate (Margolis *et al*., 2000; Apfel *et al*., 2001) and hence development has not proceeded as expected. In contrast, almost all existing classes of clinical antibiotics inhibit multiple paralogous targets (Spratt 1994; Li, 2005). GlaxoSmithKline used a genomics-derived, target-based approach to antibiotic discovery for 7 years, in which they examined more than 300 genes and employed 70 high-throughput screening campaigns, but did not develop an antibiotic into the market (Payne *et al*., 2007). The company has now returned to more conventional non-target-based methods of discovery. The disappointments of the genomics target-based approach have certainly used up large amounts of resources. The downside of this setback is that companies are further discouraged from entering the antibiotic discovery field. The upside is that we now know that this route is to be avoided for the time being; however, it is probable that in the long run, genomic information will prove to be highly useful to antibiotic discovery, but perhaps not in the way originally envisioned, as a short cut to target selection.

Forward is back

If genomic target-based discovery is not the way forward, where do we go from here?

GlaxoSmithKline has returned to more conventional whole-cell assays (Payne *et al*., 2007). Libraries with new chemical diversity are important in this approach, because most of the existing libraries have already been screened for activity against whole-bacterial cells. However, it is not entirely clear how these libraries can be improved. Combinatorial chemistry has not yet matured to fill this gap. Should libraries be small molecules, for example, small chemicals, or large molecules that are often derived from natural sources? Libraries of small chemicals that obey Lipinski's Rule of 5 often can be used particularly if an oral drug is desired. The Rule states (Walters *et al*., 1999; Lipinski *et al*., 2001) that the molecular mass should be $\leqslant$500 Da, the $Q\log P \leqslant 5$, hydrogen acceptors $\leqslant$10, hydrogen donors $\leqslant$5 and rotation bonds $\leqslant$10. Interestingly, most currently marketed antibiotics do not obey all the parameters of Lipinski's Rule of 5, and antibiotics are slightly larger and more hydrophilic than other drug classes (Payne *et al*., 2007). For example, aminoglycosides violate the Rule. Does this invalidate the Rule's usefulness for antibiotics? Only time will tell, but there is no clear reason to believe that the bactericidal property of a molecule is related to its molecular mass, for example, one that is larger or smaller than, say, 500 Da. Hence screening of libraries of chemical compounds that obey Lipinski's rule may or may not yield further compounds. Current belief by many in the field is that compound collections that adhere to the Rules have impeded antibiotic discovery selection.

Screening of natural compound libraries, although a well-trodden path, may well still have hidden treasures (Sheridan, 2006). Hence, some future antibiotics will probably come from this source. Compounds such as platensimycin (Wang *et al*., 2006) isolated from *Streptomyces platensis* look attractive. Combinatorial genetics may also yield recombinant organisms that produce novel antibacterial compounds (Walsh, 2002). Plant-derived compounds are another promising source of potential candidates (Lewis and Ausubel, 2006).

For the time being, it seems that analogues of existing antibiotics will provide the majority of new compounds that reach the market. Unfortunately, as bacteria develop increasingly sophisticated mechanisms of resistance against a particular family, over time this route will fade away. The inescapable conclusion is that, if modern medicine is to

continue in its present form, mankind will need, at regular intervals, discovery of novel families of antibiotics.

New methods that have the potential to deliver novel antibiotics

Natural compounds: non-culturable bacteria as targets

Bacteria produce antibiotics that kill or inhibit the replication of competitors. To date, marketed antibiotics such as streptomycin have been derived from bacteria that grow on artificial solid or liquid media. However, most species of bacteria will not grow on artificial media (Diaz-Torres *et al*., 2006). Marketed antibiotics have not been isolated from non-culturable bacteria, since growth on solid media has been an essential step to the development antibiotics. Now, it is possible to clone large fragments of non-culturable bacterial genomes and to express them using recombinant DNA technology (see Figure 1) (Garcia *et al*., 2006; Turnbaugh *et al*., 2006; Jones and Marchesi, 2007; Lee *et al*., 2007; Ono *et al*., 2007).

