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Review

Alternatives to Conventional Antibiotics in the Era of Antimicrobial Resistance

Chandradhish Ghosh,[1] Paramita Sarkar,[1] Rahaf Issa,[2] and Jayanta Haldar[1,*]

As more antibiotics are rendered ineffective by drug-resistant bacteria, focus must be shifted towards alternative therapies for treating infections. Although several alternatives already exist in nature, the challenge is to implement them in clinical use. Advancements within biotechnology, genetic engineering, and synthetic chemistry have opened up new avenues towards the search for therapies that can substitute for antibiotics. This review provides an introduction to the various promising approaches that have been adopted in this regard. Whilst the use of bacteriophages and antibodies has been partly implemented, other promising strategies, such as probiotics, lysins, and antimicrobial peptides, are in various stages of development. Propitious concepts such as genetically modified phages, antibacterial oligonucleotides, and CRISPR-Cas9 are also discussed.

Highlights

As bacteria grow resistant to conventional antibiotics, alternatives are being investigated, including antibodies, probiotics, bacteriophages, and antimicrobial peptides currently undergoing clinical trials.

The specificity of antibodies, and the inability of bacteria to develop resistance against them, make antibodies attractive, albeit expensive, alternative therapeutic agents.

Bacteriophages have been used for therapy in some parts of the world.

Antimicrobial peptides have long been considered as potential replacements for antibiotics but with limited success. Synthetic peptides and synthetic membrane-active agents might herald a shift.

Probiotics and fecal transplant therapy are already in practice for enhancing the human microbiota.

The use of oligonucleotides for silencing resistance genes and resensitizing resistant bacteria to antibiotics is still in the research stage.

The Problem of Antimicrobial Resistance and the Way Forward

Currently, every year, 700 000 patients die globally due to antimicrobial resistance (AMR). It has been estimated that this death toll will increase to 10 million by 2050, which would lead to a reduction of gross domestic product (GDP) by at least 2.5%[i]. The fight against AMR is constant, and the discovery of new antibiotics is critical. Together, the facts and statistics raise an important question: is the time of antibiotics up? Although antibiotics have served humanity well for the last 70 years or so, the ability of bacteria to quickly evolve has made it imperative to look for other options. In this review, we provide a brief introduction into the strategies that could be therapeutic alternatives to antibiotics. We focus on therapeutic strategies, thus, vaccines which have been widely used as preventive measures against bacterial infections such as tuberculosis, tetanus, pertussis, diphtheria, and pneumococcal diseases, are excluded from this review. Instead of including traditional antibiotics that normally target physiological processes in bacteria (e.g., replication, transcription, translation, biosynthetic processes) we have covered synthetic compounds which work using a different mechanism. A summary of the novel approaches that hold promise is detailed in Table 1 (Key Table). For simplicity, strategies have been arranged in three nonexhaustive categories: (i) naturally occurring alternatives, (ii) synthetically designed strategies, and (iii) biotechnology-based strategies. Finally, we conclude the review with our perspective on the status of the field and its future prospective.

Naturally Occurring Alternatives

Phage Therapy

Bacteriophages (phages), or viruses that 'eat' bacteria, were used to treat infected livestock before conventional antibiotics were used for this same purpose [1]. Bacteriophages propagate at the expense of bacteria. First, they anchor onto the bacterial cell surface and then inject phage genetic material into the bacterial cytoplasm. This subsequently takes over the host cell machinery, resulting in the synthesis of phage components and assembly of new phages within

[1]Antimicrobial Research Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bengaluru 560064, India
[2]Department of Infection, Immunity and Cardiovascular Diseases, The University of Sheffield, Sheffield, UK

*Correspondence: jayanta@jncasr.ac.in (J. Haldar).

Key Table

Table 1. Major Alternatives to Antibiotics and Their Advantages and Disadvantages

Strategy	Advantages over conventional antibiotics	Possible disadvantages
Phage therapy	• Self-replicating pharmaceuticals • Selective towards specific strains of bacteria • Amenable to genetic engineering	• Immunogenicity • Pharmacokinetics • Release of bacterial endotoxins • Inadequate preparations – failure to remove endotoxins and pyrogenic substances • Resistance development
Lysins	• Amenable to genetic engineering • Selective towards specific strains of bacteria • Not prone to resistance development	• Production • Lack of sufficient knowledge
CRISPR/Cas9	• Can be tuned for a variety of antimicrobial applications • Reversal of antibiotic usage • Specificity towards pathogenic strains	• Expensive large-scale production • Toxicity
Antimicrobial peptides	• Not prone to resistance development • Broad-spectrum activity is an advantage, depending upon application	• Expensive large-scale production • Susceptible to proteolysis • Toxicity
Bacteriocins	• Specificity towards pathogenic strains of bacteria • Resistance to heat and UV	• Expensive large-scale production • Susceptible to proteolysis
SMAMPs[a]	• Ease of synthesis • Not prone to resistance development • Broad-spectrum activity is an advantage, depending upon application	• Toxicity • Route of administration
IDR peptides[b]	• Work by modulating the immune system • No resistance development as no direct antimicrobial activity	• Expensive large-scale production • Susceptible to proteolysis
Probiotics	• Easy availability	• Used mostly for intestinal infections
Antibodies	• Selective towards specific strains of bacteria • Do not damage the microflora	• High cost of production • Poor shelf life

[a]Synthetic mimics of antimicrobial peptides.
[b]Innate defense regulatory peptides.

the infected bacteria. This eventually leads to bacterial lysis and the release of phage progeny that can commence a second infection cycle. Phages are known to select between mixed populations of bacteria. Thus, exploitation of the lytic cycle of bacteriophages can be used to develop an alternative but selective approach to target pathogenic bacteria over commensal bacteria (Figure 1) [2]. Despite the successful use of phage therapy in Eastern European countries and the former Soviet republics, the Western world previously failed to follow in developing phage therapy [3]. Currently, phage therapy is the subject of renewed interest due to the debilitated state of the antibiotic arsenal [4,5].

