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Alternatives to antibiotics—a pipeline portfolio review

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Antibiotics have saved countless lives and enabled the development of modern medicine over the past 70 years. However, it is clear that the success of antibiotics might only have been temporary and we now expect a long-term and perhaps never-ending challenge to find new therapies to combat antibiotic-resistant bacteria. A broader approach to address bacterial infection is needed. In this Review, we discuss alternatives to antibiotics, which we defined as non-compound approaches (products other than classic antibacterial agents) that target bacteria or any approaches that target the host. The most advanced approaches are antibodies, probiotics, and vaccines in phase 2 and phase 3 trials. This first wave of alternatives to antibiotics will probably best serve as adjunctive or preventive therapies, which suggests that conventional antibiotics are still needed. Funding of more than £1·5 billion is needed over 10 years to test and develop these alternatives to antibiotics. Investment needs to be partnered with translational expertise and targeted to support the validation of these approaches in phase 2 trials, which would be a catalyst for active engagement and investment by the pharmaceutical and biotechnology industry. Only a sustained, concerted, and coordinated international effort will provide the solutions needed for the future.

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Introduction

Given the rise of antibacterial resistance and the challenges of conventional antibacterial agent discovery and development that have led to a very small pipeline of new therapies, it would be prudent to consider the potential of non-conventional approaches.[1,2] This Review—written by 24 scientists from academia and industry, commissioned by the Wellcome Trust, and jointly funded by the Department of Health (England)—considers the prospects for alternatives to antibiotics. Although there have been technical reviews of individual alternative approaches,[3] this Review seeks to define the present state of alternatives to antibiotics at the portfolio level, prioritise approaches, and provide evidence-based expectations of their delivery to inform funding decisions and policy in this crucial area of health care.

Alternatives to antibiotics were defined by us as non-compound approaches (ie, products other than classic antibacterial agents) that target bacteria or approaches that target the host. Thus, an antibody targeting a virulence factor or quorum sensing would be included, but a compound targeting these processes would not.[4,5] Biological drugs or compounds targeting the host were included. This Review focuses on therapies that could be developed to treat systemic or invasive infections rather than superficial infections and is therefore restricted to therapies that are administered orally, by inhalation, or by injection. External topical administration is beyond the scope of this Review. The primary objective is to identify and review prospective therapeutic replacements for antibiotics. Alternatives that could be used in combination with conventional antibiotics and prophylactic approaches are also considered.

In this Review, we discuss feasibility of informative clinical trials, magnitude of medical potential, likelihood and consequences of resistance, level of current research activity, likely timeline to registration, and activities that might enable validation and progression. The review process involved the preparation of a 50-page document summarising 19 current alternatives to antibiotics within the scope of the review, a meeting to discuss and prioritise approaches, and collective preparation of a report for the funders, which is summarised in this Review. This process allowed us to compile and share broad and well informed views on the state of the art for alternatives to antibiotics with a wider community.

Portfolio of alternative approaches

We identified 19 alternatives-to-antibiotics approaches for consideration and recognised that the list might be

Key messages

- Alternatives to antibiotics: non-compound (ie, non-classic antibacterial compounds) approaches that target bacteria or approaches that target the host to treat bacterial infection
- Academics and industry have produced at least 19 approaches that need to be further assessed
- Understanding of the potential of alternatives to antibiotics will need experimental clinical medicine and not just drug discovery
- Enhanced translational expertise should be used to help validation and progression of these alternatives to antibiotics
- Model projects must be advanced to phase 2 clinical trials to enable validation of approaches
- Antimicrobial resistance needs to grow into big science to deliver new innovative therapies
- The Large Hadron Collider project cost roughly £6 billion and the International Space Station £96 billion; antimicrobial research and development to address the problem of antibiotic resistance probably needs an effort that is somewhere between these two projects

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incomplete (table 1 and panel). Projects were not reviewed in sufficient detail to make individual funding recommendations. Technical feasibility and clinical potential of the approaches were considered for all projects, but the commercial attractiveness, potential return on investment, or potential for reimbursement of specific projects were not analysed. Given the wide range of views within our group, this Review does not represent a unanimous consensus. We recognise that perspectives differ, that gaps in available data exist, and that science will continue to advance. This Review should be seen as a snapshot of alternatives to antibiotics and their perceived potential. Ten alternatives were prioritised and analysed in more detail (table 1). Nine approaches were not prioritised at this time because other projects were considered more advanced in the translational pipeline or there was insufficient peer-reviewed information to assess their potential clinical impact, feasibility, or safety (panel).

With the exception of antibiofilm peptides, which were discovered in 2013, the potential of the top ten approaches has been known for more than a decade, but has not led to therapeutic breakthroughs for systemic treatments for reasons that are not entirely clear.[104] New vaccines have been the most notable successes, but they are of course prophylactic.[105]

The top ten approaches, which our group considered merited attention, were placed into two tiers. Tier 1 focused on clinical development and tier 2 on preclinical development over the next 5 years. The main reason why peptides are not included in tier 1 is that almost all clinical trials so far were for topical treatments, whereas this Review is mostly about systemic use. Success of tier 1 projects in phase 2 and phase 3 studies could transform the perception of the alternatives-to-antibiotics portfolio. Access to funding through key preclinical and clinical development steps (eg, production and characterisation, formulation, pharmacokinetics and pharmacodynamics, toxicology, and safety pharmacology), and subsequent published reports that lent support to continued drug development were thought to be crucial to progress towards clinical validation and to build confidence in the field. Studies should define and test clear go or no-go decision points for product progression. Programmes of work that are mainly in vitro or those focused entirely on surrogate endpoints (eg, characterising cytokines rather than pathology, microbiology, or clinical response) might not be competitive for funding.