DNA is extracted from a mixture of bacteria from, for example, soil, and is inserted into a vector, such as a bacterial artificial chromosome (Rondon *et al*., 2000) that can accept large DNA fragments. Open-reading frames within the fragment are then expressed in a culturable cell such as *Streptomyces spp* and are screened for antibiotic activity. Streptomyces genetics is very sophisticated, and the industry has great experience with fermentations using these organisms. The location of the genes that are needed for the production of antibiotics is then achieved by a variety of molecule DNA techniques. For example, SelectX have found ways to capture and enrich antibiotic synthesis encoding DNA from non-cultivable organisms. Merlion and Novo-Biotics are also attempting to access non-cultivable organisms, for instance, by devising ways to grow organisms previously not grown in artificial media. These types of efforts represent possibly some of the best opportunities for antibiotic novelty. Possible problems with this approach are that the productive DNA fragments occur too infrequently to be detected by cloning. Also, DNA fragments may not contain all the genes that are required for the production of the antibiotic, and the host organism may not express the genes in the DNA fragment correctly. Culture of previously uncultivated microbes is notoriously difficult, and success may be limited. No compounds that have been produced in this way are yet in clinical trials.

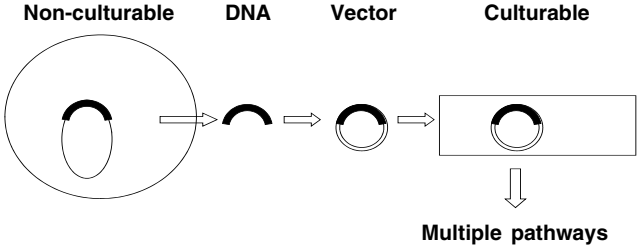

Figure 1 Non-culturable bacteria. The unfilled circle on the left of the diagram depicts the non-culturable bacterium. Within the circle is the smaller ring, which shows the bacterial genome with the thick black segment representing the coding region for synthesis of a novel antibiotic. The DNA of the coding region is extracted, inserted into a vector and this is transfected into a culturable bacterium or other host that expresses the antibiotic. Antibiotic biosynthesis often involves multiple pathways that are found on relatively large genomic segments of DNA, and cloning these pathways is often a complex task.

Bacteriophages

Bacteriophages and their fragments (Borysowski *et al*., 2006) kill bacteria. It is estimated that every 2 days, half of the world's bacterial population is destroyed by bacteriophages (Hendrix, 2002). Bacteriophages have been used as antibacterials in humans in some countries of the world (Sulakvelidze *et al*., 2001). Indeed, in the last century, just before the introduction of penicillin and sulpha drugs, phage preparations were sold in the United States of America (Fischetti *et al*., 2006). Even now, bacteriophages are used in the former Soviet Union to treat patients with infectious diseases (Sulakvelidze *et al*., 2001). In addition, bacteriophages have been developed for use in the poultry and cattle industries (Huff *et al*., 2003; Doyle and Erickson, 2006; Sheng *et al*., 2006), aquaculture (Nakai and Park, 2002) and in sewage treatment (Withey *et al*., 2005). In 2006, the FDA approved the use of bacteriophages in the treatment of *Listeria monocytogenes* contamination of meat and poultry (Fischetti *et al*., 2006). Potential advantages of this approach are that the mechanism of action is likely to be completely different to current antimicrobials. Disadvantages are that quality control and standardization are difficult. Also, when used systemically in patients, phages are likely to be immunogenic and may induce neutralizing antibodies (Dabrowska *et al*., 2005). Furthermore, massive bacterial lysis might lead to toxic shock (Matsuda *et al*., 2005). One approach that has been developed to address this problem is lysis-deficient phages, which can still kill bacteria (Matsuda *et al*., 2005). Phages can even be used to transport and target antibiotics into bacteria (Yacoby *et al*., 2006). Although penetration of such a large molecular complex into relevant tissues might be a problem, for example, as an oral drug, intravenous injection leads to bacteriophages in most organs, which suggests that they could be useful in certain infections. Bacterial resistance to phages emerges quickly and hence it is necessary to use a cocktail of phages to counter the emergence of resistance (Sulakvelidze *et al*., 2001). The patentability of groups of phages is another challenge for companies that are trying to exploit this field, but patenting of specific bacteriophage sequences is one potential patenting strategy (Thiel, 2004).