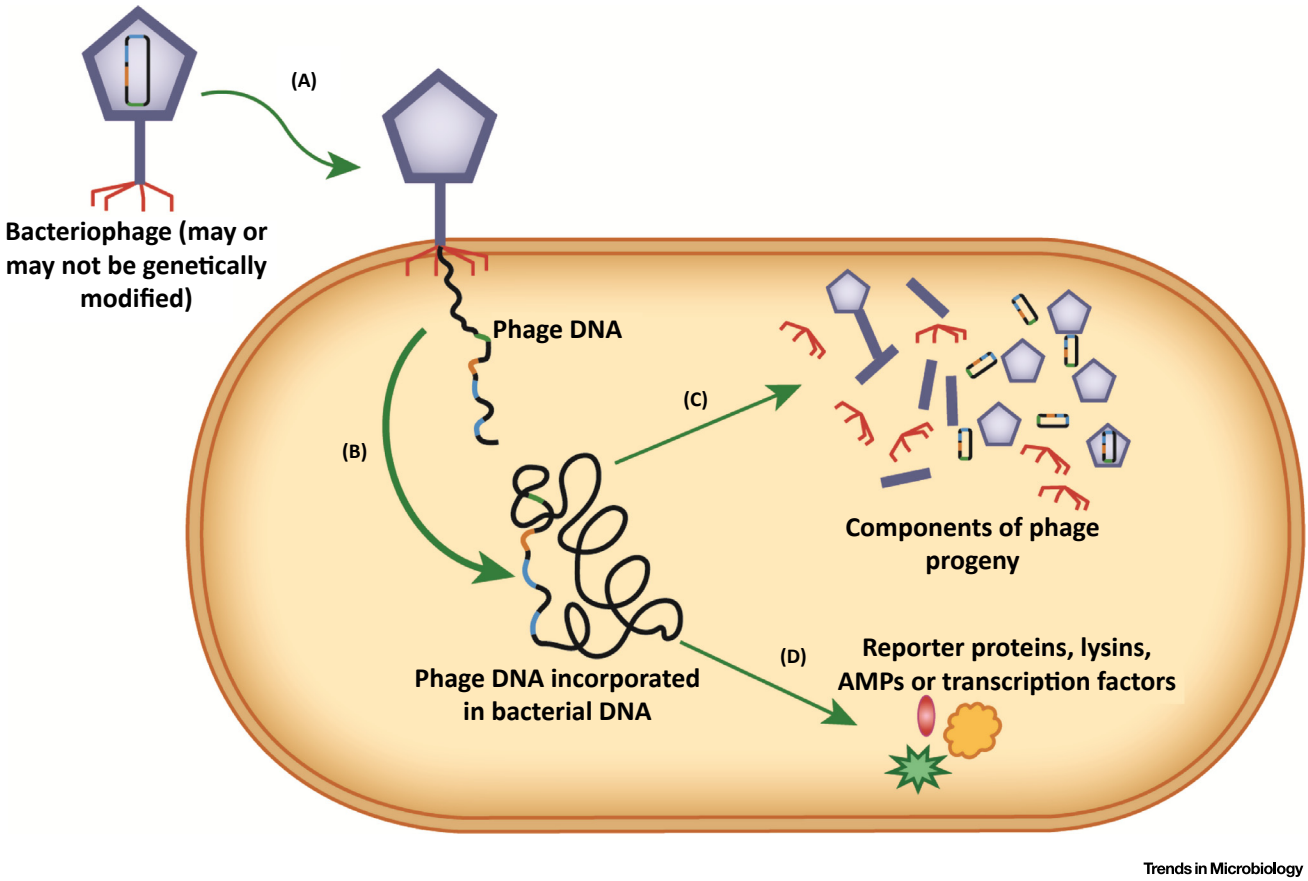

Figure 1. Bacteriophage Therapy. (A) Natural or genetically modified phages will selectively target bacteria. (B) The phage genome will be integrated into the bacterial genome, and replication will commence using host machinery. (C) Translation into progeny phage particles, which will assemble and burst the cell. (D) Expression of reporter proteins, toxins, lysins, and antimicrobial peptides (AMPs) to damage the host.

Bacteriophages can be used for the treatment of infections by various bacteria, ranging from *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella*, and *Salmonella* (Table 2). Treatment of resistant infections using bacteriophages in diseases such as cystic fibrosis has also been tested [6]. Phage therapy offers several advantages, but concerns about its usage, like any other treatment, also exist. Bacterial resistance to phages has been reported [7]. Assessing the susceptibility of bacteria to a particular phage is therefore crucial. However, the lack of rapid diagnostic platforms means that a cocktail of multiple bacteriophages is often required for treatment. Concerns regarding the release of endotoxins from the bacteria lysed by bacteriophages have also been raised as this can potentially lead to sepsis. Moreover, the pharmacokinetics of phages is a concern as they are known to easily diffuse to several organs of the body [4]. Perhaps an even greater concern is the immunogenicity of bacteriophages [4]. This possibly will limit the uses of bacteriophages beyond a single time, as they would be efficiently cleared by the body the next time they are administered. However, advances in genetic engineering have ensured that bacteriophages can be used in innovative ways to tackle bacterial infection [8]. In terms of phage preparation, inadequate methodology can hinder their development into successful therapy. The presence of endotoxins and pyrogenic substances poses a high degree of toxicity, and their removal is essential for a safe application.