Use of major pharmaceutical company development resources and expertise will be essential to validation and progression of alternatives to antibiotics in a timely manner. Reliance on academic and biotechnology communities alone might not be sufficient to provide new products within a decade. Application of best practices to define target product profiles, rigorous target validation, understanding bacterial species and strain differences, mechanisms and consequences of resistance, differences in rodent and human responses, time and resources to adequately optimise and characterise compounds as they progress through in-vitro and in-vivo efficacy, safety, and toxicology assays, will collectively contribute to increased success or at least enable definitive and evidence-based decisions to stop the investigation of unproductive approaches.

Unfortunately, and by contrast with classic antibiotics, the predictive value of preclinical studies for host-directed therapies could be restricted. Specifically, some alternatives to antibiotics act via the immune system, which could mean that increased preclinical use of non-human primates will be necessary.[106] This drawback increases risk and averts funding. However, failure of early clinical studies should not block future investigation.

On the basis of a combination of high clinical impact and high technical feasibility, the approaches anticipated to have the greatest potential to provide alternatives to antibiotics were phage lysins as therapeutics, vaccines as prophylactics, antibodies as prophylactics, and probiotics as treatments or prophylactics for *Clostridium difficile*-associated diarrhoea and antibiotic-associated diarrhoea. Bacteriophages (wild-type and engineered) were also thought to have potentially high impact as alternatives to antibiotics, but the feasibility of their introduction to the market was unclear. Selected immune stimulation approaches were thought to be feasible as broad-spectrum prophylactics or adjuncts to conventional treatments, but their clinical impact was also unclear.

Because of their potential for broad-spectrum activity, it was disappointing that antimicrobial peptides were best placed in tier 2 rather than tier 1. Antimicrobial peptides have been tested in clinical trials and failed, but the tested products were given topically and, as such, are outside the scope of this Review. The reasons for peptide failure in phase 3 clinical trials and non-progression to product registration include low efficacy, non-superiority over antibiotics, and safety; the underlying reasons for these clinical outcomes have not been reported.[107] We speculate that early attempts to develop new therapies, particularly peptides, were hampered by insufficient investment, use of peptides that had not been optimised, and insufficient drug development and clinical expertise.

Although past failure might suggest poor prospects for peptide-based therapies, as a group, we regard alternatives to antibiotics, including peptides, as an emerging field. For instance, only six pharmacology studies are published about antimicrobial peptides (two for plectasin, two for lantibiotics, and two for other peptides), and only two safety studies have been published across the topics of lysin, bacteriophage, antimicrobial peptides, host-defence peptides, and antibiofilm peptides.[108–114] Thus, the available scientific literature does not suggest an established area of research. Most preclinical characterisation of alternatives to antibiotics are proprietary with insufficient peer-reviewed evidence published to help understand the pharmacokinetic, pharmacodynamics, toxicity, and safety strengths and liabilities of

