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Review

Exploring the future of infectious disease treatment in a post-antibiotic era: A comparative review of alternative therapeutics

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ABSTRACT

Antibiotic resistance is projected to be one of the greatest healthcare challenges of the 21st century. As the efficacy of these critical drugs wanes and the discovery of new antibiotics stagnates, exploration of alternative therapies could offer a much needed solution. Although numerous alternative therapies are currently under investigation, three in particular appear poised for long-term success, namely antimicrobial oligonucleotides, monoclonal antibodies and phage therapy. Antimicrobial oligonucleotides could conceivably offer the greatest spectrum of activity while having the lowest chance of unrecoverable resistance. Bacteriophages, while most susceptible to resistance, are inexhaustible, inexpensive and exceptionally adept at eliminating biofilm-associated infections. And although monoclonal antibodies may have limited access to such recalcitrant bacteria, these agents are uniquely able to neutralise exotoxins and other diffusible virulence factors. This comparative review seeks to illuminate these promising therapies and to encourage the scientific and financial support necessary to usher in the next generation of infectious disease treatment.

Contents

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1. Introduction

Throughout history, microbial pathogens have persisted as one of humanity's most formidable adversaries. In 1900, most Americans lived only into their forties, with infectious diseases such as cholera, typhoid fever, plague and tuberculosis causing the majority of deaths [1]. The advent of antibiotics in the mid-20th century led to a drastic decline in associated morbidity and mortality [2]. For decades, antibiotics proved remarkably effective; so effective, in fact, that development and employment of alternative therapies virtually ceased following the antibiotic revolution in Western medicine [3,4]. But just as quickly as new antibiotics arrived, bacteria resistant to those antibiotics emerged. Partly, this is because antibiotics are predominately derived from nature and, as such, have been around for millennia. For as long as microbes have developed antibiotics to protect themselves from competitors, those competitors have developed resistance mechanisms to render those antibiotics ineffective. Consequently, antibiotic resistance is already widespread in nature, even to those antibiotics not yet invented [5]. Owing to horizontal gene transfer, this resistance can be easily passed between organisms via transformation, conjugation or transduction [6]. Furthermore, and perhaps most daunting, bacteria exhibit short generation times and high mutation rates enabling rapid evolution. This swift adaptability fosters a distinct capacity to withstand virtually any assault.

The function of antibiotics is to directly inhibit the growth or viability of bacteria by targeting conserved pathways such as cell wall synthesis, DNA replication, protein synthesis and metabolism [7]. This scorched earth approach is what enabled the widespread success of antibiotics for several decades and, paradoxically, what is currently leading to their demise. The trouble with antibiotics is two-fold. Because antibiotics aim to kill or inhibit the growth of organisms, this introduces a strong selective pressure to become resistant. In response, bacteria have evolved a myriad of defence mechanisms, including decreased outer membrane permeability, efflux pumps, modification of the antibiotic target, and secretion of enzymes capable of degrading the antibiotic [6]. Additionally, because of their broad-spectrum activity, antibiotics can obliterate the protective normal microbial flora of patients. This resident microflora is not only essential for development and maturation of the immune system but also competes with pathogenic organisms for space and nutrients; without it, patients are left susceptible to secondary infections by opportunistic pathogens [8]. As an example, *Clostridioides* (formerly *Clostridium*) *difficile* infection typically results from colonisation of the gastrointestinal tract following antibiotic use for an unrelated infection. This potentially fatal infection is responsible for approximately 250 000 hospitalisations in the USA alone and 14 000 deaths per year [9].

Issues surrounding antibiotic use are becoming increasingly recognised. In fact, antibiotic resistance has been declared one of the greatest global health challenges of modern time [10]. Over 2.8 million antibiotic-resistant infections occur every year in the USA with over 35 000 resultant deaths [10]. As the prevalence of resistance continues to rise, life-saving surgeries, organ transplants, chemotherapy and other medical interventions that rely on prophylactic antibiotics will become progressively more dangerous [2]. A report commissioned by the UK estimated that by the year 2050, approximately 10 million people will succumb to antibiotic-resistant infections annually, surpassing the number of deaths currently caused from cancer each year [11].

Unfortunately, the number of antibiotic agents in clinical development is woefully insufficient to keep pace with escalating antibiotic resistance [12]. Despite a considerable push to develop novel antibiotics, only a limited number of new drugs are currently in the pipeline. Compounding this problem is the fact that many pharmaceutical companies are becoming reluctant to pursue the development of antibiotics. In general, chronically-administered medications are far more profitable than antimicrobials, which are meant to cure the disease for which they are prescribed [13]. The current climate is particularly concerning for the pharmaceutical industry as escalating resistance may prohibit companies from recouping their investments [14]. As a result, many of the largest pharmaceutical companies have abandoned their antibiotic programmes [2].

The impending challenge of antibiotic resistance necessitates bold and innovative solutions. The existing literature primarily emphasises the need for novel antibiotics as well as strategies to prolong the life of current antibiotics such as enhanced infection control practices, antibiotic stewardship, and reduction or elimination of antibiotic use in agriculture. While these strategies are undoubtedly important, they are also insufficient as they do not address a future in which antibiotics are no longer effective. Researchers have begun exploring a wide array of alternative therapeutic strategies that offer a more targeted approach to treatment with less susceptibility to resistance. In particular, antivirulence strategies that aim to attenuate pathogens without impacting viability may offer a more sustainable solution; drug resistance to these therapeutic agents would not confer any survival advantage, thus eliminating any selective pressure. Despite advances in this field, the use of alternative therapies as a potential solution to antibiotic resistance is not widely discussed in the literature. This lack of attention could lead to a subsequent lack of scientific and financial support for this vital research. The present work aims to offer a fresh perspective by presenting a comparative review of alternative therapies currently in research and development. Three promising platforms were chosen based upon their ostensible advantages and clear distinction from traditional antibiotics, namely antimicrobial oligonucleotides, monoclonal antibodies and phage therapy. Through an evaluation of efficacy, sustainability (susceptibility to resistance), clinical considerations (safety and pharmacology) and economic considerations, this review will assess the potential of these alternative therapeutics as novel additions to our antimicrobial arsenal.