Table 2. Bacteriophages That Are Approved or Are Undergoing Clinical Trials for Human Use

Product	Company	Condition	Phase (status)
ListShield	Intralytix	Food industry (*Listeria monocytogenes*) Food industry (*Escherichia coli*)	Approved
EcoShield	Intralytix	Food industry (*Salmonella enterica*)	Approved
SalmoShield	Intralytix	Burn infections (*Pseudomonas aeruginosa* and *E. coli*)	Approved
ABPA01	AmpliPhi	Chronic rhinosinusitis and cystic fibrosis (*P. aeruginosa*)	Preclinical
PHOSA	Multiple centres	Treatment and prophylaxis of gastrointestinal infections (multiple organisms)	Preclinical
Phagesti	Biochimpharm	Treatment and prophylaxis of bacterial purulent–inflammatory infections (multiple microorganisms)	Approved
Phagyo	Biochimpharm	Treatment and prophylaxis of bacterial purulent–inflammatory infections (multiple microorganims)	Approved
Phagedys	Biochimpharm	Treatment and prophylaxis of dysentery (*Shigella*)	Approved
Phagetyph and Phagesal	Biochimpharm	Treatment and prophylaxis of enteric fever and salmonellosis (*Salmonella*)	Approved
Phagestaph	Biochimpharm	Treatment and prophylaxis of bacterial purulent–inflammatory infections (*Staphylococcus aureus*)	Approved
Phagepy	Biochimpharm	Treatment and prophylaxis of bacterial purulent–inflammatory infections (*P. aeruginosa*)	Approved
Pyo-Phage	Eliava Institute	Urogenital infections; pyo-inflammatory gynecologic diseases; enteric infections; dysbacteriosis, surgical infections	Approved
Intesti-Phage	Micro-gen	Bacterial dysentery; salmonellosis; dyspepsia; dysbacteriosis; enterocolitis, colitis	Approved
SES-Phage	Eliava	Pyo-inflammatory and enteric infections caused by staphylococci, streptococci, and enteropathogenic *E. coli*	Approved
ENKO-Phage	Eliava	Pyo-inflammatory and enteric infections caused by *Shigella*, *Salmonella*, different types of *E. coli*, species of pathogenic staphylococci	Approved
Fersisi-Phage	Eliava	Pyo-inflammatory and enteric infections caused by staphylococci and streptococci	Approved
ABSA01	AmpliPhi	Chronic rhinosinusitis, bacteremia, endocarditis, prosthetic joint infections, osteomyelitis, diabetic foot ulcers (*S. aureus*)	Phase I
PhagoBurn	Multiple centres	*E. coli*or *P. aeruginosa* burn wound infections	Phase I/II

Furthermore, the pH, and thus stability, of the phage preparation needs to be considered as these factors will affect the treatment's efficiency.

Antimicrobial Peptides

As the first line of defense, antimicrobial peptides (AMPs) and host-defense peptides (HDPs) are produced by many multicellular organisms against invading pathogens [9–11]. They have diverse activities ranging from antibacterial, antifungal, antiviral, anticancer, antiplasmodial, antiprotistal, insecticidal, and spermicidal to immunomodulation [12]. Although most of these peptides bear a net positive charge, anionic antimicrobial peptides have also been reported [13]. These peptides are facially amphiphilic and their cationic domain engages in electrostatic interactions with the negatively charged bacterial cell surface, while the hydrophobic domain interacts with the lipids of the bacterial membrane. This results in disintegration of the cell membrane, and finally bacterial death [14,15]. Mammalian cells are zwitterionic in nature and

hence do not interact well with the positively charged AMPs, rendering them selectively toxic toward bacteria. Aside from membrane activity, intracellular targets are increasingly being probed [16]. Reports of bacteria developing resistance against AMPs do exist [17]. However, disintegration of the bacterial cell membrane is energetically too unfavorable for bacteria to easily develop resistance [17,18]. The broad range of properties and the aforementioned advantages have prompted the scientific world to consider AMPs as future antibiotics [19,20].

Although peptide antibiotics – such as polymyxin B (a lipopeptide obtained from *Bacillus polymyxa*), colistin (polymyxin E, also from *B. polymyxa*), gramicidin (a linear polypeptide derived from *Bacillus brevis*), daptomycin (a cyclic anionic lipopeptide) – are being used in the clinic, AMPs have not yet had full clinical success [21,22]. A biomimetic peptide, enfuviritide was approved by the FDA for combination therapy (Fuzeon) against HIV [23]. Although several AMPs are undergoing clinical trials, *in vivo* toxicity and difficult industrial scalability have hindered clinical translation of this class of alternative antimicrobial agents (Table 3) [24].

Bacteriocins

In order to prevent competition and enhance survival, several bacteria produce small AMPs that act against other bacteria within the population. These ribosomally synthesized peptides, called bacteriocins, are often active against drug-resistant pathogens of clinical importance [25]. Broadly classified into two groups, bacteriocins that undergo rigorous post-translational modification belong to class I, and the unmodified belong to class II [26].

Table 3. Antimicrobial Peptides, Synthetic Mimics and Lysins of AMPs in Clinical Trials

Product	Company	Indication	Status
Locilex (Pexiganan)	Dipexium Pharma	Diabetic foot ulcers	Failed Phase III
CLS001 (Omiganan)	Cutanea Life Sciences	Rosacea, acne, atopic dermatitis, and genital human papillomavirus	Phase III–Phase II
AB103	Atox Bio	Necrotizing soft-tissue infections	Phase III
LL-37	Promore Pharma	Chronic leg ulcers	Phase II
LL-37	M. D. Anderson Cancer Centre	Melanoma	Phase II
NP213	Novabiotics	Onychomycosis	Phase II
P-113	Pacgen Lifesciences	Oral candidiasis	Phase II
SGX 942	Soligenix	Oral Mucositis	Phase II (Fast track)
Brilacidin	Innovation Pharmaceuticals	Oral mucositis Skin infections (ABSSSI)[a]	Phase II
AMC-109	Amicoat AS	Impetigo Nasal decolonization	Phase II
AP 138	Adenium Biotech	MRSA implant infections	Phase I
Avidocin and purocin	Pylum Biosciences	Narrow-spectrum antibiotic	Preclinical
HB 1345	Helix Biomedix	Acne	Preclinical
HB 1275	Helix Biomedix	*Trichophyton* infections	Preclinical
Plectasin	Adenium Biotech	Gram-positive infections	Preclinical
OG-716	Oragenics	*Clostridium difficile* infections	Preclinical
NP432	Novabiotics	Multibacterial infections	Preclincial
CSA-13	N8 Medical	Bacterial infections/fracture	Preclinical

[a]ABSSSI, acute bacterial skin and skin structure infection.