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	Comment	Probable spectrum of activity and initial use	Recommendation over the next 5 years
Tier 1 approaches (translational funding to clinical evaluation at phase 2)			
Antibodies[4,6–13]	Antibodies that bind to and inactivate a pathogen, its virulence factors, or its toxins were widely considered one of the alternative approaches most likely to have major clinical impact. Antibodies were considered a low-risk area with strong science basis, history of safe use, and a high degree of technical feasibility	Prevent Gram-positive and Gram-negative infection; possibly adjunct use	Basic research and development and translational
Probiotics[14–18]	Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit to the host organism. Defined mixtures of bacteria or the use of non-toxigenic spores of *Clostridium difficile* will probably provide therapeutic and prophylactic therapies that will improve current clinical practice for the treatment of *C difficile*-associated diarrhoea and antibiotic-associated diarrhoea. Basic research to understand the mechanism of action of probiotics in different settings and how they might be used in combination with antibiotics and other alternatives to antibiotics (eg, bacteriophages) could enable their wider use in other indications	Prevent or treat *C difficile*-associated diarrhoea or antibiotic-associated diarrhoea	Translational
Lysins[19–27]	Phage lysins are enzymes used by bacteriophages to destroy the cell wall of a target bacterium and are potential replacements for antibiotics because of their direct antibacterial action, and as adjuncts because they act to reduce bacterial burden, weaken biofilms, or both. Emphasis on lysins active against Gram-negative pathogens would be beneficial	Treat Gram-positive infection	Basic research and development and translational
Wild-type bacteriophages[28–32]	Wild-type bacteriophages that infect and kill bacteria have the potential to replace antibiotics for some indications. Bacteriophage could be used in small doses because they replicate when their host bacterium is present. During treatment of an infection they might also evolve to infect the strains causing the disease. This replication and evolution makes them unique in pharmaceutical product development. More product than was dosed will be present in the patient and that product can change over time; what is sampled after dosing is not exactly what was given to the patient	Treat Gram-positive and Gram-negative infection	Basic research and development and translational
Engineered bacteriophages[33–36]	The ability to genetically engineer phages with new properties for therapeutic use is potentially advantageous. Many of the challenges associated with mixtures of wild-type phages, such as breadth of strain coverage, development of resistance, and rapid elimination after systemic administration, could be addressed. In-dose selection could be an advantage of this approach, exposure to the larger doses of non-replicating phage required to treat infection might be a drawback	Treat Gram-positive and Gram-negative infection	Basic research and development and translational
Immune stimulation[37–45]	Successful antimicrobial therapy depends on an appropriate immune response. Immune stimulation has been proposed as a potential adjunct approach in conjunction with antibiotic therapy. Repurposing of phenyl butyrate and vitamin D to enhance expression of innate antimicrobial peptides seems feasible Oral bacterial extracts are registered and used in clinic to reduce the incidence of respiratory tract infections in some at-risk groups in some regions. If successful, additional clinical trials to substantiate their efficacy in other populations would encourage wider use. The mechanisms by which these extracts might work are unclear but might involve TLRs—eg, TLR2 and TLR9. Targeted interventions could be devised once these mechanisms are understood The working group focused on assessment of repurposed drugs for immune stimulation rather than assessment of early translational research in this specialty. Generally, there was insufficient target validation for bacterial infection, a high potential for side-effects, variable responses and polymorphisms in patient populations, and responses specific to bacterial species and strain. The clinical development path for host-targeted therapies will probably use non-human primates during product development	Prevent or provide adjunct therapy for Gram-positive and Gram-negative infection	Basic research and development and translational
Vaccines[46–60]	The long established investment in vaccines for new targets should continue given their potential to substantially reduce the incidence of infection and, therefore, the need for antibiotics. In view of the ageing human population, we need better knowledge of the potential for vaccination in the elderly and how best to dose immune compromised individuals	Prevention, Gram-positive more than Gram-negative infection	Basic research and development, especially new adjuvants
Tier 2 approaches (strong support for funding while monitoring for breakthrough insights regarding systemic therapy)			
Antimicrobial peptides[61–72]	The advantages of antimicrobial peptides are their broad spectrum activity, which includes most major Gram-positive and Gram-negative bacteria, their bactericidal and rapid action, low target-based resistance, and low immunogenicity. Detailed scientific literature and early clinical trials have not yet led to a therapeutic breakthrough for systemic treatments. Studies will be needed that aim to establish why they have largely not been used systemically (eg, toxicity, cost, lability to proteases, etc) and how to overcome these deficiencies (eg, formulation, redesign or use of non-natural aminoacids, etc). In some instances topical application (eg, by aerosol) might supplement systemic therapy. The reasons why projects were stopped are not in the public domain. Public–private partnerships that fund and test the potential of antimicrobial peptides in well designed clinical trials and publish the outcomes will be necessary to inform future investment into this approach	Treat or adjunct for Gram-positive and Gram-negative infection	Translational
Host defence peptides and innate defence peptides[37,73–76]	Host defence peptides (small, natural peptides) and innate defence regulators (small, synthetic peptides) have indirect antimicrobial effects. They primarily act by increasing expression of anti-inflammatory chemokines and cytokines, and reducing the expression of proinflammatory cytokines. Additional resources are needed to accelerate their preclinical assessment and progression into clinical trials to provide validation of the approach. Targeting host responses could carry an increased risk of side-effects and make it more difficult to distinguish and understand immunological differences between rodents and humans at the population level	Adjunct for Gram-positive and Gram-negative infection	Basic research and development
Antibiofilm peptides[77,78]	Peptides that specifically inhibit bacterial biofilm formation have been identified and are in preclinical development. Their use as adjunctive therapy could improve outcomes	Adjunct for Gram-positive and Gram-negative infections	Basic research and development

Basic research and development describes the provision of support for fundamental research and preclinical proof of concept studies to validate approaches and extend into early translational work to characterise efficacy, pharmacology, pharmacodynamics, and preliminary toxicology so that potential liabilities can be defined. Translational, in this context, means a focus support to bring products into the clinic. TLRs=toll-like receptors.

Table 1: **Prioritised alternative approaches**

***Panel:* Additional alternative approaches**

Immune suppression[37,79–82]
Bacterial infection can lead to an excessive host innate immune response (ranging from the systemic inflammatory response syndrome to septic shock), in which the injury to the host is made much worse by the host's proinflammatory cytokine response. Selective manipulation of this cytokine response could potentially be used in combination with antibiotics to reduce pathogen-induced tissue damage mediated by cytokines and neutrophils, and to accelerate patient recovery. The medical need is high, but past failures of phase 3 clinical trials, despite promising preclinical, phase 1, and phase 2 data, suggests that manipulation of the cytokine response in sepsis and septic shock carries a great risk and has, therefore, not been prioritised. New approaches are needed to develop small-molecule and large-molecule drugs for these infections that cause high mortality with increasing incidence. By contrast with antibiotics, the health-care sector would pay a large premium for a drug that was effective at reducing morbidity and mortality. The immune system is complex, and changing the balance of proinflammatory and anti-inflammatory activities in bacterial infection to achieve a therapeutic benefit will need new thinking in systems biology. A detailed academic review of this topic was beyond the scope of this Review.

Anti-resistance nucleic acids[83–88]
Antibiotic resistance genes are often spread by highly transmissible plasmids, particularly in Gram-negative pathogens. Effective removal of resistance genes could sensitise bacteria to conventional antibiotics. Some researchers believe that this approach might not reach all resistance targets in a complex environment (eg, gut or abscess) in the absence of selection, while containment of a transmissible genetically modified vector that delivers the anti-resistance nucleic acid in an open system could face substantial regulatory challenges.

Antibacterial nucleic acids[87–89]
Use of nucleic acids to directly kill bacteria is being investigated in both academia and biotechnology companies. Studies are at an early stage. At the very least, these methods will continue to be developed to support fundamental microbial genetics studies.

Toxin sequestration using liposomes[90]
Pathogens often secrete toxins that damage mammalian cells and cause inflammation. Administration of liposomes to act as decoys for toxin binding has been shown to reduce damage to cells and reduce disease severity.