2. Antimicrobial oligonucleotides

2.1. Mechanism of action

Short synthetic nucleic acid sequences known as oligonucleotides may be used therapeutically to silence the expression of deleterious genes. While several oligonucleotide-based platforms exist, the two primary approaches for modulating gene expression include antisense oligonucleotides (ASOs) and short interfering RNAs (siRNAs). Both ASOs and siRNAs possess complementary sequences to their target mRNA. Hybridisation of the oligonucleotide

inhibits translation of the corresponding gene via degradation of the target mRNA or obstruction of ribosomal binding. Alternatively, ASOs that target sequences within pre-mRNA can modulate alternative splicing to induce production of favourable isoforms [15–17]. In the context of infectious disease treatment, antimicrobial oligonucleotides can be used to knock down essential genes required for survival or non-essential genes required for virulence. Targeting virulence genes is an approach that renders a pathogen less capable of successful infection while reducing the risk of resistance. A similar approach involves silencing antibiotic resistance genes to restore susceptibility to a co-administered antibiotic [18,19].

Delivery of these oligonucleotides presents the greatest challenge to their therapeutic success [20]. Chemical modifications are necessary to prevent degradation by serum nucleases, to avoid clearance by the kidneys and to facilitate cellular uptake [15]. To reach their intracellular targets, antimicrobial oligonucleotides must cross the bacterial cell wall. High-molecular-weight oligomers are only capable of reaching the cytoplasm when conjugated to another compound that can permeate the cell wall, such as cell-penetrating peptides. Delivery of oligonucleotides poses an even greater challenge when considering intracellular bacteria because, in this case, both host and pathogen membranes must be traversed. Some of the most popular chemical modifications designed for ASOs include first-generation phosphorothioates and third-generation locked nucleic acids (LNAs), peptide nucleic acids (PNAs) and phosphorodiamidate morpholino oligomers (PMOs) [18,20]. In the case of siRNAs, delivery technologies typically involve complexing the RNA with cationic and neutral lipids [15].

2.2. Efficacy

Oligonucleotide therapies have the potential to treat a wide variety of diseases. At present there are therapeutic candidates ranging in application from genetic disorders, metabolic disorders, oncology and infectious diseases [21]. From an infectious disease standpoint, oligomers could potentially target any pathogen whether it be bacterial, viral, fungal or parasitic in nature as long as the base sequence of the target gene is known [18]. While there are currently no US Food and Drug Administration (FDA)-approved oligonucleotide drugs on the market for the treatment of infections, there are a handful of antisense therapies in clinical trials for the treatment of viral infections. Oligonucleotides for the treatment of bacterial infections have yet to reach clinical trials; however, there is a promising collection of in vivo and in vitro research making headway in this area [22] (Table 1). Although this therapeutic class is only in its infancy and considerable work is still required, results thus far unequivocally highlight the immense potential of oligonucleotide-based therapies.

2.3. Sustainability

Antimicrobial oligonucleotides are not susceptible to traditional antibiotic resistance mechanisms [18]. Any acquired resistance obtained via mutation of the target sequence can be readily overcome by redesigning the antisense molecule. Adjusting the base sequence can be accomplished rapidly and has no impact on the pharmacology of the drug [20,32]. Furthermore, oligonucleotides are several times larger than their small-molecule antibiotic counterparts; while this makes cellular uptake inherently more challenging, oligonucleotides have demonstrated greater cellular retention. In comparison with some antibiotics, which may be rapidly expelled from bacteria via efflux pumps, oligonucleotides are retained for several hours leading to enhanced accumulation and a long post-antibiotic effect [8,33].

Perhaps the most probable mechanism of resistance to contend with is modification of the entry mechanism. Experiments for induced resistance against PMOs and PNAs have resulted in mutations of sbmA, the inner membrane transporter of these ASOs

Table 1
Efficacy of therapeutic antimicrobial oligonucleotides in various stages of research and development.

Oligonucleotide	Indication	Mechanism of action	Stage	Results of efficacy studies
Fomivirsen (phosphorothioate ASO)	Cytomegalovirus (CMV) retinitis	Silences the expression of CMV immediate-early 2 protein required for viral replication	FDA-approved but no longer on the market as of 2017	Highly efficacious in treating CMV retinitis but treatment was obsolete following the development of high-activity antiretroviral therapy [23]
Miravirsen (LNA ASO)	Hepatitis C virus (HCV) infection	Silences liver-expressed miRNA-122 required for viral stability and propagation	Phase 2a clinical trials as of 2013	Treatment of patients with chronic HCV infection resulted in a statistically significant dose-dependent reduction in HCV-RNA levels [24]
AVI-6002 (PMO ASO)	Ebola virus post-exposure prophylaxis	Silences mRNA sequences encoding viral proteins	Phase 1 clinical trials as of 2014	Studies in mouse, guinea pig and non-human primate lethal challenge models resulted in high levels of survival in the treatment groups [25]
FtsZ ASO (peptide-conjugated LNA)	Bacterial infection	Silences *ftsZ* gene required for bacterial cell division and viability	In vivo experiments as of 2015	In a murine model of sepsis with MRSA, treatment with 3 mg/kg of FtsZ ASO increased survival by 60% [26]
MexB siRNA	*Pseudomonas aeruginosa* infection	Silences *mexB* gene encoding a component of the efflux pump that expels many antibiotics	In vivo experiments as of 2014	In a murine model of *P. aeruginosa* lung infection, treatment with siRNA rescued meropenem activity to significantly reduce bacterial load [27]
NDM-1 ASO (peptide-conjugated PMO)	Gram-negative bacterial infection	Silences bla_{NDM-1} gene encoding a carbapenemase conferring resistance to β-lactam antibiotics	In vivo experiments as of 2017	In a murine model of sepsis with NDM-1-positive *Escherichia coli*, treatment with the ASO restored meropenem activity and increased survival by 75% [19]
Coagulase siRNA	*Staphylococcus aureus* infection	Silences expression of coagulase, a virulence factor protecting the bacterium from phagocytosis	In vivo experiments as of 2006	In a murine model of pulmonary infection with MRSA, treatment with siRNA significantly reduced the number of viable organisms [28]
CTX-M-15 ASO (lipid-conjugated phosphorothioate)	Gram-negative bacterial infection	Silences $bla_{CTX-M-15}$ gene encoding resistance to third-generation cephalosporins	In vitro experiments as of 2020	An *E. coli* strain positive for $bla_{CTX-M-15}$ subjected to 5 μM of the ASO yielded a 25-fold decrease in the MIC of ceftriaxone [29]
RNA analogue 2′-OMe PGO	*Mycobacterium tuberculosis* infection	Silences the *ald* gene that likely plays a role in cell wall synthesis	In vitro experiments as of 2019	Treatment with 2′-OMe PGO led to a statistically significant growth reduction of the intracellular model organism *Mycobacterium smegmatis* [30]
2′-*O*-methyl phosphorothioate ASO with carrier	*Clostridioides* (*Clostridium*) *difficile* infection	Silences the *dnaE* gene encoding a DNA polymerase important for DNA replication	In vitro experiments as of 2018	Exposure of *C. difficile* to the ASO complex led to a significant reduction in MIC with no deleterious effect on common intestinal flora [31]