The development of phage gene products is another potential route for new antibacterials. Phage lysins, which are cell wall hydrolases and are produced late in the viral infection cycle, bind to peptidoglycan and disrupt the cell wall of Gram-positive bacteria that results in hypotonic lysis (Fischetti *et al*., 2006). Lysins have potential uses as antibacterials for human use. For example, they could clear mucous membranes of pathogens, such as *S. pyogenes* group A, *S. agalactiae* and *S. pneumoniae* (Nelson *et al*., 1997; Loeffler *et al*., 2001). Systemic use of lysins has also been described

(Fischetti *et al*., 2006), and may have advantages over whole bacteriophages because, in preclinical studies, they do not induce resistance, neutralizing antibodies or toxic shock. A particularly interesting finding is that lysins may be active against non-multiplying bacteria and biofilms (Balaban *et al*., 2004; Entenza *et al*., 2005). This could help in the treatment of, for example, catheter-associated infections.

Other small genome viral components, such as Qβ and φX174, and the phage Dna^2 have also been proposed as novel antibacterials (Bernhardt *et al*., 2001; Liu *et al*., 2004; Bugg *et al*., 2006). Currently, there is a lack of good human clinical trial results, although animal studies suggest that in certain circumstances, bacteriophage therapy may be useful.

Non-multiplying bacteria as targets

Bacteria exist in two different states in a clinical infection, such as tuberculosis, bacterial endocarditis, biofilms and streptococcal sore throat. The states are described as multiplying (logarithmic phase) and non-multiplying (sometimes called stationary phase, dormant or latent) (Coates *et al*., 2002). Biofilms, for example on indwelling catheters, are complex mixtures of bacteria, which contain both multiplying and non-multiplying bacteria (Costerton *et al*., 1999). From a clinical perspective, it has been suggested that 60% of human bacterial infectious disease contain a significant proportion of non-multiplying bacteria (Galli *et al*., 2006). The practical significance of an infection that has non-multiplying bacteria is that these organisms cannot be easily killed by antibiotics (Coates and Hu, 2006). Although multiplying bacteria die in the presence of an antibiotic (Figure 2), non-multiplying ones survive. Currently marketed antibiotics are bacteriostatic for non-multiplying bacteria, although some of them, such as the penicillins (Wright and Wilkowske, 1987), are highly bactericidal for multiplying organisms. After a period of time, for example, when antibiotic levels in the blood fall below an effective therapeutic level, non-multiplying bacteria spawn multiplying ones. This means that if the antibiotic is withdrawn at this stage, the multiplying bacteria increase, which, in turn, leads to clinical relapse, for instance, a recurrence of the symptoms of sore throat. Therefore, additional doses of antibiotic have to be administered, which kill any multiplying bacteria that emerge from the pool of non-multiplying ones. Because the pool is not limitless, all the target bacteria are eventually killed and cure is achieved. This takes different periods of time for different infectious diseases. For example, the duration of therapy for tuberculosis is months to years (Mitchison, 2005), for bacterial endocarditis several weeks (Alestig *et al*., 2000; Elliott *et al*., 2004) and for streptococcal sore throat up to 10 days (Cohen and Green, 2004). Biofilms on prosthetic implants are particularly difficult to cure. Rifampicin is the standard antibiotic and is used as part of a combination regimen to attempt to clear such infections when the material cannot be removed for medical reasons The reasons for the emergence of bacterial resistance are complex, but it is more likely to emerge, partly as a result of selection of bacterial mutants by antibiotics over time (Craig *et al*., 1993; Epstein *et al*., 2004; Cottagnoud *et al*., 2005; Firsov *et al*., 2006) and partly as a result of reduced patient compliance (Mitchison, 1998; Kardas *et al*., 2005; Niederman, 2005), which leads to periods of sub-optimal levels of antibiotics that, in turn, trigger bacterial resistance. It has been proposed (Coates *et al*., 2002) that antibiotics could be developed against non-multiplying bacteria, perhaps for use in combination with a drug that kills multiplying bacteria. No current marketed antibiotics have been developed primarily against non-multiplying bacteria and hence it is not surprising that they have either no or only limited activity against organisms in this state.

An example of this approach is the one being pursued by Helperby Therapeutics Group plc, which has screened a