The mechanism of action of bacteriocins is similar to that of AMPs in that they target the cell membrane. Other than causing membrane pore formation, these compounds can have several other mechanisms of action, for example, inhibition of peptidoglycan biosynthesis by targeting lipid II. Nisin, a commercially used bacteriocin in the food industry, acts by targeting the cell membrane and inhibiting peptidoglycan biosynthesis [25]. Lactococcin A, a class II bacteriocin, binds to the pore-forming receptor mannose phosphotransferase system (Man-PTS) [27]. Other bacteriocins, including thiopeptides and bottromycin, are known to target translation [28,29]. Microcin B17 (MccB17), MccJ25, and MccC7-C51 are bacteriocins that traverse through the outer and inner membrane of Gram-negative bacteria to affect metabolism of DNA, RNA, and proteins [30–32].

Bacteriocins suffer from the same problems as AMPs, but their use in certain cases can offer more advantages. Conventional antibiotics and AMPs rarely select between commensal and pathogenic bacteria. However, like bacteriophages, bacteriocins can also have selectivity against particular bacterial strains. As an example, the bacteriocin thuricin does not affect commensal bacteria but kills *Clostridiodes difficile* (*Clostridium difficile*) [33]. Like AMPs, resistance against bacteriocins is slow but imminent. Resistance development has been observed in the laboratory, yet in practice this is not always the case. Nisin, for example, has been used for decades, yet widespread resistance has not been reported. Perhaps the biggest advantage of bacteriocins is their resistance to harsh conditions of heat and exposure to UV. Thus, they find applications in the food industry easily [25]. Given the advantages mentioned above, bacteriocins have the potential to be alternative sources of treatment of infection.

Alteration of the Gut Microbiota

Probiotics

The mammalian gut microbiota comprises over 1000 microbial species, including bacteria and yeast. Bifidobacteria, lactobacilli, streptococci, and nonpathogenic strains of *E. coli*, *Bacteroides* sp., *Clostridiodes* sp., *Fusobacterium* sp., *Eubacterium* sp., *Peptococcus* sp., *Peptostreptococcus* sp., and *Veillonella* sp. are some of the predominant bacterial genera found in the intestines [34]. Recent studies have indicated that these bacteria play a crucial role in energy metabolism and immune function and thereby maintain human health [35]. Antibiotic treatment usually perturbs the composition of the human gut microbiota. This promotes the growth of drug-resistant pathogens and often leads to secondary infections, for example, *C. difficile*-induced colitis. Further use of antibiotics to treat these infections increases the chances of recurrence of the disease. Manipulating the gut microbiota to treat and prevent systemic dissemination is an alternative to treatment with antibiotics. Probiotics and prebiotics have thus been indicated in the treatment of various gastrointestinal infections such as pseudomembranous colitis caused by *C. difficile* and *Helicobacter pylori* [36,37].

Although bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium* have been used for the treatment of various gastrointestinal infections, the potency of the yeast *Saccharomyces boulardii*, and of nonpathogenic strains of *E. coli* and *Bacillus* spp., has also been explored [38]. The supernatants of *S. boulardii* cultures were shown to reduce the damage to epithelial cells caused by the toxins of *C. difficile* [39]. Further, *S. boulardii* could also reduce the inflammatory response to *C. difficile in vivo* [40]. The concept governing the use of probiotics is that, upon restoration of balance in the gut microbial flora, the commensal bacteria can outgrow and competitively exclude pathogenic strains (Figure 2). These commensal bacteria promote resistance to pathogens either directly, through interactions with other bacteria, or indirectly through induction of host immune defenses [41]. Treatment with probiotics and prebiotics is

advantageous over antibiotics as these agents are safe for long-term consumption and do not cause side effects or allergies. The probiotics under clinical trials for the treatment of infections are included in Table 4.

Fecal Transplant Therapy

Fecal transplant treatment (FTT) is an alternative strategy involving the introduction of the microbiome from a healthy donor into the diseased gut. It is used to treat bacterial infections or other cases involving gastrointestinal dysbiosis [42]. Although the mechanism of action of this technique is not clearly understood its use for the treatment of *C. difficile* infections (CDIs) has been widely explored. The cure rate for patients undergoing colonoscopic FTT for recurrent CDI (RCDI) was ~90%. The Food and Drug Administration Agency (FDA) has approved the use of FTT for the treatment of patients suffering from CDI that is unresponsive to standard therapy. It has also shown promise in clearing colonization with multidrug-resistant Enterobacteriaceae such as *E. coli*, *Salmonella*, *Klebsiella pneumoniae, Acinetobacter baumannii*, methicillin-resistant *S. aureus*, and vancomycin-resistant enterococci (VRE). However, the investigation of the efficacy of FTT against these pathogens in humans is limited.