Antibiotic-degrading enzymes to reduce selection of resistance[91–94]
When antibiotics are eliminated via the gut, exposure of the normal gut bacteria to the antibiotic may lead to development of resistance and drive *Clostridium difficile*-associated diarrhoea or antibiotic-associated diarrhoea. Phase 2 studies show that oral β-lactamase can destroy β-lactams in the faeces. Demonstration of a clinical benefit of degrading enzyme administration at phase 3 could be challenging.

Metal chelation[95–99]
Bacterial pathogens need zinc, manganese, and iron ions to fully express their pathogenicity or virulence, biofilm formation, and multiple essential enzymatic and metallo-β-lactamase activities. Metal chelation could prevent these key processes in pathogens. Pharmacologists and toxicologists suggest that this approach is speculative and could present safety concerns.

Alphamers[100]
Alphamers are immune modifiers consisting of a galactose-α-1,3-galactosyl-β-1,4-N-acetyl-glucosamine (Gal) epitope fused to a bacterial pathogen binding aptamer to redirect endogenous anti-Gal antibodies to the pathogen and enhance immune clearance.

Apheresis of protective antibodies[101]
In some patients with *Pseudomonas aeruginosa* lung infection, antibodies bind to the pathogen and protect it from serum-mediated killing. Depletion of these antibodies restores the ability of serum to kill bacteria and initial clinical data suggest improved clinical outcome.

Immune stimulation by P4 peptide[102,103]
Phagocytic killing of bacteria can be enhanced by P4 peptide—a chemically synthesised 28 aminoacid peptide derived from the *Streptococcus pneumoniae* surface exposed virulence factor PsaA. P4 peptide stimulates opsonophagocytic uptake and killing in invasive disease models of *S pneumoniae* infection in mice. The combination of P4 given intranasally and IgG given intraperitoneally resulted in 100% survival in the mouse model and significantly reduced bacterial burden. A therapy based on P4, IgG, and antibiotic is proposed. However, additional evidence might be required to support the use of intravenous IgG in severe pneumonia. In 2015, the project received funding from the UK Medical Research Council Developmental Pathway Funding Scheme to progress to phase 1 studies.

these approaches. Revisiting past programmes and applying new methods of pharmacokinetic and pharmacodynamic modelling might offer improvements in dosing regimens that could alter the outcome of new clinical trials.[115] Development of narrowly focused products can be surprisingly difficult, but regulatory pathways continue to develop and additional approaches to such products are being sought.[116]

Alternatives to antibiotics portfolio analysis

To enable an evidence-based review of the current state of development and likelihood of success of the prioritised alternative approaches, detailed internet searches and knowledge of the members of our group were used to define the breadth (number of projects and targets) and depth (phase of development) of the alternatives-to-antibiotics portfolio. Company websites and news releases were used to identify projects that were in progress as of January–March, 2015 (table 2). Because companies quickly announce positive news, but might announce negative news of project cessation less quickly, the list of alternatives-to-antibiotics projects is considered inclusive and likely to overstate rather than understate the active project portfolio.

Industry-standard timelines for clinical development phases (phase 1 [1 year], phase 2 [2 years], phase 3 [3 years], and registration [1 year]) were used to estimate the earliest possible date of product registration.[1,130–132] The estimated year of registration might therefore differ from the sponsor company's estimates or project timelines. Host defence peptides and antibiofilm peptides were excluded because they were too early in development for analysis.

Similarly, industry-standard probabilities of success across projects in different phases of development (preclinical to phase 1 [23%], phase 1 [45%], phase 2 [47%], phase 3 [71%], and registration [90%]) were applied.[1,130–132] Estimates of the probability of success for individual projects in each developmental phase were added together. Values greater than 100% for a given category suggest that there are sufficient project numbers, project maturity, or both, to expect at least one product to be registered, if sufficient funding and skilled development resources are provided.

Industry-standard costs for clinical development phases (phase 1 [£6 million], phase 2 [£10 million], phase 3 [£45 million], and registration [£1·3 million]) were used to estimate the cost of portfolio projects.[1,130–132] Similar to timing projections, the estimated costs will probably differ from sponsors' estimates.

This uniform approach was used because similar levels of project planning data are not available for all projects; when available, project-specific timelines developed by sponsors often change; and use of standard timelines allows uniform recalculation of the data as needed. This type of analysis removes personal bias, but it is almost always incorrect in the specifics of its details.

Analysed by approach, the pipeline for antibodies, probiotics, and vaccines suggests success because the combined probability of registration is greater than 100%. However, for the other approaches, few projects are in progress, those in progress are early in development, and typical attrition rates thus lead to an estimate that a successful product is unlikely. For instance, on the basis of the portfolio discussed in this Review, we cannot assume that lysins, bacteriophages, or antimicrobial peptides will be developed into new therapies.

Most new research and development activity is focused on *C difficile*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The timeline analysis suggests that, if successful, the following registrations might take place in the next decade: antibodies (2017), probiotics (2018), vaccines (2019), immune stimulants (2021), lysins and antimicrobial peptides (2022), bacteriophages (2023), and host defence and antibiofilm peptides (from 2027 onwards).

When analysed by pathogen, the probability-of-success analysis suggests that if the alternatives-to-antibiotics portfolio is adequately funded, we could expect two new products for *C difficile*-associated diarrhoea and antibiotic-associated diarrhoea (antibody, probiotic, or vaccine) by 2019, one for *P aeruginosa* (antibody or vaccine) by 2021, and one for *S aureus* (antibody, lysin, or vaccine) by 2022. The portfolio lacks sufficient breadth and depth to predict success of multiple new products for these pathogens in this timeframe. That there is little activity on the other ESKAPE pathogens (ie, enterococcus, klebsiella, acinetobacter, or enterobacter) or on other Enterobacteriaceae is of concern. Therefore, it is unlikely that alternatives to antibiotics for these life-threatening pathogens, and others, will be developed in the next 10 years.