ASO, antisense oligonucleotide; FDA, US Food and Drug Administration; LNA, locked nucleic acid; PMO, phosphorodiamidate morpholino oligomer; MRSA, methicillin-resistant *Staphylococcus aureus*; siRNA, short interfering RNA; MIC, minimum inhibitory concentration; PGO, phosphoryl guanidine oligonucleotide.

[32]. Kotil and Jakobsson reason that because the path of evolution is dictated by the relative fitness of mutations, by increasing the relative advantage of rescuable mutations over mutations of the entry mechanism, the effectiveness of therapy could be prolonged. Thus, they proposed a therapeutic protocol aimed to manipulate bacterial evolution towards easily recoverable mutations of the antisense target. Using a computer simulation, they estimated that a thoughtfully designed oligonucleotide therapy could potentially remain effective in the order of centuries before succumbing to resistance [32].

2.4. Clinical considerations

Gene silencing therapies have the potential for faster and longer-lasting activity than protein inhibition by conventional therapies [17]. Additionally, antisense therapies can readily silence the expression of molecular targets that are inaccessible to existing small-molecule therapies [34]. Nevertheless, research and development of this therapeutic class is still in its infancy; the precise pharmacological profiles of antimicrobial oligonucleotides are yet to be determined. However, information may be extrapolated from comparable drugs since the behaviour of antisense oligonucleotides is similar among classes [20,35]. While efficacy and specificity are directly related to the base composition, the backbone chemistry is what determines an oligonucleotide's safety and pharmacokinetic profile [25].

Because of their high molecular weight, oligonucleotides are not easily absorbed into the bloodstream following oral administration. Thus, all antisense drugs currently available or undergoing clinical trials are intravenous or locally administered via subcutaneous or intrathecal injection [20,33]. Oligonucleotides have broad biodistribution into most tissues, although concentrations vary and are highest in the liver and kidney. For most oligonucleotides, size and charge prohibit their crossing of the blood–brain barrier; however, intrathecal injection into the cerebrospinal fluid allows distribution throughout the central nervous system [35].

Safety concerns with antisense therapies in clinical and preclinical phases include pro-inflammatory effects, kidney and liver toxicity, and thrombocytopenia [36]. It remains to be seen whether these safety concerns are relevant to oligonucleotide therapies for the treatment of infectious diseases. Clinical trial results for the antiviral oligonucleotides miravirsen and AVI-6002 (Table 1) were supportive of continued development. These therapies were determined to be safe and well tolerated at the doses studied, and treatment side effects were generally mild [24,25].

One general concern related to antisense therapies is the potential suppression of non-target mRNAs. However, because antimicrobial oligonucleotides are directed against sequences specific to microbial pathogens, the presence of human homologues is unlikely and interference with human gene expression should be minimal [16,19]. Furthermore, bioanalytic screening enables a rigorous vetting process in which molecules with greater chances of cross-reactivity are weeded out [36]. In comparison with traditional small-molecule therapies, gene silencing agents could actually have the most potential for a tailored spectrum of activity since the degeneracy of the genetic code creates greater variation at the genetic level than the phenotypic level [8]. Thus, the specificity of this therapeutic approach has the benefit of sparing beneficial commensal bacteria [18]. On the other hand, broad-spectrum antibiotics can be used empirically to treat urgent life-threating infections, whereas the success of antimicrobial oligonucleotide therapies will rely heavily on rapid and accurate diagnostic testing to identify the relevant pathogen [8].

2.5. Economic considerations

The so-called 'small white pill' has traditionally been the mainstay of the pharmaceutical industry. These low-molecular-weight medications are typically administered orally with once-daily dosing [34]. The introduction of oligonucleotide therapies would require a paradigm shift. Realising the potential of this novel therapeutic class will certainly require a large initial investment as researchers continue to ascertain the pharmacological properties of these agents and learn how to optimise them.

However, once the initial obstacles are overcome, this platform technology has the potential to be comparably more efficient and cost effective. In the case of small-molecule drugs that modulate the function of their protein targets, each individual agent has a unique pharmacological and toxicity profile that must be established prior to pre-clinical testing. Consequently, each new small-molecule drug requires a significant investment. Whereas with a platform technology, the same framework may be applied to multiple therapies and information obtained from the development of one drug may be used for the next. Because the pharmacokinetics and toxicities are very similar among oligonucleotide classes, there is no need to expend additional resources characterising these properties. Furthermore, commercial manufacturing may be less costly as the same facility could manufacture multiple drugs [34].

Finally, the amount of time associated with drug discovery and early development is anticipated to be markedly reduced. As long as the base sequence of the corresponding mRNA is known, potential targets can be easily identified using bioinformatics, and new antisense molecules can be rapidly designed and synthesised [18,32]. While identification and development of a small-molecule drug may take up to 6 years before it is equipped for clinical trials, oligonucleotide therapies may take as little as 1 year [34].

3. Monoclonal antibodies

3.1. Mechanism of action

Another promising platform technology yet to reach its prime is monoclonal antibody (mAb) therapy. mAbs have their roots in serum therapy, which was widely used prior to the antibiotic revolution. In the early 1900s, passive administration of animal sera was the primary means of treating numerous infections including diphtheria, tetanus, scarlet fever and pneumococcal pneumonia. Today's antibody therapies have advanced considerably with humanised and fully human therapeutic mAbs that offer a high degree of specificity and reduced toxicity [3].