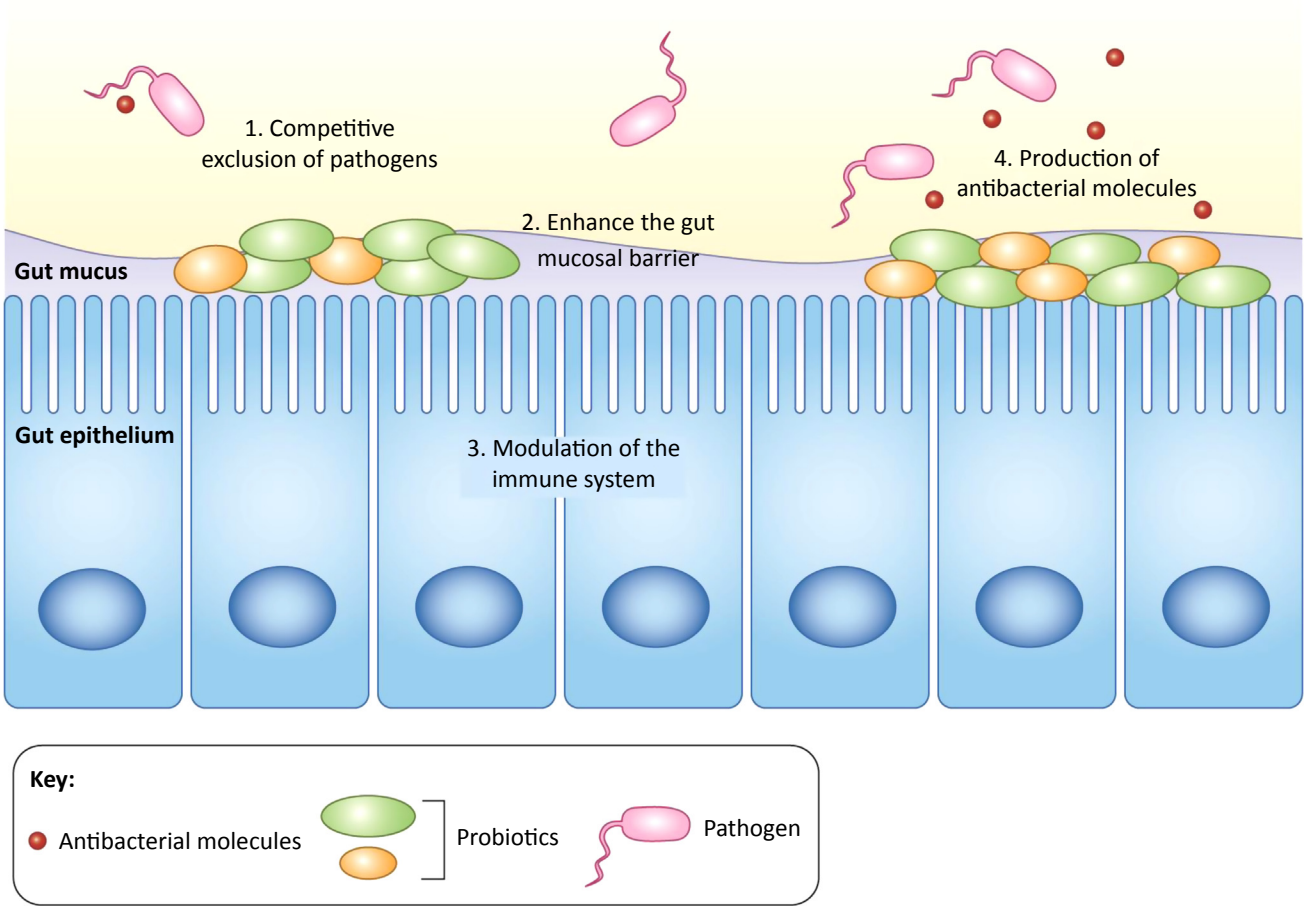

Figure 2. Illustration of the Mechanism of Protection of Probiotics against Infections.

Table 4. Probiotics That Are Approved or Are Undergoing Clinical Trials for Human Use

Product	Company	Condition	Phase (status)
SER-109	Seres	Recurrent *Clostridium difficile* infection	Phase III
RBX2660	Rebiotix	Recurrent *C. difficile* infection	Phase III
VP20621	Shire (Viropharma)	*C. difficile*	Phase II
FIN 403	Finch therapeutics	Recurrent *C. difficile* infection	Phase II
SER 262	Seres	Recurrent *C. difficile* infection	Phase Ib
RBX 7455	Rebiotex	Recurrent *C. difficile* infection	Phase I

Predatory Bacteria

The use of predatory bacteria such as *Bdellovibrio* and like organisms (BALOs) has been considered a promising alternative to antibiotics [43]. These organisms are δ-proteobacteria that multiply only upon entering other Gram-negative pathogens such as *E. coli*, *Salmonella*, *Legionella, Pseudomonas* [44]. BALOs degrade prey cells by lysing them with various hydrolytic enzymes (such as DNAses and proteases). Such enzymes can also penetrate into bacterial biofilms, giving them an advantage over conventional antibiotics [43]. They enter the periplasm of prey bacteria by forming a localized pore. Upon entry into the prey, they form a hybrid called a bdelloplast. These then often deform into spherical cells due to degradation and remodeling of the peptidoglycan. The lipopolysaccharide (LPS) of *Bdellovibrio* lacks the negatively charged phosphate group, resulting in reduced binding affinity to the LPS receptors in human immune cells. Thus, they exhibit minimal inflammatory response (TNF-α and IL-6) [43]. The failure of these bacteria to multiply within mammalian cells indicates their potential in the treatment of bacterial infections. Upon oral administration of *Bdellovibrio* to chicks infected with a gut-colonizing *Salmonella enterica* serovar Enteritidis phage type 4, Atterbury *et al*. found a significant reduction in the bacterial load in bird gut cecal contents and reduced cecal inflammation [45]. This indicated the potential of *Bdellovibrio* for treatment of bacterial infections. The activity against a wide range of bacteria, low immunogenicity, low toxicity, and negligible propensity to induce resistance make this bacterium a promising candidate for the treatment of infections.