As the approaches in the portfolio advance through the later development phases, costs will increase and innovative funding arrangements will be needed to maintain momentum given that most pharmaceutical companies have stopped developing new antibiotics. We found that by 2018–19, success could be achieved in multiple projects in phase 2 trials and this could encourage greater investment in the sector. New projects starting in 2018–19 might reach registration by 2030.

Our group found that alternatives to antibiotics have the potential to deliver clinical benefit, but the scale of current activity and availability of funding will need to increase substantially to achieve that benefit.

What will the portfolio cost?

Named projects were budgeted to 2025 using industry standard costs for clinical development phases to estimate funding needs (table 2). Although some organisations might aim to deliver with smaller budgets, standard costs are based on real projects, are more suggestive of reality, and remove bias.

The funds for the current phase of the project are assumed to be in place and confirmed. The risk-adjusted funds needed for registration were calculated by addition of the cost of subsequent stages of each project before registration and use of risk estimates at each stage of development.

Additional funding is needed to strengthen the portfolio of approaches for which the portfolio is too small or early in development to expect success. A key objective should be to test alternatives to antibiotics at phase 2 to validate the approach. To adequately understand the clinical potential of an approach, it might be necessary to progress several different projects using one approach into phase 2. Lysin, bacteriophage, and antimicrobial peptide approaches have projects that are advancing, but adequate testing of each approach cannot take place because too few projects exist. These approaches will need additional investment to increase capacity and translational expertise to exploit their full potential.

To plan for project attrition, a pipeline to support the assessment of a single project at phase 2 would need nine preclinical projects at a cost of £12·5 million per project over 5 years, which would lead to two phase 1 and potentially one phase 2 study with a total budget of £135 million. Any funding should be dependent on the results being peer reviewed and accessible via open access publications to provide the necessary evidence base to inform future research and development.

The host defence and antibiofilm peptide approaches are appealing because of their broad spectrum potential. It might be necessary to advance the first wave of these innovative projects beyond phase 2 to validate the approaches and to convince pharmaceutical companies, investors, and clinicians. A large investment of £604 million would be needed to develop a pipeline of host defence peptide and antibiofilm peptide projects because they are in an early stage of development at present. An estimated 34 preclinical projects are needed to provide eight phase 1 studies and four phase 2 studies to get at least one project to phase 3 studies and product registration (table 3). There are several natural and synthetic host defence peptides and antibiofilm peptides that could be potential starting points. Chemical modifications, hybrid peptides, and chemical mimetics could be explored.[133,134] Project creation and translational research in this research area could be accelerated by committing £85 million per year for 5 years. This

	Target	Product name, reference	Phase as of January–March, 2015	Earliest anticipated registration	Probability of registration by 2025	Risk-adjusted cost of projects; current phases, subsequent phases (£ million)	Pipeline investment needed for additional phase 2 validation (£ million)
Antibodies							
Merck	*Clostridium difficile*	Bezlotoxumab[117,118]	Phase 3 ongoing	2017	..	..	..
MedImmune	*Staphylococcus aureus*	MEDI4893[6,118]	Phase 2 ongoing	2021	..	..	..
Aridis	*Pseudomonas aeruginosa*	AR-101[119]	Phase 2a complete	2021	..	..	..
Aridis	*S aureus*	AR-301[119]	Phase 2a ready	2022	..	..	..
MedImmune	*P aeruginosa*	MEDI3902[9]	Phase 1 ongoing	2023	..	..	..
XBiotech	*S aureus*	514G3[13]	Phase 1 ongoing	2023	..	..	..
Aridis	*P aeruginosa*	Aerucin[10]	IND ready	2025	..	..	..
Combined	..	..	..	..	183%	60, 120	..
Probiotics							
Seres	*C difficile*	SER-109[120]	Phase 3 ready	2018	..	..	..
Rebiotix	*C difficile*	RBX2660[121]	Phase 2 ongoing	2019	..	..	..
Shire (Viropharma)	*C difficile*	VP20621[122]	Phase 2 ready	2022	..	..	..
Combined	..	..	..	..	124%	52, 53	..
Lysins							
Intron Biotechnology	*S aureus*	SAL200[27]	Phase 1 ongoing	2022	..	..	..
ContraFect	*S aureus*	CF-301[19]	Phase 1 ongoing	2022	..	..	..
Combined	..	..	..	..	26%	12, 28	135
Bacteriophages							
Wild-type bacteriophages							
AmpliPhi	*C difficile*	AmpliPhage-004[123]	Pre-phase 1	2023	..	..	..
AmpliPhi	*P aeruginosa*	AmpliPhage-001[123]	Pre-phase 1	2023	..	..	..
Engineered bacteriophages							
Phico Therapeutics	*P aeruginosa*	PT-3.1[34]	Pre-phase 1	2023	..	..	..
Combined	..	..	..	..	9%	13, 57	135
Immune stimulation							
Akthelia	*C difficile*	Phenylbutyrate/vitamin D[38,40]	Phase 2 ready	2021			
Various	Various	Bacterial extracts[43]	Phase 1 ready	2022			
Combined	..	..	..	..	43%	0, 55	..
Vaccines							
Sanofi Pasteur	*C difficile*	*C difficile* toxoid vaccine[124]	Phase 3	2019	..	..	..
Valneva	*P aeruginosa*	IC43[125,126]	Phase 2 and Phase 3 ongoing	2019	..	..	..
Valneva	*C difficile*	IC84[126]	Phase 2 ongoing	2021	..	..	..
Pfizer	*S aureus*	SA4Ag[127]	Phase 2 ready	2021	..	..	..
Combined	..	..	..	..	188%	74, 66	..