Antibodies are high-molecular-weight glycoproteins, roughly ten times larger than oligonucleotides, and function solely extracellularly [33]. The pharmacodynamic action of antibodies depends on the nature of the target and its role in pathogenesis as well as the specific class of antibody [37]. Antibodies can directly target cell surface components such as proteins and polysaccharides or target diffusible virulence factors essential for pathogenesis such as exotoxins, signalling molecules and proteases [38,39]. When the Fab region of an antibody binds to a soluble antigen, it blocks the ability of that antigen to bind to its corresponding ligand, thus neutralising the antigen [40]. Neutralisation of virulence factors represents an indirect mechanism of suppressing pathogenic activity, which relies on a competent host immune system to subsequently clear the infection [39]. Direct mechanisms of action are typically mediated by the Fc region and result in the targeting of pathogens for destruction. Binding of the Fc region can induce antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity and antibody-dependent cellular phagocytosis [40].

While most antibodies synchronise their activity with other components of the immune system, there are occasional cases in which antibodies can be directly bactericidal. Binding of therapeutic bactericidal antibodies may trigger lysis of bacterial cells directly, circumventing the need for a competent host immune system [37]. An alternate approach is to target host factors rather than the pathogen itself. Host-directed therapies can augment the immune system and/or control excessive inflammation and prevent associated tissue damage. Interest in immunomodulatory mAbs has increased as this type of therapy could benefit treatment for a number of diseases [37,41].

3.2. Efficacy

More than 40 mAbs are currently approved for use by the FDA; they are an integral part of therapy for many conditions including several autoimmune and inflammatory disorders and numerous types of cancer [42,43]. As a treatment for infection, mAbs have the capacity to target a broad range of microbial pathogens including bacteria, viruses, parasites, fungi and any associated toxins [38]. All currently licensed antibacterial mAbs neutralise exotoxins, which are well suited to antibody therapy owing to their presence in the extracellular milieu. Such antibodies may be used therapeutically as adjuncts to antibiotics or prophylactically providing protection to high-risk groups [44].

Antibacterial antibody research has faced some failures in the recent past. Aridis halted development of its AR-105 mAb targeting *Pseudomonas aeruginosa* alginate for the adjunctive treatment of ventilator-associated pneumonia after phase II trial failure. Similarly, Arsanis ended production of its ASN-100 mAb against *Staphylococcus aureus* α-toxin that failed to prove efficacy in mechanically ventilated patients with *S. aureus* pneumonia [45].

Despite these setbacks, important lessons have been learned. First, simply using one antibody targeting a single epitope may be insufficient, as bacteria have hundreds of antigenic targets with presumed roles in virulence. Second, bacteria exhibit a wide variety of lifestyles (e.g. biofilm-associated, encapsulated, intracellular, etc.), which leads to variability in protein secretion and surface protein expression [46]. One of the greatest challenges to antibody therapy will be ensuring a sufficient protective effect against a broad range of bacterial serotypes and disease states [47]. Even within the same bacterial species, there is a limited number of surface antigens conserved across bacterial serotypes that can be targeted by mAbs [39]. Gram-negative organisms pose an even greater challenge because conserved surface antigens are often masked by highly variable polysaccharides [48].

The immense diversity among bacterial pathogens must be carefully considered in order to devise effective mAb therapies. One solution is the administration of multifunctional mAb cocktails [37]. Advancements in antibody engineering have also enabled the creation of bispecific antibodies that individually have two antigenic targets and allow researchers to adjust parameters such as size, half-life and stability. Furthermore, modifications to the Fc terminus of antibodies via adjustments in amino acid sequence or glycosylation pattern improve binding affinity to Fc receptors on effector cells [43]. As of September 2020, there are nearly 50 clinical trials involving mAbs for the treatment of bacterial infections and hundreds more for the treatment of viral

Table 2
Efficacy of monoclonal antibody therapies in various stages of research, development and approval.

Antibody	Indication	Mechanism of action	Stage	Results of efficacy studies
Bezlotoxumab	Adjunct therapy for *Clostridioides* (*Clostridium*) difficile infection	Protects against recurrent infection by neutralising enterotoxin B	FDA-approved as of 2017	*C. difficile*-positive patients receiving a combination of bezlotoxumab and antibiotics had a 38% lower re-infection rate compared with those receiving antibiotics alone [49]
Obiltoxaximab	Inhalational anthrax	Acts therapeutically in combination with antibiotics or prophylactically by neutralising the anthrax toxin	FDA-approved as of 2017	In a rabbit model of lethal inhalational anthrax, survival was markedly increased (90%) in the group given a combination of obiltoxaximab and antibiotic compared with antibiotics alone [50]
MEDI3902	*Pseudomonas aeruginosa* pneumonia	Binds the exopolysaccharide Psl as well as the PcrV antigen, a type III secretion system component	Phase II clinical trials as of 2019	Pre-clinical studies in murine models demonstrated that with an antibody dose of 1 mg/kg, 90% of mice survived a lethal challenge with *P. aeruginosa* [47,51]
MEDI4893	*Staphylococcus aureus* pneumonia	Binds to the α-toxin preventing it from adopting a lytic transmembrane conformation [52]	Phase II clinical trials as of 2019	Among mechanically ventilated ICU patients colonised with *S. aureus*, a single dose of antibody provided a 31.9% relative risk reduction for *S. aureus* pneumonia [53]
514G3	Adjunct therapy for *S. aureus* bacteraemia	Enhances opsonisation by binding to surface-expressed protein A	Phase II clinical trials as of 2018	In a pre-clinical murine model of MRSA bacteraemia, 60% of mice pre-treated with 514G3 survived the lethal infection compared with none of the mice in the control group [54]
DSTA4637S mAb–antibiotic conjugate	*S. aureus* bacteraemia	mAb portion opsonises *S. aureus*; phagocytosis releases antibiotic, which kills intracellular bacteria	Phase I clinical trials as of 2019	In a murine model of *S. aureus* bacteraemia, treatment with the mAb plus vancomycin led to significantly enhanced bactericidal activity compared with vancomycin alone [55]
Infliximab	*Helicobacter pylori* infection	Neutralises cytokine TNFα, which may reduce gastric tissue damage and neoplastic transformation	Clinical case report from 2001	A host-directed mAb typically used in Crohn's disease was administered to two patients positive for *H. pylori* infection, resulting in a significant reduction in gastrin levels [41,56]
A1102	*Klebsiella pneumoniae* ST258	Neutralises endotoxin by targeting the conserved LPS *O*-antigen D-galactan-III	In vivo experiment as of 2017	In a rabbit model of lethal *K. pneumoniae* bacteraemia, prophylactic immunisation with A1102 afforded a significant level of protection at a dose as low as 2 mg/kg [57]
mAb binding LPS antigen O25b	Multidrug-resistant *Escherichia coli* ST131-*H*30	Facilitates complement-mediated cytotoxicity, opsonisation and endotoxin neutralisation	In vivo experiment as of 2017	In a murine model of lethal *E. coli* ST131 bacteraemia, 90% of mice immunised with an antibody dose of ~0.5 mg/kg survived [48]
mAb binding BamA epitope	Proof-of-concept[a]	Directly bactericidal by targeting the essential β-barrel assembly machine (BAM) in *E. coli*	In vitro experiment as of 2018	Researchers demonstrated that antibody binding to an exposed BamA epitope inhibited its folding activity, compromising membrane integrity and killing bacteria [58]

