Narrative review

Current landscape in the discovery of novel antibacterial agents

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ABSTRACT

Background: Standard treatments against bacterial infections are becoming ineffective due to the rise of antibacterial resistance worldwide. Classical approaches to develop new antibacterial agents are not sufficient to fulfill the current pipeline, therefore new strategies are currently being devised in the field of antibacterial discovery.

Objectives: The objective of this narrative review is to compile the most successful strategies for drug discovery within the antibacterial context that are currently being pursued.

Sources: Peer-reviewed publications from the MEDLINE database with robust data addressing the discovery of new antibacterial agents in the current pipeline have been selected.

Content: Several strategies to discover new antibacterials are described in this review: (i) derivatives of known antibacterial agents; the activity of a known antimicrobial agent can be improved through two strategies: (a) the modification of the original chemical structure of an antimicrobial agent to circumvent antibacterial resistance mechanisms and (b) the development of a compound that inhibits the mechanisms of resistance to an antibacterial agent; (ii) new antibacterial agents targeting new proteins; (iii) inhibitors of virulence factors; (iv) nanoparticles; (v) antimicrobial peptides and peptidomimetics; (vi) phage therapy and enzybiotics; and (vii) antisense oligonucleotides.

Implications: This review intends to provide a positive message affirming that several different strategies to design new antibacterial agents are currently being developed, and we are therefore confident that in the near future some of the most promising approaches will come to fruition.

Introduction

The development of new therapeutic strategies seems to have reached a dead end. Despite the urgent need to find new antibacterial products, many pharmaceutical companies, including a significant number of large companies, have abandoned new antibiotic research programmes, investing their research and development resources in other therapeutic areas [1]. Besides private efforts, research groups at the hospital or academic level outside the industry may play an important role in discovering new antibiotics. This narrative review describes the major strategies implemented to design and develop new antibacterial agents.

Improving known antibacterial agents

The activity of a known antimicrobial agent can be improved through two strategies. First, modification of the basic chemical structure of an antimicrobial agent, such as tigecycline, which circumvents antibacterial resistance mechanisms. It is a derivative of minocycline with a 9-tert-butyl-glycylamido side chain added to the D ring at the ninth position of the molecule, which avoids the effect of specific tetracycline efflux pumps or ribosomal protection, two of the mechanisms of tetracycline resistance [2]. Cefiderocol can also be considered a cephalosporin-derivative as it has been linked to a siderophore that helps to reach the periplasmic space and has enhanced stability to β-lactamases. It shows good activity against Enterobacteriaceae and non-fermenters such as Pseudomonas aeruginosa and Acinetobacter baumannii, and it is currently in Phase III studies (Table 1). Second, compounds inhibiting the mechanisms of resistance to an antibacterial agent; in this regard,
several approaches such as new β-lactamase inhibitors are being used [3]. Two main groups of β-lactamase inhibitors are being developed: the diazabicyclococtane group (i.e. avibactam or relebactam) and the boronate β-lactamase inhibitors group. The diazabicyclococtane group inhibits class A, class C and some class D enzymes, but does not show inhibition of metallo-β-lactamases, class B β-lactamases; however, the combination with aztreonam also covers metallo-β-lactamase-producing Enterobacteriaceae, because aztreonam has activity against the bacteria producing metallo-β-lactamases. The main example of the boronate β-lactamase inhibitors group is vaborbactam, which also does not show inhibition of class B and D β-lactamases and is combined with meropenem. Inhibitors of efflux pumps allowing the antibiotic to accumulate in the bacterial cell are being developed. Some examples include phenylalanine-arginine-β-naphthylamide or the most recent indole-2-carboxamides. Nevertheless, none of these inhibitors have reached the clinical trial stage, mainly due to toxicity. Another area of research is the development of inhibitors of RecA, which plays an important role in the SOS response and has been shown to potentiate antibiotic activity and block the evolution of antibiotic resistance [4,5].

New antibacterial agents targeting new proteins

Although 30 antibacterial agents are currently in the pipeline [6], few are actually considered new (Table 1). It was thought that the advent of bacterial genomics would open the door to the discovery of new antibiotics. However, although it is true in part that the search for essential targets using computational analysis is feasible, finding an in vitro inhibitor of these protein targets is difficult and faces development hurdles such as limitations in penetrating the bacteria. Therefore, there has been no success with this approach.