(Table 2 continues on next page)

	Target	Product name, reference	Phase as of January–March, 2015	Earliest anticipated registration	Probability of registration by 2025	Risk-adjusted cost of projects; current phases, subsequent phases (£ million)	Pipeline investment needed for additional phase 2 validation (£ million)
(Continued from previous page)							
Antimicrobial peptides							
Roche	*P aeruginosa*	POL7080[128]	Phase 2 ongoing	2022	..	..	..
Novacta Biosystems	*C difficile*	NVB302[129]	Phase 1 ongoing	2022	..	..	..
Adenium	*S aureus*	AP-138[64]	Pre-phase 1	2023	..	..	..
Adenium	Urinary tract infection	AP-139[64]	Pre-phase 1	2023	..	..	..
Adenium	*C difficile*	AP-114[64]	Pre-phase 1	2023	..	..	..
Combined	..	..	..	..	52%	16, 104	135
Other peptides							
Various	Gram-negative and Gram-positive	..	Preclinical	2027	..	..	604*

The alternatives-to-antibiotics portfolio contains 25 projects representing eight alternative approaches from preclinical to phase 3 studies at 18 companies from six countries. The probability of registration for a single project is estimated by multiplying the probabilities of success through each stage of development from its current position to successful registration. The probability of registration of a product from an approach is estimated by adding the individual project probabilities. An approach with a probability of registration greater than or equal to 100% suggests that if adequate funding and expertise is applied across the projects, then successful registration is anticipated from that category. The risk-adjusted cost of each project was estimated by applying standard project risks at each stage to the standard costs for each phase of development. The risk-adjusted cost for the approach was estimated by summing the individual project risk-adjusted costs. The cost per approach assumes that companies have the funds for the current phase of the project, but have to justify the need for additional funds for further product development. The pipeline investment is an estimate of the funding needed to enable new project activity to expand those approaches with insufficient activity to adequately test the concept of the approach at phase 2. The current phase of the projects, and if successful, the earliest date that registration can be anticipated using standard metrics is shown. The group note that funding of roughly £160 million into these projects has been secured during the past 12 months.[13,26,120,123] IND=Investigational New Drug application. *To progress at least one project to registration (table 3).

Table 2: **Alternatives to antibiotics portfolio review as of January–March, 2015**

investment would provide a powerful incentive to build capacity and progress towards clinical validation of these peptide-based approaches.

Our analysis assumes that funding of £227 million for named projects is available to complete their progression through their current project phase. Additional risk-adjusted funding of £48 million will be needed for subsequent phases and should support development of one new product for *P aeruginosa*, *C difficile*, and *S aureus* by 2022. This investment would enable validation of antibodies, probiotics, and novel vaccines as alternatives to antibiotics.

The lysin, bacteriophage, and antimicrobial peptide portfolios should be expanded to adequately test these approaches in a timely manner. This could be achieved with risk-adjusted investment of £405 million. Building an adequate host defence peptide and antibiofilm peptide portfolio will need £604 million. We therefore estimated that £1·5 billion of risk-adjusted funding is needed to validate and investigate the ten high priority alternatives-to-antibiotics approaches in a timely manner. We did not calculate the funding needed for the nine additional approaches or for additional blue-sky activity to add to the pipeline in the future.

	Preclinical	Phase 1	Phase 2	Phase 3	Registration	Total
Stage probability of success	23%	45%	47%	71%	90%	..
Number of projects	34	8	4	2	1	..
Cost of phase (£ million)	12·5	6	10	45	1·3	..
Portfolio cost (£ million)	425	48	40	90	1·3	604

The calculation used to estimate the costs of funding a relatively new alternative approach to provide sufficient number of preclinical projects to survive standard rates of attrition and to have a reasonable chance of product registration is shown.

Table 3: **Estimate of the project pipeline cost for host defence and antibiofilm peptides**

Challenges of development and use of alternatives to antibiotics

The innovators in this space (largely academics and biotechnology companies) often do not have industry-level development and clinical skills. Increased funding should, therefore, be partnered with investment in translational skills development. Alternatives-to-antibiotics programmes could benefit from greater access to expertise in pharmacokinetics and pharmacodynamics, formulation, toxicology, and manufacturing. Provision of adequate funding for multidisciplinary teams and costs associated with the preclinical characterisation and delivery of competitive lead candidates for clinical development will be a crucial factor for success. Precompetitive partnerships and the creation of development hubs might be one way to support this area. Calls for tender and purchase of research and development activities from contract research organisations and pharmaceutical companies on behalf of the academic and biotechnology small and medium-sized enterprise community is another way to support innovation. Such activities might encourage the pharmaceutical industry to become involved in a