Antibodies

To fight the invasion of pathogens, the immune system produces antibodies – proteins that recognize specific components of the pathogen and neutralize them. Antibodies are thus useful alternatives for the treatment of intractable bacterial infections. They could be used to treat bacterial infections either by directly targeting the bacterial surface or indirectly by neutralizing the bacterial toxins and the virulence factors that are responsible for infection. Numerous antibodies against staphylococci, *P. aeruginosa*, *Bacillus anthracis*, and *C. difficile* are in various stages of clinical development and, in fact, some of them have already been approved by the FDA (Table 5) [46,47]. A major drawback of using antibodies for antibacterial therapy is the cost of the production and poor shelf life.

Synthetically Designed Strategies

Synthetic Mimics of Antimicrobial Peptides (SMAMPs)

Scientists have also designed molecules in an attempt to mimic the properties of antimicrobial peptides [48]. Broadly, there have been three different approaches towards designing synthetic mimics of antimicrobial peptides. They are either polymeric mimics of AMPs, peptidomimetic

Table 5. Antibodies Approved or in Clinical Phases for the Treatment of Bacterial Infections

Antibody name	Company	Pathogen	Target	Condition	Clinical studies
Bezlotuxumab	Merck	*Clostridium difficile*	Toxin B	*C. difficile*-associated diarrhea CDAD	FDA approved
Raxibacumab	GlaxoSmith Kline	*Bacillus anthracis*	Protective antigen	Anthrax	FDA approved
Anthim	Elusys therpaeutics	*B. anthracis*	Protective antigen	Anthrax	FDA approved
Pagibaximab	Biosynexus	Staphylococci	Lipoteichoic acid	Staphylococcal sepsis	Phase III
Aurexis	Bristol-Meyrs Squibb	Staphylococci	Clumping factor A	Staphylococcal infections	Phase II
Shigamabs	Taro pharmaceuticals	Shiga-toxin-producing *Escherichia coli*	Shiga toxin (tx1 and tx2)	Shiga-toxin-producing bacterial infection	Phase II
Pseudomonas aeruginosa immune globulin (MEP IGIV)	National Center for Research Resources	*Pseudomonas aeruginosa*	Specific target unknown	Cystic fibrosis	Phase II
Aerumab (AR-101)	Aridis Pharmaceuticals	*P. aeruginosa*	LPS[a]	Pneumonia/ ventilator-associated pneumonia	Phase I/Phase II
Anti-*Pseudomonas* IgY	Immunsystem AB	*P. aeruginosa*	Specific target unknown	Cystic fibrosis/*P. aeruginosa* infections	Phase I/II
Aerucin	Aridis Pharmaceuticals	*P. aeruginosa*	Alginate on cell surface	Pneumonia	Phase II
Salvecin (AR 301)	Aridis Pharmaceuticals	*Staphylococcus aureus*	α-toxin	Sepsis Pneumonia	Phase II Phase I
514G3	XBiotech	*S. aureus*	Immunomodulator	Bacteremia	Phase II
MEDI 3902	MedImmune Inc.	*P. aeruginosa*	Psl and PcrV	Noscocomial pneumonia	Phase II
MEDI 4893	MedImmune Inc.	*S. aureus*	α-toxin	Hospital-acquired pneumonia	Phase II
Valortim	Pharm-Athene	*B. anthracis*	Protective antigen	Anthrax	Phase I

[a]Lipopolysaccharide.

oligomers, or small molecules [21]. Most of these strategies try to overcome the problems of protease lability, toxicity, and the high cost of manufacture of AMPs. Although each of them constitutes broad fields of its own, they are briefly described here.

The earliest synthetic strategies used to overcome protease stability focused on modification of the peptide backbone, keeping the cationic and amphiphilic designs steady. Promising peptidomimetic designs include β-peptides [49–51], oligoureas [52], peptoids [53], oligoacyl-lysines [54], and α-AA peptides [55]. These compounds are oligomeric and thus able to form secondary structures necessary for potent antimicrobial activity. Due to the presence of abiotic moieties these peptides are usually resistant to degradation.

Incorporation of hydrophobic and cationic domains into polymers results in antimicrobial polymers [56–58]. These polymers, classified into segregated monomers, same-centered polymers, and facially-amphiphilic polymers, show moderate activity against bacterial

pathogens [59]. Synthetic antimicrobial polymers have also yielded excellent results, with some able to resensitize drug-resistant bacteria to conventional antibiotics [60–64]. The designs and activities of antimicrobial polymers are not covered here but are well reviewed elsewhere [56–58,65].

In the third strategy, small molecules which could mimic the properties of AMPs were designed. This involves alternative molecular designs and synthetic strategies [21,66]. Initial designs focused on integrating facial amphiphilicity into small molecules using H-bonding motifs. Brilacidin is one of the success stories of this strategy and is currently undergoing clinical trials (Table 3) [67–69]. Truncation of natural antimicrobial peptides and subsequent synthetic modification also yielded AMC-109, which has entered clinical trials as an antistaphylococcal agent (Table 3) [70,71]. Ceragenins, which are at the preclinical stage, are cholic acid derivatives that also possess promising antimicrobial properties [72]. Incorporation of short peptides/amino acids on hydrophobic moieties also results in compounds with significant antimicrobial activity, such as binaphthyl-based dicationic peptides and xanthone derivatives [73,74]. We have also contributed significantly to the field by designing aryl-alkyl-lysines [75–77]. These compounds were synthetically simple, rapidly bactericidal compounds that were also active against fungi, the malaria parasite (*Plasmodium*), and Ebola virus [78,79]. Simple lipidated lysines were also designed with potent activity *in vivo* [80]. Biphenyl-based lysine derivatives could act on intracellular bacteria as well as other manifestations of bacterial infections [81]. In other designs, a library of norspermidine-based compounds was designed with potent activity against Gram-positive and Gram-negative bacteria [82–86]. Extremely selective cationic amphiphiles were also designed that could act on bacterial biofilms [87–89]. Although relatively young, success in clinical trials might prove this field to be a good source of alternative antibacterial drugs.