The traditional pathway of identifying microorganisms from a rich ecological niche that produce an antibiotic as a secondary metabolite still has potential for the discovery of new antibiotics. Moreover, some authors are trying to find microorganisms producing antibiotics from recondite niches such as marine samples (invertebrates or algae), insects and invertebrate organisms (e.g. symbionts and plants) [7]. An alternative to this approach is searching for new antibacterial compounds from the metabolism of microorganisms present in the human microbiota or from the microbiome of different sources; in this regard lurgdin, a macrocyclic thiazolidine peptide antibiotic produced by Staphylococcus lugdunensis, has been shown to be active against a group of Gram-positive pathogens including Staphylococcus aureus [8]. However, the mode of action is unknown. Regarding the microbiome, there are two approaches: (a) the capture of biosynthetic gene clusters from whole metagenomic DNA, and (b) the prediction of natural product structures from primary sequence data by means of bioinformatic tools and their production by chemical synthesis. These approaches have led to the discovery of two molecules: tetarimycin and humimycin. The former is a tetracyclic antibiotic that is active against methicillin-resistant S. aureus from the soil microbiome and the latter inhibits lipid II flipase and shows activity against Gram-positive bacteria including S. aureus and Streptococcus pneumoniae and interesting synergy with some β-lactam antibiotics [9,10].

Virulence blockers

An alternative to the classical approach of drug development is to affect pathogenicity by targeting specific virulence factors involved in this process. This strategy aims to prevent the bacterium developing resistance and so contain the spread. Molecules interfering with virulence factors will disarm the pathogen, thereby allowing bacterial clearance by the host immune system. There is a myriad of factors involved in bacterial virulence that are being investigated as targets for new agents including the following categories:

1. Determinants involved in host cell attachment inhibiting access and translocation into the host tissue. Molecules targeting fimbra, such as the FinH antagonist mamosides and the antibody scFv-Fc KP3 targeting type 3 fimbrial subunit [11–13], have shown good in vivo effects in mouse models (Table 2). Pilicides, pili formation inhibitors, and the glycosylated molecules mucins are in the discovery phase [11,12,14].

2. Actors involved in host immune modulation. Lipid A inhibitors include LpxC-1 [15], the substituted sulphone-based hydroxamates with good in vitro efficacy [16] and ACHN-975, having failed Phase I [17] (Table 2). Another molecule, erianin, a Sortase A inhibitor that interferes in host immune recognition and attachment in host surfaces, affects virulence in S. aureus murine infections [18].

3. Biofilm modulation (limiting adhesion, affecting the extracellular matrix and disturbing mature biofilm). A number of small molecule inhibitors have been identified and recently reviewed [19]. Agents of natural origin such as flavonolignans and streptorubin B [20], cyclosporine and its derivative valspodar, have been shown to be good antibiofilm agents [14,21]. AR-105 entered the Phase II clinical phase as an adjunctive treatment [6,22,23] (Table 2).

4. Global regulators of virulence. These include anethole and SE-1, tested in vivo and in vitro, respectively [24,25] (Table 2). Inhibition of two-component systems has also been shown to block the pathogenesis of clinically relevant bacteria [14], although only savirin and LED209 have shown good in vivo results [26,27] (Table 2).

5. The quorum sensing network, which mediates bacterial communication and is key in the infection process. Quorum sensing quenching includes the acyl-homoserine lactone lactonases effective against P. aeruginosa. The main advantage of this approach is