Search strategy and selection criteria

The Review benefited from expert summaries and non-confidential information on approaches and projects provided by its members and comprehensive scientific literature and database searching, which was used to identify approaches, projects, companies, and publications to inform the group. All projects in progress identified that were within scope were included in the portfolio review. Historic projects informed the review, but were not included in the portfolio analysis. Preclinical and clinical projects were identified through a series of searches of PubMed, the internet using Google, and the ClinicalTrials.gov database up to Feb 27, 2015, by use of key terms such as "antibody", "probiotic", "lysin", "bacteriophage", "vaccines", "antimicrobial peptide", "lantibiotic", "host defense peptide", "innate defense peptide", "antibiofilm peptide", "immunomodulation", "immune stimulation", "immune suppression", "vaccine", "liposome", "chelation" and, if necessary, their use with "*E coli* OR *P aeruginosa* OR *K pneumoniae* OR *A baumannii* OR *C difficile* OR *S aureus* OR infection OR bacteria" before review of the papers and top 30 websites listed. Once proteins or compounds (table 1 and panel 1) and the organisation developing them had been identified, their names were used for additional searches—eg, "Merck", "MedImmune", "Aridis", "Seres", "Rebiotix", "Shire", "Viropharma", "Intron Biotechnology", "ContraFect", "Ampliphi", "Phico", "Akthelia", "Sanofi Pasteur", "Valneva", "Pfizer", "Roche", "Novacta", "Adenium", and the associated company website overview, pipeline, and news pages. The state of alternative project pharmacology was assessed by PubMed searches for articles published up to Feb 27, 2015, using the terms "pharmacokinetic OR safety" with "human OR mouse OR rat" in combination with "host defense peptide"; "antibiofilm peptide"; "lantibiotic"; "bacteriophage"; "lysin" and in the case of antimicrobial peptides "antimicrobial peptide" with "pharmacokinetic OR safety" and "*E coli* OR *P aeruginosa* OR *C difficile* OR *S aureus*" before review of the 238 papers listed. We also reviewed studies cited in articles identified by this search and included them when relevant. The primary focus of the review was on non-compound approaches that target bacteria and any approaches that target the host to provide alternatives to antibiotics and to address antibiotic resistance. Projects using compounds to directly target bacteria were excluded. Therefore, compound-based approaches targeting efflux pumps, regulators of transcription, and antibiotic resistance breakers were excluded from this Review. The searches were rerun on Oct 14, 2015. Scientific literature about potential modulators of innate immunity was identified by PubMed search using the terms "TLR2, TLR4, NLRP3, AIM2, C5-cleavage" each in combination with "*E coli* OR *P aeruginosa* OR *K pneumoniae* OR *A baumanii* OR *C difficile* OR *S aureus* OR infection OR bacteria". The titles of the first 500 papers for each search were inspected for relevance and selected papers reviewed in detail. Additionally, searches were refined by adding keywords "agonist OR inhibitor OR monoclonal OR polyclonal OR knockout" and the first 200 paper titles reviewed for relevance. Further searches including the list of bacteria with "innate immunity antibiotic resistance" with or without the keywords "TLR2, TLR4, NLRP3, AIM2, C5-cleavage" were done and the first 200 paper titles reviewed for relevance. Group members also suggested key relevant references on modulating innate immunity.

manner that develops both critical mass (ie, the minimum amount of resources needed for projects across the portfolio) and sustainability.

Careful clinical trial design will be essential. Projects need to ensure that endpoints are relevant to both the patient and the physician, which are often but not necessarily exclusively based on endpoints grounded in how patients feel, function, and survive. Unless the clinical signal is strong, there is a risk that the size and cost of clinical trials needed to show an incremental benefit will be too large to support. Thus, developers must be willing to terminate projects if it becomes clear that the product either has a low chance of success or will not have a big impact, which could have been one reason for the cessation of previous peptide trials. As with trials of new antibiotics, surrogate endpoints that are predictive of clinical efficacy should be included as secondary endpoints (eg, changes in cytokine levels or changes in imaging of infections), but are unlikely to be acceptable as the basis for registration of drugs for life-threatening infections.

In addition to adequate funding and expertise, development and deployment of alternative antibacterial medicines is dependent on a return on investment; therefore economic models for this therapy area should be improved. We did not consider the economics of alternatives to antibiotics, but noted that replacing antibiotics will be a major challenge. Many of the alternatives-to-antibiotics approaches are specific to pathogens or strains of pathogens. By comparison, most modern antibiotics have a broad spectrum of activity. For example, the approved combination of ceftolozane and tazobactam, for complicated intra-abdominal infections, complicated urinary tract infections, and pyelonephritis, has clinical efficacy data for ten pathogens including *Klebsiella* species, *Escherichia coli*, and *P aeruginosa*, with clinical microbiology data suggesting potential efficacy against 20 other pathogens.[135,136] Multiple alternative therapies would be needed to provide a similar spectrum of coverage. In the first instance, alternatives to antibiotics are likely to focus on the most prevalent infections and might provide sufficient clinical benefit to ensure a return on investment. At best, they will be a partial replacement for antibiotics.

The future role of innovative diagnostics, their use in combination with innovative targeted therapies, and the likelihood and timescale of their delivery and costs were not within the scope of this Review. However, we recognise that for therapies that target single species, these diagnostics will be crucial for widespread clinical use and patient benefit and their introduction into clinical practice would support improved antibiotic stewardship.[137]

Innovative therapies might need innovative regulation.[116] Bacteriophage therapies in development are an example of products that drive innovative regulatory approaches. Broad conversations about options for the unique challenges of each alternative are needed. A workshop on the therapeutic use of bacteriophages hosted by the European Medicines Agency (June 8, 2015)[138] is one example of how this work should progress.

Some alternatives to antibiotics could be delivered by methods different to those used for traditional antibiotics. Instead of a single global manufacturing pipeline, the development of localised services similar to blood transfusion or stem-cell harvesting and transplantation could benefit patients and should be considered. Production of bacteriophage therapeutics at the point of care is an example of a model that might be appropriate for some products.