Innate Defence Regulatory Peptides

AMPs are also known to modulate the immune system for defense against invading pathogens [90]. In an interesting concept, peptides with no antibacterial activity but with antiendotoxin and immunomodulatory activities were designed [91]. Called ‘innate defence regulatory’ (IDR) peptides, they were shown to protect mice from succumbing to severe bacterial and malarial infections, without having any direct antimicrobial activity [10,90,92–94]. IDR peptides are a promising alternative to conventional antibiotics, and one of them has completed Phase I clinical trials for the treatment of bacterial infection (SGX 942, Table 3).

Antibacterial Oligonucleotides

Gene silencing therapy has been explored for the treatment of numerous diseases including infectious diseases. Silencing of essential and resistance-causing genes by the use of antisense oligonucleotides with sequences complimentary to the target mRNA is an alternative strategy for tackling multidrug-resistant bacteria. The backbone of oligomers is often modified to contain morpholino and phosphorodiamidate groups (termed as phosphorodiamidate morpholino oligomers, PMOs) in order to confer resistance to nuclease degradation. These oligomers are further conjugated with cell-penetrating peptides (PPMOs) to help transport the oligomers across the negatively charged bacterial membrane. PPMOs have shown antibacterial activity against a variety of bacteria such as *E. coli*, *S. enterica*, and *A. baumannii* both *in vitro* and *in vivo* [95]. Alternatively, phosphorothioate oligodeoxynucleotides (PSODNs) are known to activate RNase H activity to degrade mRNA and hence lead to antibacterial activity. To enhance penetration across the bacterial cell wall, PSODNs were encapsulated in polyanionic liposomes [96].

Howard *et al*. designed PPMOs targeting the essential genes of *acpP*, *lpxC*, and *rpsJ* in *P. aeruginosa*. The PPMOs exhibited growth inhibition in the presence of sublethal concentrations of colistin and polymyxin B [97]. They inhibited and disrupted biofilms of *P. aeruginosa* in combination with β-lactams and tobramycin, the combination of the PPMO targeting *rpsJ* with tobramycin being the most effective. They also significantly reduced the bacterial load in the lungs of mice in a mouse model of *P. aeruginosa* pneumonia infection. PSODNs that inhibit the expression of the *mecA* gene (which encodes modified penicillin-binding protein 2a, PBP2a) in methicillin-resistant bacteria have also been developed. Anti-*mecA* PSODNs were found to restore the susceptibility of methicillin-resistant staphylococci to oxacillin both *in vitro* and *in vivo* [98]. This could also sensitize resistant staphylococci to other existing β-lactam antibiotics (cefatoxin, cephalothin, cefoperazone, cefoxitin, and oxacillin). Anti-*mecA* PSODN-PEI complexes were then further encapsulated within anionic liposomes and shown to enhance the survival rate of mice suffering from sepsis induced by methicillin-resistant *Staphylococcus aureus* (MRSA) [98]. Recently, Sully *et al*. developed a PPMO that could inhibit the expression of the New Delhi metallo-β-lactamase (NDM-1) gene. This was found to reduce the MIC of meropenem in NDM-1-producing pathogens and also reduce the bacterial burden in a murine model of *E. coli* sepsis [99].

Inhibitors of Bacterial Virulence

In order to establish infection, bacteria produce both extracellular and cell-surface molecules, also known as virulence factors. The inhibition of expression of virulence factors, which interferes with the interaction between the bacterium and its host, is another strategy for tackling infection. Since the strategy does not directly target bacterial cellular processes, the propensity to develop resistance is reduced. Numerous antibodies which inhibit various virulence factors are undergoing clinical trials (Table 4). Recently, liposome-based virulence inhibitor, CAL-02, was introduced for clinical trials to prevent deterioration in patients with pneumococcal pneumonia. This first-in-class drug was found to neutralize virulence factors (toxins) produced by a broad spectrum of bacteria such as *S. aureus*, *S. pneumoniae*, *P. aeruginosa* and various other streptococcal or clostridial strains. Although it was not antibacterial itself, it exhibited synergism with antibiotics[ii].

Biotechnology-Based Approaches

Genetically Modified Bacteriophages

The advancement in the field of genetically modified phages has been very well reviewed recently [8,100]. Herein, we mention some of the pioneering work done in the field. In a landmark paper, Lu *et al*. engineered bacteriophages to produce bacterial-biofilm-degrading enzymes upon infection [101]. Resultantly, upon treatment with these engineered phages, both the biofilms and the bacteria that were embedded within the biofilms were lysed. In another concept, bacteriophages were engineered to overexpress *lexA3* that represses the bacterial SOS DNA repair system [102]. *E. coli* treated with bacteriophages, engineered to suppress the SOS network, became more susceptible to bactericidal antibiotics such as ofloxacin [102]. In another approach, temperate bacteriophages were used to deliver genes *rpsL* and *gyrA* that rendered *E. coli* susceptible to two antibiotics, streptomycin and nalidixic acid [103]. In a similar way, phagemids, plasmids carrying an ORI from a phage, were used to deliver small regulatory RNAs inside drug-resistant bacteria. This, too, resulted in resistant bacteria such as *P. aeruginosa* becoming susceptible to conventional antibiotics [104]. As the bacterium evolved resistance to bacteriophage OMKO1, changes in its efflux pump mechanism was observed which, in turn, made it susceptible to antibiotics [105]. Phagemids were also engineered to express antimicrobial peptides and toxins. After phagemid infection, expression of AMPs/toxins interfered with bacterial processes, ultimately leading to death [106]. This rapid

growth of genetic engineering is expected to bring about swift changes in this field of antimicrobial therapy. Numerous bacteriophages are currently used clinically or under clinical trials, and have been summarized in Table 2. Like most other fields, phage therapy also has several problems to address (as mentioned earlier, Table 1) but the incorporation of newer technologies might be a way forward.