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**Table 1**

<table>
<thead>
<tr>
<th>Antibacterial agent</th>
<th>Type of drug</th>
<th>Target</th>
<th>Phase</th>
<th>Pharmaceutical Co.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRS3123&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Diarylamine</td>
<td>Methionyl-tRNA synthetase</td>
<td>I</td>
<td>Crestone Inc.</td>
</tr>
<tr>
<td>MGB-BP-3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Distamycin</td>
<td>DNA minor groove binder</td>
<td>I</td>
<td>MGB Biopharma</td>
</tr>
<tr>
<td>CG400549&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Benzyl-piridinone</td>
<td>Biosynthesis fatty acids (FabI)</td>
<td>II</td>
<td>Crystal Genomics</td>
</tr>
<tr>
<td>Afabicina&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Benzofuran naphthyridine</td>
<td>Biosynthesis fatty acids (FabI)</td>
<td>II</td>
<td>Debilpharm Int.</td>
</tr>
<tr>
<td>Murepavadin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Peptidomimetic</td>
<td>LpxD</td>
<td>III</td>
<td>Polyphor AG</td>
</tr>
<tr>
<td>Cefiderocol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Siderophore-cephalosporin</td>
<td>PBP</td>
<td>III</td>
<td>Shionogi Co.</td>
</tr>
</tbody>
</table>

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<sup>a</sup> These drugs are not active against bacteria from the ESKAPE group.
<sup>b</sup> This drug is specific for Pseudomonas aeruginosa and is discussed in the subheading concerning peptides and peptidomimetics of this review.
<sup>c</sup> Hybrid molecule active against bacteria in the ESKAPE group. It is not actually a new antibacterial agent.
Table 2
Description of virulence blockers in the research pipeline of new antibacterial agents