FDA, US Food and Drug Administration; ICU, intensive care unit; MRSA, methicillin-resistant *Staphylococcus aureus*; mAb, monoclonal antibody; TNFα, tumour necrosis factor-alpha; LPS, lipopolysaccharide.

[a] Because LPS is known to prevent antibody binding to outer membrane proteins, the researchers used an *E. coli* strain with a truncated LPS allowing maximal access to cell surface epitopes. Thus, the present study does not directly translate to the clinic, as this LPS-deficient strain would not be typical amongst clinical isolates [58].

and other infectious diseases [22]. Table 2 showcases the potential scope of antibody therapy by providing examples of mAbs with diverse modes of action and indications.

3.3. Sustainability

Because the antibacterial mechanisms of antibodies are distinct from conventional small-molecule antibiotics, cross-resistance between the two therapeutic classes is unlikely to develop [37,39]. However, mAbs are subject to resistance by alternate means. The host immune system has applied selective pressure to bacteria for thousands of years, leading to the evolution of a variety of bacterial defence mechanisms that can similarly provide protection against therapeutic mAbs. For example, several bacterial pathogens including *Streptococcus pyogenes*, *S. aureus*, *P. aeruginosa*, *Streptococcus pneumoniae* and *Haemophilus influenzae* secrete antibody-cleaving proteases that separate the Fc and Fab termini. The resulting antibody fragments compete for binding of the antigenic target, thus impeding the action of functional intact antibodies [37]. Alternatively, some bacteria possess membrane-bound, antibody-neutralising proteins that bind the Fc region. This prevents effector functions such as opsonisation and complement activation. Examples include protein A of *S. aureus* and protein G of *Streptococcus*. Other mechanisms of immune evasion include intracellular life cycles, acquisition of capsules and formation of biofilms, which render bacterial cells comparatively inaccessible to the action of antibodies [37,39].

Similarly, antibodies can be rendered ineffective by the emergence of escape variants that do not retain the antibody binding site. Escape variants may arise as a result of either epitope masking or epitope switching. Epitope masking occurs when bacteria downregulate expression of a particular antigenic target. Epitope switching involves a drastic change in the amino acid composition of an antigenic target that reduces antibody affinity without impacting fitness or pathogenesis. This occurs via recombination with externally-acquired DNA or via mutation of the corresponding gene [59].

Despite the vast array of bacterial defence mechanisms, there are potential strategies to overcome this resistance. The high specificity of antibodies makes them particularly vulnerable to escape variants, but administration of mAb cocktails and bispecific antibodies could meet this challenge by widening the range of protection [38]. Therapeutic mAb development could be further improved upon with the assistance of 'omics' technologies and systems biology to identify optimal antigen targets [59]. But regardless of carefully chosen antigenic targets and improved antibody designs, targeting the bacterium directly will always prove a difficult task because of the continuous pressures imposed on bacteria to become resistant. Therefore, neutralisation of toxins and other diffusible virulence factors is perhaps the most rational and sustainable application of therapeutic antibodies since these antigenic targets are easily accessible, highly conserved and critical for pathogenesis [39]. Furthermore, selection for escape variants would be unlikely since mutations and/or downregulation of these targets would not confer any survival advantage [39,46].

3.4. Clinical considerations

Antibodies may be administered via intravenous, subcutaneous or intramuscular injection. Oral administration of antibodies is hindered by their high molecular weight, limited membrane permeability and susceptibility to gastrointestinal proteases [42]. The high potency and long half-life of antibodies offer the potential for infrequent dosing and long-term prophylaxis [37,40,57]. Prophylactic use of antibodies may provide the greatest benefit to patients based upon current studies of efficacy. Passive immunisation, unlike vaccines, offers immediate protection, which may be of particular importance in high-risk immunocompromised patients [59].

Therapeutic mAbs are generally well tolerated with no known drug interactions [47,60]. The most important safety concern is immunogenicity, which has been significantly reduced over the years with the transition to humanised and fully human antibodies. However, immunogenicity is not simply a function of percent homology; other factors such as route of administration, dose and duration of therapy can influence immunogenic potential [42,61]. Adverse events may range from local injection site reactions to potentially life-threatening anaphylaxis [40]. Recent studies of FDA-approved antibodies and those in clinical trials exhibited favourable safety profiles; side effects were generally mild or moderate in severity, although some instances of acute hypersensitivity reactions and generation of anti-drug antibodies did occur [49–51,53,62].

Like antimicrobial oligonucleotides, the high specificity of antibodies affords a low potential for off-target effects and minimal impact on the human microbiota [38,59]. It also means that rapid diagnostics are critical for therapeutic success. In addition, because antibodies are biological molecules with diverse and complex mechanisms of action that often work synergistically with the host's immune system, in vitro predictive testing will be more difficult compared with traditional antimicrobial susceptibility testing [63].