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All of the alternatives to antibiotics have potential uses in animal health and evidence of efficacy in companion and agricultural animals could be important in de-risking an approach before clinical development in human beings. The anticipated costs for many of the approaches could, however, be prohibitive for animal use. Commitment to substantial subsidies might be needed to incentivise development of alternatives to antibiotics for animal health, in which their use could contribute to reduction in antibiotic use. Epibiome is an example of a company targeting animal health with bacteriophages before human use, but their programmes are too early in development to be included in this Review.[139]

At least initially, many of the alternatives to antibiotics will be trialled and used as adjuncts to antibiotics because their activities might not provide sufficient therapeutic benefit as a single therapy. As long as effective antibiotics are still available, superiority over standard of care when comparing an antibiotic with an antibiotic and an alternative-to-antibiotics adjunct treatment might prove difficult to confirm. If a patient develops resistance to the antibiotic, then its use in combination therapies will be compromised. Alternatives to antibiotics that are primarily adjunctive therapies might have a narrow window of opportunity in which to show benefit. In the longer term, combinations of alternatives-to-antibiotics therapies could possibly be used without antibiotics.

We expect that use of alternatives to antibiotics will be reliant on improved and faster diagnostic technology to enable targeting of individual bacterial species, or even strains of species, rather than clinical indications; be used for prophylaxis more often than for treatment; need to be used with other products to replace a single antibiotic; have substantially higher developmental costs than traditional antibiotics; and need access to sufficient and sustained funding to enable timely research and development and prompt clinical assessment.

Future outlook

The objective of this Review was to find out which alternatives to antibiotics are most likely to deliver new therapies of clinical use. Our group found that academic researchers and the pharmaceutical industry have successfully generated a diverse portfolio of potential alternatives-to-antibiotics projects from preclinical optimisation to phase 3 studies and prioritised ten approaches for more detailed review. Results from studies of these approaches are still emerging and these approaches hold promise provided that adequate funding is available for researchers to build capacity and create a preclinical evidence base to enable prioritisation and progress of optimised drugs to crucial phase 2 validation.

Little doubt exists that research activity might deliver new drugs for *P aeruginosa*, *S aureus*, and *C difficile* infections. However, other than probiotics for *C difficile* infection, this first wave of new approaches will probably best serve as adjunctive or preventive therapies. Therefore, traditional antibiotics will still be needed.

If we have to depend on alternatives to antibiotics in the future, we need to build capacity and substantially increase the number of projects now.[140] We estimated that the priority alternatives-to-antibiotics approaches alone need an investment of at least £1·5 billion, committed in the next 5 years and spent within 10 years, to initiate a pipeline of translational projects that would develop new therapies. An investment of this scale will provide a better understanding of which approaches are most likely to be successfully registered and those that are not. Additional investment is needed to bring products to market and into clinical use. Longer term substantial and sustainable funding will be needed to advance and make use of the wider alternatives-to-antibiotics portfolio. Policy and funding should now be linked. Without sufficient funding we can assume that new treatments to replace or supplement antibiotics will not be available, and the consequences of such a prolonged delay for global health-care systems needs to be considered now. Our analysis of just a subset of all activity that could contribute towards the fight against antimicrobial resistance suggests that funding is now the key limiting factor that is stalling a global response. Antimicrobial resistance has to become a major international science programme to provide the solutions needed now by society. By comparison, the Large Hadron Collider project cost about £6 billion and the International Space Station cost £96 billion.[141,142] Antimicrobial research and development to address the problem of antibiotic resistance probably needs an investment that is somewhere between the two.

Contributors

LC formed the alternatives-to-antibiotics group and defined the focus of the Review. LC and JHR co-chaired the group and prepared the first draft of the Review. All members contributed to provision of scientific literature searching, analysis, and interpretation of findings, especially in their areas of expertise. A subgroup (LC, BFG, IRH, BVJ, AK, SO, SS, TJT, and JHR) edited the draft of the Review before wider input from the group. LC and JHR finalised the manuscript taking the editorial suggestions of the group into account. LC had final responsibility for the content of the manuscript and for submission.

Declaration of interests

Given the aims of this review process, many of the contributors were selected because they had (and will continue to develop) conflicts of interest with respect to companies and products mentioned in this report. Connections to related companies based on employment at the time the report was prepared are as noted in the summary of authors. LC is a Director of Chemical Biology Ventures and Abgentis, and an employee of Persica Pharmaceuticals. MC consults and collaborated with AmpliPhi Biosciences Corporation. MD is a director and shareholder of Novacta Biosystems and a director of Cantab Anti-infectives. HF is a director and shareholder of Phico Therapeutics. VAF consults and is a shareholder of ContraFect. SF is a director, shareholder, and consultant for Absynth Biologics. REWH has licensed projects to Elanco, is on the SAB of Adenium Pharmaceuticals, and is the founder and major shareholder of ABT Innovations. DH is a former employee and a current shareholder of AmpliPhi Biosciences Corporation. KH is a director of TiKa Diagnostics. BVJ is on the scientific advisory board of Hutman Diagnostics. DK is chairman of

Absynth Biologics and Procarta Biosystems. SO is a former shareholder of Akthelia. DP is an employee and shareholder of GlaxoSmithKline. SP is an employee of MedImmune and a shareholder of AstraZeneca. SS is a founder and shareholder of Abzenza. JS is a non-executive director of Auspherix. CMT is a director of Plasgene. PW is an employee of Evotec a contract research organisation that performs work with alternatives-to-antibiotics companies. JHR is an employee and shareholder of AstraZeneca, a consultant for F2G, and a consultant for Advent Life Sciences (an investor in F2G). Of note, AstraZeneca owns MedImmune, but SP manages the MedImmune products mentioned in table 2 and JHR was not otherwise connected with them at the time of this Review. JHR accepted an offer to become a non-executive director of Adenium Biotech ApS after the Review had been accepted for publication. LC and JHR received remuneration from the Wellcome Trust and the Department of Health for chairing the group and managing the preparation of the report and this Review. RB, BFG, IRH, AK, and TJT declare no competing interests.

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