<table>
<thead>
<tr>
<th>Virulence categories</th>
<th>Agent</th>
<th>Action</th>
<th>Bacterial target</th>
<th>Infectious disease targeted</th>
<th>Current stage</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell attachment</strong></td>
<td></td>
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<tr>
<td>Pilicides (bicyclic 2-pyridones)</td>
<td>Inhibition of pilus formation/ biogenesis and regulation Host receptor analogues inhibiting FimH of type 1 fimbriae</td>
<td>Uropathogenic Escherichia coli (UPEC) Uropathogenic E. coli</td>
<td>Urinary tract infections caused by UPEC Urinary tract infection caused by UPEC</td>
<td>Discovery</td>
<td>[11,12]</td>
<td></td>
</tr>
<tr>
<td>Mannosides (FimH antagonist)</td>
<td>Targeting type 3 fimbrial subunit in Klebsiella pneumoniae</td>
<td>K. pneumoniae</td>
<td>K. pneumoniae infections</td>
<td>Preclinical[1]</td>
<td>[74]</td>
<td></td>
</tr>
<tr>
<td>scFv-Fk K3 (synthetic antibody)</td>
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<tr>
<td>Mucins</td>
<td>Interference with bacterial adhesins (mimic host cell receptor glycosylation)</td>
<td>E. coli, Salmonella spp., Helicobacter pylori, Staphylococcus aureus and Bacillus subtilis Acinetobacter baumannii</td>
<td>Several Gram-positive and Gram-negative infections</td>
<td>Discovery</td>
<td>[14]</td>
<td></td>
</tr>
<tr>
<td><strong>Immune modulation of the host</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LpxC-1</td>
<td>Inhibition of the lipid A biosynthetic enzyme LpxC</td>
<td>K. pneumoniae, Pseudomonas aeruginosa and E. coli</td>
<td>K. pneumoniae, P. aeruginosa and E. coli infections</td>
<td>Phase I (interrupted)[4]</td>
<td>[17]</td>
<td></td>
</tr>
<tr>
<td>ACHN-975</td>
<td>Inhibition of the lipid A biosynthetic enzyme LpxC</td>
<td>K. pneumoniae, E. coli, Enterobacter aerogenes and Citrobacter freundii</td>
<td>Infections caused by K. pneumoniae, E. coli, Enterobacter aerogenes and Citrobacter freundii</td>
<td>Preclinical[4]</td>
<td>[16]</td>
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</tr>
<tr>
<td>Substituted sulphone-based hydroxamates</td>
<td>Inhibition of the lipid A biosynthetic enzyme LpxC</td>
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<tr>
<td>Eriain</td>
<td>Sortase A inhibitor (Sortase A anchors cell surface molecules involved in pathogenesis in Gram-positive bacteria)</td>
<td>S. aureus</td>
<td>S. aureus infections</td>
<td>Preclinical[4]</td>
<td>[18]</td>
<td></td>
</tr>
<tr>
<td><strong>Biofilm modulators</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Hydnocarpin-type flavonolignans (isolated from Silybum marianum)</td>
<td>Inhibition of the icaADBC-dependent biofilm formation pathway</td>
<td>S. aureus</td>
<td>S. aureus-mediated biofilm infections</td>
<td>Discovery</td>
<td>[75]</td>
<td></td>
</tr>
<tr>
<td>Streptorubin B (isolated from actinobacteria)</td>
<td>Unknown</td>
<td>Methicillin-resistant S. aureus Streptococcus pyogenes</td>
<td>S. aureus-mediated biofilm infections Streptococcus pyogenes infections</td>
<td>Discovery</td>
<td>[20,21]</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine and valsoprodar (cyclosporine-derivative)</td>
<td>Inhibition of the Rgg2/Rgg3 regulatory system</td>
<td>P. aeruginosa</td>
<td>Ventilated-acquired pneumonia caused by P. aeruginosa (Adjuvantive treatment)</td>
<td>Phase II</td>
<td>[6,22,23]</td>
<td></td>
</tr>
<tr>
<td>AR-105 (monoclonal antibody)</td>
<td>Blockage of the polysaccharide alginate (surface polysaccharide of P. aeruginosa involved in biofilm formation and adhesion</td>
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<tr>
<td><strong>Global regulators</strong></td>
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<tr>
<td>Anethole (natural compound)</td>
<td>Repression of the production of the cholera toxin and the toxin co-regulated pilus</td>
<td>Vibrio cholerae</td>
<td>Vibrio cholerae infections</td>
<td>Preclinical[4]</td>
<td>[24]</td>
<td></td>
</tr>
<tr>
<td>SE-1</td>
<td>Inhibition of VirF expression</td>
<td>Shigella flexneri</td>
<td>Shigellosis</td>
<td>Discovery[4]</td>
<td>[25]</td>
<td></td>
</tr>
<tr>
<td>Savirin</td>
<td>Inhibition of the transcriptional regulator AgrA (affects the regulatory cascade including hla, psm alpha, pvi, flhK, agrA and agrC)</td>
<td>S. aureus</td>
<td>Skin and soft-tissue infections caused by S. aureus</td>
<td>Preclinical[4]</td>
<td>[26]</td>
<td></td>
</tr>
<tr>
<td>LED209</td>
<td>Blockage of autophosphorylation of the sensor kinase QseC (involved in the regulation of virulence gene expression as motility via flhDC operon in E. coli or regulation of the pathogenicity island LEE in enterohaemorrhagic E. coli and involved in virulence in Salmonella Typhimurium and Franciscella tularensis)</td>
<td>E. coli, Salmonella Typhimurium and Franciscella tularensis</td>
<td>Infections caused by E. coli, Salmonella Typhimurium and Franciscella tularensis</td>
<td>Preclinical[4]</td>
<td>[14,27,76,77]</td>
<td></td>
</tr>
<tr>
<td><strong>Quorum-sensing network</strong></td>
<td>Acyl-homoserine lactone lactonases</td>
<td>Targeting the acyl-homoserine lactones (quorum sense signals)</td>
<td>P. aeruginosa and A. baumannii P. aeruginosa -mediated biofilm infections</td>
<td>Discovery</td>
<td>[78]</td>
<td></td>
</tr>
<tr>
<td><strong>Toxins</strong></td>
<td>MED14693 (monoclonal antibody)</td>
<td>Binding to α-toxin of S. aureus</td>
<td>Diabetic foot ulcers infected by S. aureus</td>
<td>Completed Phase II[4]</td>
<td>[29]</td>
<td></td>
</tr>
</tbody>
</table>
that modulation of one quorum sensing system allows interference in other systems [28].

6. Toxins secreted by pathogenic bacteria required for bacteria-host interactions and evasion of the immune system. The anti-z-toxin antibody S. aureus (MEDI4893) that completed the Phase II trial in 2018 (results not yet available) is promising [29].

7. Bacterial functional membrane microdomain-associated proteins related to signalling networks. Small molecules interfering with the metabolic pathway of polysaccharide lipid biosynthesis have been shown to attenuate bacterial virulence. Zaragozic acid alters oligomerization of the penicillin-binding protein PBP2a in methicillin-resistant S. aureus reverting the resistant phenotype [28].