3.5. Economic considerations

Therapeutic mAbs represent another platform technology with the capacity for faster and more efficient drug discovery and development [17]. Manufacturing antibodies is a generic and well-established process, and due to their modular nature antibodies can be modified by genetic engineering with relative ease [44,63,64]. However, progress to commercial production is still a lengthy and technically complex process that may require at least 18 months to complete [43]. Unlike conventional antibiotics and oligonucleotides, mAbs are not chemically synthesised [17]; rather, they are produced batch-wise in living cells. Variability between batches is a legitimate concern, so tightly controlled environments during cell culturing, product processing and purification are essential [42].

Development of mAbs is expensive; pre-clinical development through market approval can cost approximately one billion dollars [43]. This leads to an increased cost for the consumer. Unquestionably, anti-infective mAbs will be more costly than conventional antibiotics and other alternative therapeutic agents [64]. The difference in cost may be especially jarring considering the price of antibiotics is currently quite low compared with other life-saving medications [65]. But with therapeutic options quickly dwindling, the public may need to reconsider what it is willing to pay for anti-infectives [60].

4. Phage therapy

4.1. Mechanism of action

Like antibody therapy, phage therapy has a long history that pre-dates the arrival of antibiotics [4]. Phages are viruses that exclusively infect bacteria. Exploiting the bactericidal action of these viruses offers a therapeutic approach with a high level of specificity. While certain phages are capable of infecting a range of bacterial genera, the majority of phages are specific to a particular species or strain. The host range is determined by the type of bacterial receptor [66]. Following recognition and attachment to the receptor, phages inject their genetic material into the host cell.

Lytic phages commandeer bacterial replication machinery to manufacture their own phage progeny within the bacterial cytosol. When a particular threshold of progeny is reached, phage-encoded lytic enzymes are activated to hydrolyse the peptidoglycan cell wall. This allows the newly synthesised phages to escape and reinitiate the lytic cycle. In this manner, lytic phages can act as self-amplifying therapeutic agents with highly targeted bactericidal activity [1,67].

Several approaches exist for the use of phages in therapy. Phages may be used singly or in combinations known as cocktails. Personalised phage cocktails are created by testing a particular bacterial isolate against a vast collection of phages, whereas fixed phage cocktails have a predetermined composition of lytic phages that target specific bacterial species [45]. For example, a fixed phage cocktail widely used in Eastern Europe known as Intestiphage is designed to target approximately 23 different enteric pathogens [6]. Phages have also been shown to act synergistically with antibiotics. Sublethal concentrations of antibiotics that inhibit cell division without causing cell death may increase the biosynthetic potential of bacteria leading to an increase in production of lytic phages. Such synergy has been demonstrated with β-lactams, quinolones and tetracyclines [6].

In addition, phages may be used as vehicles to deliver RNA-guided nucleases genetically engineered to target antibiotic resistance or virulence genes [68]. The archetype RNA-guided nuclease system is the clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) system. Engineered sequences encoding a type II CRISPR-Cas9 system can be inserted into the capsid of inert phages [69]. Following delivery into the host, CRISPR RNAs guide the Cas9 endonuclease to induce site-specific cleavage of target sequences. If the target sequence is chromosomal, nuclease activity is cytotoxic [68]. By simultaneously cleaving all chromosomal copies, double-strand break repair through homologous recombination is not possible and the bacterial cell cannot recover [70]. If the target sequence is located on a plasmid, nuclease activity will lead to either cell death or plasmid loss. One advantage of this technique is that it may be multiplexed by targeting several genetic signatures at once [68].

Finally, purified phage products such as lysins may themselves be used therapeutically. Lysins are enzymes that degrade peptidoglycan [45]. While these are particularly useful in treating infections caused by Gram-positive bacteria, the outer membrane of Gram-negative bacteria largely protects them from the action of exogenously administered lysins [71]. That said, lysins capable of accessing the peptidoglycan layer of Gram-negative bacteria have been described in the literature [72]. Furthermore, lysins can be genetically engineered or complexed with adjuvants to improve activity [72].

4.2. Efficacy

Despite extensive experience and commercial availability in other countries, there are no therapeutic phages currently approved for use in the USA. Empirical evidence from other countries supporting the efficacy of phage therapy often does not meet FDA standards, which typically require randomised controlled clinical trials. Thus far, clinical trials have failed to demonstrate suitable efficacy. A recent example was the termination of Phagoburn, a phase I/II clinical trial for the use of phage therapy in treating infected burn wounds [45].

In the meantime, physicians may be permitted to use phage therapy as a form of compassionate care when all other approved therapeutic options have been exhausted. Some patients have made the decision to travel abroad for treatment at centres such as

Table 3
Efficacy of phage-based therapies in various stages of research and development.