Lysins (Endolysins, Exolysins, and Autolysins)

Bacteriophages burst the bacterial cell open using enzymes (mostly peptidoglycan hydrolases) termed endolysins that digest the bacterial cell wall prior to release [107,108]. Phage endolysins are similar to bacterial exolysins (produced by bacteria to kill cells of other strains or species) and autolysins (functional during remodeling of peptidoglycans during cell growth and division) [109–111]. These are attractive candidates for alternative therapy because of their direct antibacterial activity. Since these enzymes are genetically encoded, they are amenable to production using genetic engineering. A detailed description of such enzymes is beyond the scope of this review; however, interested readers will find several papers describing structure and function of such lysins for antimicrobial therapy [107,108,112,113]. Indeed, two of the leads in this category are undergoing clinical trials. Intron Biotech's N-Rephasin® SAL200 and ContraFect's CF-301 are in Phase IIa and Phase I, respectively, for treating *S. aureus* bacteremia.

CRISPR-Cas 9

Ever since its discovery, the CRISPR (clustered, regularly interspaced, short palindromic repeats)-Cas9 (CRISP-associated protein 9) system has taken the world by storm [114,115]. These are key components of a bacterial immune system wherein a 20 nt small RNA acts as a guide for Cas9 to cleave foreign genetic elements, such as those present in plasmids and phages, at specific sites. The CRISPR-Cas9 system has been used in a variety of biological applications which can be found in some excellent reviews [114,116,117]. The benefits of the CRISPR system have also been extrapolated to the field of antimicrobial therapeutics [118,119].

In a landmark paper, Citorik *et al*. used the CRISPR-Cas9 system to target multidrug-resistant bacteria [120]. They used bacteriophages or bacterial plasmids to deliver novel RNA-guided nucleases (RGNs) to DNA sequences encoding virulence and antibiotic resistance in carbapenem-resistant Enterobacteriacae and enterohemorrhagic *E. coli*. In a *Galleria mellonella* infection model, this system significantly increased survival of worms infected with enterohemorrhagic *E. coli*. The introduction of RNA-guided nucleases also silenced antibiotic-resistant bacteria in a complex bacterial population, which potentially allows for programing or remodeling of the microbiota [120].

Bikard *et al*. reported sequence-specific antimicrobials by exploiting phagemid delivered CRISPR-system that could selectively kill virulent, but not avirulent, strains of *S. aureus* [121]. In this pioneering work, staphylococcal plasmids containing drug-resistance genes were selectively destroyed, thereby preventing horizontal transfer of virulence. This strategy was also validated in a murine model of skin infection. In another strategy, temperate phages were used to deliver the CRISPR-Cas system into the genome of antibiotic-resistant bacteria [122]. The delivered CRISPR-Cas system destroyed not only the antibiotic-resistance-conferring plasmid but also genetically modified lytic phages. This strategy has potential uses in surfaces prone to bacterial contamination/adhesion, such as hospital worktops. Overall, CRISPR-Cas-based systems offer an excellent alternative to antibiotics [123,124]. However, their industrial scalability remains a question.

Antibiotic Inactivators

The use of antibiotics disrupts the gut microbiome, thereby providing an opportunity for the outgrowth of pathogenic strains. In order to reduce this possibility, antibiotic inactivators could be used. Ribaxamase is an engineered β-lactamase which can degrade all classes of β-lactam antibiotics. It was designed to be administered orally during treatment with intravenous β-lactam antibiotics and is currently under clinical trials (Phase IIb) to prevent *C. difficile* infections [125].

Concluding Remarks

Numerous alternatives to conventional antibiotics have been developed to combat antimicrobial resistance and treat bacterial infections. Some of the approaches have progressed while others are still at the laboratory level. The approval of bacteriophages for treatment and prophylaxis of infections, and for use in the food industry, exhibits potential for use as an alternative to antibiotics. Progress in genetic engineering offers significant options for further improvement. Antimicrobial peptides have attracted a lot of attention in the last two decades but have not lived up to the expectations. Although the synthetic membrane-active agents bear promise as topical agents, the real challenge is to have candidates for systemic infections. Antibodies have been approved for the treatment and prophylaxis of few common bacterial infections. Their extensive use is, however, limited by the cost of production and limited shelf life. Most of the alternative approaches are strain- or species-specific, as opposed to the broad-spectrum activity of conventional antibiotics. Multiple therapeutics would therefore be needed for the treatment of different infections. This poses a major drawback to their further development, which is also hindered by the poor return on investment. Although some progress has been made, the current status indicates that alternative approaches can only partially replace antibiotics. Future studies must investigate the efficacy of combining conventional antibiotics with one of these alternative therapies to fully assess their potential. In closing, the field is in its nascent stage and further research must be invested for development of next-generation anti-infectives.

Outstanding Questions

Can genetically modified bacteriophages be produced on a commercial scale to tackle a broad range of bacterial infections?

Can the advancement in gene editing technologies, such as CRISPR-Cas9 technology, circumvent some of the limitations of bacteriophage therapy and open up new avenues?

Would any synthetic mimic of the antimicrobial peptides, possessing ideal pharmacokinetic properties, that can treat systemic bacterial infections ever be approved for clinical use?

Can antibiotics be used in combination with immunomodulatory agents for tackling bacterial infections?

Is there a way to increase the shelf life and decrease the cost of production of antibacterial antibodies?

Resources

[i]https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

[ii]www.combioxin.com/projects.html

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