8. Type three secretion system, a major Gram-negative virulence factor that allows secretion of effector proteins involved in pathogenicity. Inhibitors of this system include licoflavonol in Salmonella Typhimurium [31] and salicylidenec acylhydrazides active against infections of Chlamydia trachomatis [32] (Table 2).

9. Liposomes interfering in the progression of infection. CAL02 has completed the Phase I trial [33] and improved outcomes were shown as a combination therapy in mice [34] (Table 2). One of the advantages of anti-virulence agents is the preservation of the host’s microbiome as commensal bacteria often lack the features targeted by these agents. In terms of drug development, although to date no anti-virulence agent has entered clinical study phases, it is most likely that larger clinical trials will be needed to prove their therapeutic efficacy as adjuvants (as may be the case for other agents under other approaches also discussed in this work) of current antibiotic treatments when effective treatments are available. Additionally, it is expected that the administration of a combination of several anti-virulence agents will be required and will effectively attenuate the bacteria. Another hurdle lies in the fact that administration of the anti-virulence drug must be in concordance with the time at which the targeted factor is expressed. Finally, as one of the features of these molecules is that the effectiveness is dependent on the immune host response, these therapies will not be adequate to treat immunocompromised patients.

### Nanoparticles

Nanoparticles (NPs) are defined as particles or materials within the nanometer scale [35]. Although some metals like silver or copper have antibacterial activity in their bulk form, others only have it as NPs against bacteria. The mechanisms of action of these particles have not been completely described, but three processes are hypothesized to occur concomitantly: induction of oxidative stress, non-oxidative mechanisms and in a minor way, interaction of released metal ions with functional groups of proteins and nucleic acids [36,37].

Specific factors such as size, zeta potential (electrokinetic potential), charge, surface morphology and crystal structure determine metal NP antimicrobial activity [37]. NPs can both disrupt bacterial membranes and hinder the formation of biofilms. Smaller NPs provide greater biofilm inhibition (e.g. Ag, ZnO, Mg or NO NPs).
and rod-shaped NPs are better at inhibiting biofilms than spherical NPs [38]. Cytotoxicity of NPs is a drawback and must be carefully regarded. ZnO and Ag NPs have been described as cytotoxic at bacterial inhibitory concentrations. To overcome this issue, it has been proposed that NPs must be delivered locally at the infection site to confine the NPs and their harmful effects to eukaryotic cells [36].

Antimicrobial peptides and peptidomimetics

Antimicrobial peptides (AMPs) are ubiquitous immune effectors that aid the host in fighting pathogens. Although the classically proposed mechanism of action is membrane permeabilization, other mechanisms, including inhibition of protein, DNA and RNA synthesis, and gene material degradation, also take place. Their proposed mechanism of action is membrane permeabilization, that aid the host in fighting pathogens. Although the classically proposed mechanism of action is membrane permeabilization, other mechanisms, including inhibition of protein, DNA and RNA synthesis, and gene material degradation, also take place. Their activity is based on their composition and secondary structure [39].

The AMPs can be classified based on their secondary structure into α-helical AMPs (e.g. cathelicidins), β-sheet-containing AMPs (e.g. α- or β-defensins), AMPs with a β-hairpin or loop stabilized by a single disulphide bond or cyclization of the peptide chain (e.g. thanatin) and short AMPs with extended conformations (e.g. indolicidin) [40].

As a result of their mechanism of action, it has been proposed that these molecules are synergistic in combination with antibiotics that have difficulty in penetrating bacteria or when the resistance mechanism to that antibiotic is related to membrane modification [41].

Antimicrobial peptides usually fail preclinical studies because of low stability or high in vivo toxicity. In the last decades few natural AMPs have been commercialized, none of which was a linear peptide [40]. Most of the AMPs that continue in clinical trials are for topical use. The following examples represent promising AMPs that have undergone clinical trials with different applications: OP-145 completed Phase II [40,42–44], two AMPs targeting C. difficile, surotomycin which was discontinued after two Stage III studies [45–47] and NVB-302, has completed Phase I [40,48–50].