Phage therapy	Indication	Mechanism of action	Stage	Results of efficacy studies
Recombinant phage lysin exebacase	*Staphylococcus aureus* bacteraemia and endocarditis	Hydrolyses the bacterial cell wall and degrades biofilm	Phase II clinical trial as of 2019	A single dose of exebacase co-administered with standard antibiotics to treat MRSA bacteraemia led to a 42.8% higher response rate compared with antibiotics alone [74]
Commercial phage cocktail Pyophage	Treatment of catheter-associated UTIs	This cocktail of phages infects and lyses a variety of uropathogenic bacteria such as *Escherichia coli*	Pilot study preceding clinical trial as of 2018	Following phage instillation via suprapubic catheter for 7 days, bacterial titres decreased by 1–5 log in 6/9 patients who underwent transurethral resection of the prostate [75]
Personalised three-phage cocktail	Disseminated *Mycobacterium abscessus*	Phages were screened for efficacy against the clinical isolate and genetically modified to optimise lysis	Clinical case report from 2019	Intravenous phage therapy combined with topical phage treatment was associated with surgical wound closure, healing of skin nodules and improved liver function [76]
Fixed phage cocktail	Chronic *Pseudomonas aeruginosa* lung infection	A combination of ten bacteriophages specifically infects and lyses *P. aeruginosa*	Ex vivo experiment as of 2014	Addition of phages to sputum samples from cystic fibrosis patients led to a statistically significant decrease in *P. aeruginosa* bacterial load [77]
Phage lysin PlyF307	*Acinetobacter baumannii* infection	Hydrolyses the bacterial cell wall and degrades the extracellular polymeric matrix of biofilms	In vivo experiment as of 2015	In a murine model of lethal *A. baumannii* bacteraemia, a single dose of PlyF307 resulted in 50% survival in the treatment group compared with 10% in the control group [72]
Phage-delivered CRISPR-Cas9 pDB121::aph	*S. aureus* infection	Cleaves the *aph-3* gene conferring resistance to kanamycin	In vivo experiment as of 2014	Mice were colonised with a 1:1 mixture of kanamycin-susceptible/-resistant *S. aureus*. Post-treatment, kanamycin-resistant cells accounted for only 11.4% of the population [78]
Commercial phage cocktail Stafal®	Staphylococcal infections	Phages in Stafal® infect *S. aureus* bacteria and degrade the exopolysaccharides of biofilms	In vitro experiment as of 2019	A phage concentration of 10^8 PFU/mL killed both planktonic and biofilm-associated *S. aureus* cells of several strains after 24 h of treatment [79]
Chimeric phage lysin Ply187N-V12C	Staphylococcal and streptococcal infections	Fusing the cell-binding domain of one lysin with the catalytic domain of another broadens the lytic range	In vitro experiment as of 2018	Ply187N-V12C demonstrated lytic activity against all 25 *S. aureus* strains tested in addition to other clinically relevant staphylococcal and streptococcal species [80]
Phage-delivered ΦRGNndm-1/*shv-18*	Infections with β-lactam resistant bacteria	Induces double-strand breaks in bla_{SHV-18} and bla_{NDM-1} genes encoding resistance to β-lactams	In vitro experiment as of 2014	Treatment of *E. coli* containing bla_{SHV-18} or bla_{NDM-1} plasmids with ΦRGNndm-1/*shv-18* resulted in 2–3 $\log_{10}$ reductions in viable cells without affecting wild-type *E. coli* [68]
plv-dCas9-R(0-6) CRISPRi system	Multidrug-resistant bacteria	Represses the gene for a mobile class 1 integron that facilitates antibiotic resistance gene transfer	In vitro experiment as of 2020	Following induction of the CRISPRi system in *E. coli*, plasmid-mediated resistance to trimethoprim and sulfamethoxazole decreased 8- and 32-fold, respectively [81]

MRSA, methicillin-resistant *Staphylococcus aureus*; UTI, urinary tract infection; CRISPR, clustered regularly interspaced short palindromic repeats; CRISPRi, CRISPR interference.

the renowned Eliava Institute in Tbilisi, Georgia [73]. However, phages sourced from distant geographic regions may be of questionable applicability. Studies have shown that phages often exhibit strain specificity to the bacteria in the geographic localities from which they are isolated. Thus, not only must phage repositories be constantly updated to keep pace with evolution, but phages may also need to be locally sourced to ensure efficacy against bacterial strains of a particular region [1].

The research highlighted in Table 3 demonstrates the ability of phages to eradicate a wide range of bacterial pathogens in a variety of infection sites. Perhaps the most valuable use of phages is for the removal of biofilms. While antibiotic therapy has a high rate of success in eliminating planktonic bacteria, curing biofilm-associated bacterial infections proves considerably more difficult. Even with high antibiotic doses, complete eradication of biofilm is rare, and re-growth often follows discontinuation of antibiotic treatment [1]. Phages, on the other hand, are naturally adept at degrading the exopolysaccharide matrix of biofilm to reach their bacterial hosts. Many phages express enzymes such as proteases, polysaccharases and depolymerases to cut through biofilm [66]. Harnessing this unique advantage may have particular relevance in the maintenance of implanted medical devices [1].

4.3. Sustainability

In terms of phage-based therapies, lysins may offer the greatest sustainability. Studies have shown that repeated exposure to lysins did not lead to the emergence of resistant mutants, even after chemicals were added to increase bacterial mutation rates. This likely indicates that lysins target essential cell wall components that cannot be modified by the host [82]. On the other hand, resistance to therapeutic phages is inevitable. In fact, evolution of phage resistance can occur in mere hours [45]. Bacteria can resist phages via spontaneous mutations that modify surface structures serving as phage receptors [82]. In addition, bacteria possess viral defence mechanisms such as restriction–modification systems that degrade phage nucleic acids as well as CRISPR-Cas systems that serve as a form of adaptive immunity [66,82]. Much like antibiotic resistance, horizontal gene transfer facilitates the spread of this resistance [82].

The key to sustainable phage therapy is to anticipate this resistance and either thwart it or capitalise on it. Bacterial surface molecules targeted by phages typically serve important functions such as motility, membrane integrity or nutrient transport. Therefore, mutation or downregulation of these surface structures to inhibit phage adsorption would likely be associated with a fitness cost [83]. By purposefully selecting therapeutic phages known to bind bacterial targets associated with antibiotic resistance or virulence, development of phage resistance would likely reduce pathogenicity or increase susceptibility to antibiotics. For example, modification or downregulation of an efflux pump following phage exposure could re-sensitise a bacterium to antibiotics typically expelled by that efflux pump [67]. In this manner, phage resistance can actually be harnessed to make bacteria more vulnerable to the action of antibiotics or the host immune system.

If the goal is rather to minimise phage resistance, simultaneous exposure to a combination of phages targeting different receptors is likely the best approach [82,83]. Due to strong selective pressure, mutations conferring resistance against a single phage usually occur at high frequency and rapidly spread through a bacterial population [6,83]. In contrast, resistance to a cocktail of phages is less probable as it would require several different resistance mutations to occur within the same cell [83].

It should also be noted that unlike antibiotics, phages are biological entities with the same capacity to evolve and adapt as bacteria. With this natural coevolution in mind, continual isolation of novel phages from the environment may help keep pace with the development of resistance [66,67]. It remains to be seen whether phage resistance would pose as much of a challenge as antibiotic resistance. It is clear, however, that we must be very intentional in our approach to phage therapy to have any chance at success.

4.4. Clinical considerations

One of the advantages of phage therapy is versatility in route of administration [6]. Phages may be administered parenterally, topically, orally, via inhalation, as lavages or instillations, and in drop form for eyes and ears [67,84]. Topical administration has demonstrated considerable success in healing wounds [84]. One novel innovation from Georgia is a phage-infused bandage that gradually releases the preparation upon application to a wound [85]. The parenteral route is likely the most efficacious, offering maximum distribution and bioavailability of phages [84]. Intravenous administration of phages dates back almost a century and historical reports consistently tout safe and successful results [86].