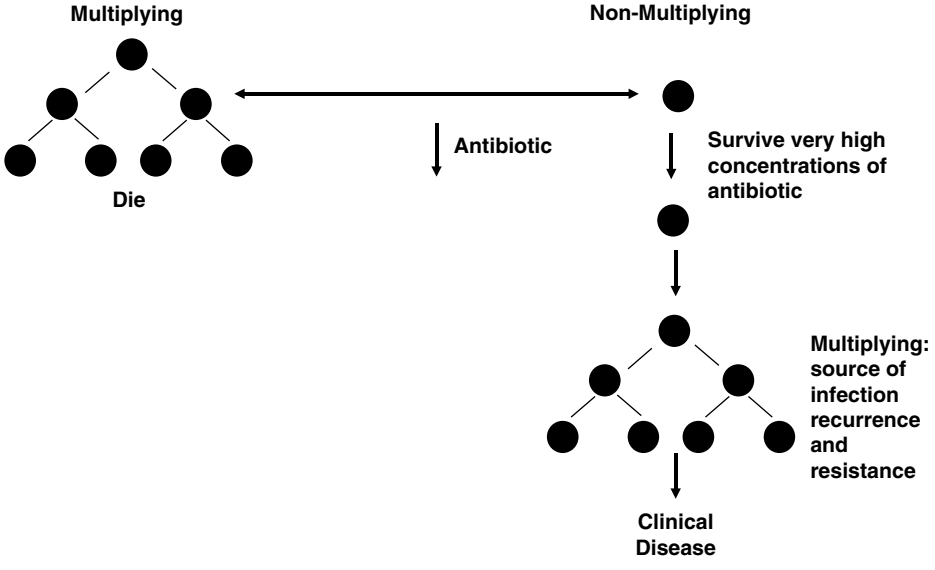

Figure 2 Non-multiplying bacteria. On the left of the figure are multiplying bacteria that die when treated with antibiotic. On the right of the diagram are non-multiplying bacteria that can survive high concentrations of antibiotic. Non-multiplying bacteria can spawn multiplying ones, which are a source of recurrence of clinical infectious disease and may also give rise to resistant mutants.

number of compound libraries that have yielded bactericidal compounds, one of which is due to enter Phase I clinical trials in 2007.

The advantage of an antibiotic that is bactericidal for non-multiplying bacteria is that the duration of therapy may be shortened. This presumes that all the multiplying and non-multiplying target bacteria are quickly killed by an antibiotic or by a combination of compounds. Short-course therapy would increase patient compliance, and this, in turn, particularly in diseases such as tuberculosis, would increase the efficiency of disease control (Murray, 1991; Yew, 1999). In addition, for many bacterial infectious diseases, shortening the duration of therapy could lead to a decrease in the rate of emergence of bacterial resistance (Guillemot *et al*., 1998), particularly if the treatment period could be reduced to one dose and if the compound was metabolized rapidly. All the target bacteria in a patient with an infectious disease, such as respiratory tract infection, meningitis, endocarditis, osteomyelitis and infections in the neutropenic host, should be eradicated because failure to eradicate bacteria may promote the emergence of resistant strains of bacteria (Dagan *et al*., 2001).

Disadvantages of this approach are that all antibacterial targets may already be known; in other words, the repertoire of naturally occurring antibiotics that are well documented may represent all the targets. This situation may be compounded by the possibility that non-multiplying bacteria may have fewer molecular targets than multiplying ones, as so many of their genes are downregulated (Hu *et al*., 1998; Hu and Coates, 1999; Beenken *et al*., 2004; Johansen *et al*., 2006). So, in practice, it may be impossible to develop antibiotics against spore-like non-multiplying bacteria. Novel antibiotic discovery has had difficulties identifying compounds against multiplying organisms, hence it might be imagined that non-growing bacteria would present an even greater problem. An additional problem with this approach could be that there may be many different types of non-multiplying bacteria, each of which may be susceptible to different compounds. For example, in tuberculosis, it is thought that there are at least three different subpopulations of non-multiplying bacteria (Hu *et al*., 2003). This would suggest that more than one compound would be needed to kill all non-multiplying bacteria in a clinical infection. In certain situations, such as biofilms, other factors may be important for the destruction of non-multiplying bacteria, such as effective penetration of the antibiotic, or a huge local concentration of bacteria that require a high level of antibiotic, which cannot be achieved therapeutically (Smith and Brown, 2003; Fux *et al*., 2005; Lewis, 2007).

Conclusion

If modern medicine is to continue in its present form, novel families of antibiotics must enter the marketplace at regular intervals. Although analogues of existing families, which kill resistant bacteria, prolong the life of each family for a number of decades, eventually this well of discovery dries out. Within the next 10 years, screening of whole bacteria against novel natural and chemical compound libraries may produce new antibiotics. Genomics, non-culturable bacteria, bacteriophages and non-multiplying bacteria may also be a source of novel compounds.

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Conflict of interest

ARMC is a director of Helperby Therapeutics Group plc, and YH receives financial support from the same company.

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