Peptidomimetics are defined as sequences purposely designed to mimic a peptide or its function but no single α-amino acid makes up the backbone structure. These sequences usually have enhanced in vivo stability and lower toxicity than usual α-helical AMPs [40]. Among the different peptidomimetics, ceragenins are resistant to proteases and are easy to produce on a large scale [51]. Two of the most active are: CSA-131, which is active against colistin-resistant A. baumannii, P. aeruginosa and Klebsiella pneumoniae strains and anaerobic bacteria [52–55] and CSA-13 with good antibiofilm activity [56,57] (Table 4).

Murepavadin, which belongs to a novel class of outer membrane protein [6], is of special interest, although it was recently halted in Phase III [41,58–60] (Table 4).

Although it has been suggested that there is little to no resistance to AMPs (and/or to peptidomimetics), cross-resistance can arise when experimentally exposing S. aureus against pexiganan [61].

Table 3 Description of antibacterial agents in the research pipeline following alternative strategies

<table>
<thead>
<tr>
<th>Category</th>
<th>Agent</th>
<th>Action</th>
<th>Bacterial target</th>
<th>Infectious disease targeted</th>
<th>Current stage</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobial peptides and peptidomimetics</td>
<td>OP-145 (AMP60.4Ac or P60.4Ac; based on LL-37)</td>
<td>Surotomycin Membrane depolarization</td>
<td>Clostridium difficile</td>
<td>Chronic middle ear infection</td>
<td>Phase II</td>
<td>[40,42–44]</td>
</tr>
<tr>
<td></td>
<td>NVB-302 (lantibiotic; polycyclic peptide containing thioether amino acids)</td>
<td>Inhibition of cell wall biosynthesis by lipid II binding</td>
<td>C. difficile and wide range of Gram-positive bacteria</td>
<td>Infectious diarrhoea associated with C. difficile</td>
<td>Discontinued Phase III a</td>
<td>[45–47]</td>
</tr>
<tr>
<td></td>
<td>Murepavadin (POL7080; cyclic protegrin analogue)</td>
<td>Outer membrane biogenesis</td>
<td>Pseudomonas aeruginosa</td>
<td>Ventilator-associated bacterial pneumonia (Pseudomonas infections)</td>
<td>Completed Phase I</td>
<td>[48–50]</td>
</tr>
<tr>
<td></td>
<td>CSA-13</td>
<td>Antibiofilm activity; bacterial membrane</td>
<td>Mixed P. aeruginosa and Staphylococcus aureus biofilm and streptococci biofilms</td>
<td>Infections caused by P. aeruginosa, S. aureus and streptococci biofilms</td>
<td>Discovery</td>
<td>[52–55]</td>
</tr>
<tr>
<td>Antisense oligonucleotides (Gene expression inhibitors)</td>
<td>CPP-PMO conjugate</td>
<td>Gene expression inhibition of gyrA</td>
<td>Enterococcus faecalis and S. aureus (gyrA) and Listeria monocytogenes (rpoA)</td>
<td>Enterococcus faecalis and S. aureus infections</td>
<td>Discovery</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td>CPP-PNA conjugate</td>
<td>Gene expression inhibition of rpoA</td>
<td>Enterococcus faecalis and S. aureus (rpoA) and Listeria monocytogenes (rpoA)</td>
<td>Enterococcus faecalis and S. aureus infections</td>
<td>Preclinical</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td>PNA</td>
<td>Gene expression inhibition of polA</td>
<td>Brucella suis (polA)</td>
<td>B. suis infection</td>
<td>Discovery</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td>PNA conjugate</td>
<td>Gene expression inhibition of ftsZ</td>
<td>S. aureus (ftsZ)</td>
<td>S. aureus infection</td>
<td>Discovery</td>
<td>[72]</td>
</tr>
</tbody>
</table>

a Surotomycin did not show superiority for clinical response or sustained clinical response versus vancomycin and failed to achieve non-inferiority for clinical cure at end of treatment [46,47].

b Two Phase III studies were suspended due to renal toxicity [59,60].
Phage therapy and enzybiotics

The use of lytic phages has been restricted to Eastern European countries, particularly Georgia and Poland where phage cocktails are commercially available (Table 4). Regarding Western European countries, a study called Phagoburn was conducted in Belgium, France and Switzerland from 2013 to 2017 to evaluate phage therapy for treating burn wounds infected with *E. coli* and *P. aeruginosa* [62] (Table 4). Additionally, the ambitious Phage 4 cure project, is currently ongoing in Germany and includes the development of inhalable bacteriophages to treat *P. aeruginosa* infections from manufacturing to preclinical studies following international quality standards [63,64] (Table 4).