Because phages do not infect mammalian cells, they are generally regarded as safe. However, phages are naturally immunogenic [45]. The production of neutralising antibodies against phages or other detrimental immune responses may inhibit successful treatment, particularly with systemic administration [66]. Despite concerns over the potential for serious immunological reactions, no documented cases of anaphylaxis associated with phage therapy have been reported in humans [6]. Another legitimate concern is the rapid release of endotoxins during lysis of bacterial cells, although bactericidal antibiotics carry this same risk [75]. As phage therapies begin to make their way through clinical trials in the USA, more concrete determinations regarding the safety of this therapeutic strategy can be made.

Another significant advantage to phage therapy is the unique capacity of phages to replicate at the site of infection, potentially enabling lower doses [6,82]. On the other hand, the pharmacokinetic profiles of phages will not resemble those of any other traditional medication. Therefore, studies to better understand the pharmacokinetic and pharmacodynamic behaviour of phages are required [82].

4.5. Economic considerations

The extreme biodiversity and ubiquity of phages in nature ensures a limitless source of these antibacterial agents [6,67]. Isolation of phages is a simple process that has remained largely unchanged for decades [85]. Sewage remains a lucrative environmental source of phages [87]. Following extraction from such natural reservoirs, phages are filtered and purified [85]. Large-scale production of purified phages is relatively simple and inexpensive, making this therapeutic strategy particularly fitting for implementation in developing nations [6,87].

Despite the ease of discovery and production, pharmaceutical companies have been reluctant to pursue research and development of phage-based therapies. This is partly because regulatory pathways are somewhat unclear [88]. Approval processes for such dynamic therapies will likely require alternate pathways than more traditional chemically synthesised drugs [87]. Moreover, the delivery of gene-editing systems such as CRISPR-Cas9 will inevitably be met with legislative and societal pushback [89]. But perhaps the most significant issue giving the pharmaceutical industry pause is the challenge of ensuring intellectual property protection for a biological agent. While antibiotics are formulated

Table 4
Advantages and disadvantages of alternative therapies compared with antibiotics.

Therapy	Advantages	Disadvantages
Antimicrobial oligonucleotides	• Spares commensal bacteria [18] • Limited potential for resistance [18] • Reduced drug discovery time [34] • Wide-ranging applications [21] • Faster and longer-lasting activity [17]	• Successful delivery is a challenge [20] • Parenteral administration only [20,33] • Rapid diagnostic testing is essential [8] • Safety profile yet to be determined
Monoclonal antibodies	• Spares commensal bacteria [38,59] • Reduced drug discovery time [17] • Wide-ranging applications [42,43] • Infrequent dosing [37] • Prophylaxis offers instant protection [59] • Generally favourable safety profile [47,60] • Adept at toxin neutralisation [40]	• Subject to resistance [37] • Expensive [64] • Serotype specificity limits coverage [47] • Parenteral administration only [42] • Rapid diagnostic testing is essential • Variability between batches [42]
Phage therapy	• Spares commensal bacteria [6] • Rapid discovery; unlimited supply [6,67] • Self-amplifying; lower doses [6,67,82] • Adept at eradicating biofilm [66] • Low toxicity [1] • Versatility in route of administration [6] • Inexpensive [6,87]	• Resistance is inevitable [67] • Rapid diagnostic testing is essential [6] • Regulatory pathways are unclear [88] • Profitability is questionable [85] • Potential for release of endotoxin [67] • May be cleared by immune system [64] • Unable to penetrate eukaryotic cells [67]
Antibiotics	• Empirical use to treat urgent infections • Versatility in route of administration • Relatively inexpensive [65] • Clear regulatory pathway	• Disrupts the microbiome • Resistance is inevitable [5] • Difficulty in eradicating biofilm [1] • Slow discovery process [67] • Potential for toxicity [1] • Maintenance of therapeutic range [67]

with unique chemical compounds suitable for patenting, biological entities or constituents thereof have historically been declined patents. It is possible that in the future companies may obtain rights to genetically modified phages or specific combinations of phage cocktails [88]. Still, the profitability of therapeutic phages, even if patented, is questionable [85]. Thus, academic institutions and government agencies may have to lead the way by creating publicly-funded phage repositories and granting access to medical centres and other approved institutions [88].

In summary, the advantages and disadvantages of all previously discussed alternative therapeutic agents is presented in Table 4.

5. Conclusion

For each of these alternative therapeutic agents, many obstacles will need to be overcome before widespread implementation. In general, since these therapies are highly specific to the particular pathogen being treated, rapid diagnostic testing with high specificity is essential. Furthermore, novel methods to predict the in vivo efficacy of a particular agent will need to be devised since minimum inhibitory concentrations (MICs) are only applicable to those agents that are directly bactericidal or static. A fascinating, albeit hypothetical, approach would be to design diagnostic and therapeutic panels in parallel. For example, a rapid multiplex nucleic acid assay could be designed to detect the same genes targeted in a panel of antisense- or CRISPR-Cas-based therapies. This could rapidly and simultaneously accomplish a probable identification and susceptibility. Such a technology would be most readily applicable to the diagnosis and treatment of infections in sterile body sites such as blood or spinal fluid in which the vast majority of infections are caused by a well-defined group of pathogens, polymicrobial infections are rare, and timely results are critical.

It is worth noting that this review of alternative therapeutic agents is far from comprehensive. A select group of therapeutic classes are included that by initial estimation seemed the most promising. Vaccination remains incredibly important in the prevention of disease and, as such, is a vital component of the multi-tiered approach to combating antibiotic resistance. Other areas of therapeutic research include small-molecule antivirulence agents, antimicrobial peptides, antibiotic adjuvants, probiotics and/or supplementation of the microbiome, immunomodulation, bacterial nutrient starvation and predatory bacteria.

Alternative therapeutic agents have faced considerable scepticism, and rightfully so; significant challenges have yet to be overcome. But with each passing year, important strides are made. It is crucial that we support and amply fund this research so that progress can continue. We have unquestionably exited the golden age of antibiotics. How we proceed from here will determine to what extent we relegate ourselves to a pre-antibiotic era: a world where a seemingly innocuous cut of the finger could lead to an untreatable life-threatening infection. Hopefully, this work and the work of countless other scholars and researchers will inspire others to take action. The urgency of the situation cannot be overstated.

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