Another antibacterial approach, the so-called enzybiotics, involves the use of phage-derived enzymes to specifically attack different species or even bacterial serotypes. These lysins were first described in the 1960s and act by degrading peptidoglycan and inducing bacterial lysis by osmotic imbalance and have shown good antibacterial activity. Endolysins, in particular Cpl-711, have shown good results when administered in mice previously challenged with *Streptococcus pneumoniae* [65] (Table 4). Alternatively, poly-saccharide depolymerases are also currently being studied as they degrade the carbohydrates of bacterial membranes. Hence, the use of this family of enzymes in the disruption of biofilms and against encapsulated bacteria has generated enormous interest [63,65,66].

Although phage therapy is seen as a potentially promising alternative to fight against antimicrobial-resistant pathogens, there are still several hurdles to overcome. One is pharmacokinetics, as high doses of phages are needed to eliminate a bacterial population (even small communities) because they have to replicate inside the host cell to exert their bactericidal effect. In terms of host response, considerations regarding immune reaction, through neutralizing antibodies, derived from the action of bacteriophages, must also be considered. Finally, the threat of the rise of bacterial resistance to bacteriophages should be taken into account and one strategy to overcome this issue lies in the combination of phages with classical antibiotics. Regarding enzybiotics, the main limitation is their weak stability and lack of solubility, requiring the need for chemical engineering.

### Antisense oligonucleotides

Oligonucleotides can be used to inhibit gene expression both in eukaryotes and prokaryotes. These molecules act on different levels in the gene expression regulation pathways. Depending on their mechanism these molecules are classified as transcription process inhibitors (e.g. triplex-forming oligonucleotides aimed against DNA), translation process inhibitors (e.g. antisense oligonucleotides, small interfering RNAs, ribozymes and microRNAs; aimed at mRNA) and oligonucleotides blocking protein activity (e.g. aptamers or decoy oligonucleotides for transcription factors).

Antisense oligonucleotides are single-stranded DNA mimicking oligomers of around 20 nucleotides that bind mRNA to modulate gene expression but do not affect nucleotide translation [67]. The most commonly investigated antisense oligonucleotides are: (a) phosphorothioate oligodeoxynucleotides (S-oligos); (b) locked nucleic acids; (c) peptide nucleic acid (PNAs); and (d) phosphorodiimidate morpholino-oligomers (PMOs) [68]. Antisense oligonucleotides can be used to fight antimicrobial resistance by inhibiting essential gene expression through RNA silencing. The main drawback of this strategy is achieving high enough concentrations inside the bacterium, which has been addressed using cell-penetrating peptides (CPPs) that aid in the effective intracellular delivery of the oligomers.

The potential of antisense oligonucleotides as antimicrobials has been shown by different research groups (e.g. CPP-PMO [69], CPP-PNA [70], PNA targeting poIA [71] and PNA conjugates [72]) (Table 3).

### Conclusion

Although as seen in this review there are currently several strategies being carried out for the discovery of new antibacterial agents, the time is not yet ripe for complacency. Therefore, more traditional and non-traditional approaches are needed to ensure a future with effective treatments against infectious diseases caused by multidrug-resistant bacteria. To make this possible, more funding opportunities are needed for public research in the field (current programmes such as Carb-X and ENABLE have been shown to be insufficient) and new incentives are necessary to induce the industry to return to the discovery of antibacterial agents. In this sense, a ‘subscription’ style payment model, such as the one that the United Kingdom recently announced [73], could be an interesting strategy to be followed up.

### Transparency declaration

The authors declare that they have no conflicts of interest.

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