Current landscape in the discovery of novel antibacterial agents

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Running heading: Discovery of novel antibiotics

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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 fulfil the current pipeline, therefore new strategies are currently being ~~conducted~~ devised in the field of antibacterial discovery.

Objectives: The objective of this narrative review is to compile the most successful strategies in which research on drug discovery within the antibacterial context is currently ongoing.

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 discovery new antibacterial are described in this review, such as: i. Derivatives of known antibacterial agents. The activity of a known antimicrobial agent can be improved through two strategies: a) Modification of the original chemical structure of an antimicrobial agent which circumvents antibacterial resistant mechanisms, and b) 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 (AMPs) 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 programs, investing their R&D 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: i) 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 since it has been linked to a siderophore which 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). ii) Compounds inhibiting the mechanisms of resistance to an antibacterial agent. In this regard, several approaches such as new β-lactamases inhibitors (BLIs) are being used [3]. Two main groups of BLIs are being developed: firstly, the diazabicyclooctane group (i.e., avibactam or relebactam) which

does not show inhibition of metallo-β-lactamases (MBLs); however, the combination with aztreonam cover also MBL-producing *Enterobacteriaceae,* since aztreonam has activity against the bacteria producing MBLs; secondly, the boronate BLI group. The main example of this group is vaborbactam, which also does not show inhibition of MBLs 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-b-naphthylamide (PAβN) 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 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, while 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.

However, the traditional pathway of identifying microorganisms from a rich ecological niche producing 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 human microbiota or from the microbiome of different sources; in this regard lugdunin, a macrocyclic thiazolidine peptide antibiotic produced by *Staphylococcus lugdunensis,* has 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: i. The capture of biosynthetic gene clusters from whole metagenomic DNA, and ii. 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 active against methicillin-resistant *S. aureus* (MRSA) from soil microbiome and the latter inhibits lipid II flipase and shows activity against Gram-positive bacteria including *S. aureus* and *Streptococcus pneumonia* 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 , prvent the bacterium to develop resistance and thus 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 fimbria, such as the FimH antagonist

mannosides , and the antibody scFv-Fc KP3 targeting type 3 fimbrial subunit [11–13], have shown good *in vivo* effects in mice models (Table 2). Pilicides, pili formation inhibitors and the glycosylated molecules mucins are in discovery phases [11,12,14].

2. Actors involved in host immune modulation. Lipid A inhibitors include LpxC-1 [15], the substituted sulfone-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 which 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 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 have also 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 (QS) network, which mediates bacterial communication and is key in the infection process. QS quenching includes the acyl-homoserine lactone lactonases effective against *P. aeruginosa.* The main advantage of this approach is that modulation of one QS 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 α–toxin antibody *S. aureus* (MEDI4893) that

completed the Phase II trial in 2018 (results not yet available) is promising [29] (Table 2). Another interesting case is the mAb targeting toxin B from *Clostridiodes difficile* (Bezlotoxumab) the first FDA-approved anti-virulence agent to be used in combination with current therapy to prevent recurrent *C. difficile* infections[30].

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

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

9. Liposomes interfering in the progression of infection. CLA02 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 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, since 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 yet, but three processes are hypothesized to occur concomitantly: induction of oxidative stress, non-oxidative mechanisms and in a minor way, interaction of released metal ion 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].

NP cytotoxicity 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 (AMPs) and peptidomimetics***

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 activity is based on their composition and secondary structure[39].

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 cyclisation of the peptide chain (e.g., thanatin) and short AMPs with extended conformations (e.g., indolicidin) [40].

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

AMPs usually fail preclinical studies due to 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 Phase 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].

Amongst 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 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).

While 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].

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 bacteria. Endolysins, in particular Cpl-711, have recently shown good results when administered in mice previously challenged with *S. pneumoniae*[65] (Table 4). Alternatively, polysaccharide depolymerases are also currently being studied as they degrade carbohydrates of bacterial membranes. Thus, 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 bacterial population (even small communities) since 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 oligos 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: i) phosphorothioate oligodeoxynucleotides (S-oligos); ii) locked nucleic acids; iii) peptide nucleic acid (PNAs); iv) phosphorodiamidate 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 concentrations high enough 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 *polA* [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 programs such as Carb-X and ENABLE have demonstrated 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 [84] could be an interesting strategy to be followed-up.

Transparency declarations.

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Table 2. Description of virulence blockers in the research pipeline

Virulence categories	Agent	Action	Bacterial target	Infectious disease targeted	Current stage	References
Cell attachment	Pilicides (bicyclic 2-pyridones)	Inhibition of pili formation/biogenesis and regulation	Uropathogenic *Escherchia coli* (UPEC)	urinary tract infections caused by UPEC	Discovery	Greene_2014; Pinkner_2006
	Mannosides (FimH antagonist)	Host receptor analogues inhibiting FimH component of type I fimbriae	Uropathogenic *Escherichia coli* (UPEC)	urinary tract infection casued by UPEC	Preclinical a	Klein_2010
	ScFv-Fc KP3 (synthetic antibody)	Targeting type 3 fimbrial subunit in *Klebsiella pneumoniae*	*Klebsiella pneumoniae*	*K. pneumoniae* infections	Preclinical b	Wang_2016
	Mucins	Interference with bacterial adhesins (mimic host cell receptor glycosylation)	*Escherichia coli, Salmonella, Helicobacter pylori, Staphylococcus aureus and Bacillus subtilis*	Several Gram-positive and Gram-negative infections	Discovery	Mühlen_2016
Immune modulation of the host	LpxC-1	Inhibition of the lipid A biosynthetic enzyme LpxC	*Acinetobacter baumannii*	*A. baumannii* infection	Preclinical c	Lin_2012
	ACHN-975	Inhibition of the lipid A biosynthetic enzyme LpxC	*Klebsiella pneumoniae, Pseudomonas aeruginosa and Escherichia coli*	*K. pneumoniae, P. aeruginosa and E. coli infections*	Phase I (interrupted) d	Erwin_2016
	Substituted sulfone-based hydroxamates	Inhibition of the lipid A biosynthetic enzyme LpxC	*Klebsiella pneumoniae, Escherichia coli, Enterobacter aerogenes, and Citrobacter freundii*	Infections caused by *K. pneumoniae, E. coli, Enterobacter aerogenes* , and *Citrobacter freundii*	Preclinical e	Brown_2012
	Erianin	Sortase A inhibitor (Sortase A anchors cell surface molecules involved in pathogenesis in Gram-positive bacteria)	*Staphylococcus aureus*	*S. aureus* infections	Preclinical	Ouyang_2018
Biofilm modulators	Hydnocarpin-type flavonolignans (isolated from Silybum marianum) ref. Vimberg_2015)	Inhibition of the icaADBC-dependent biofilm formation pathway	*Staphylococcus aureus*	*S. aureus* -mediated biofilm infections	Discovery	Vimberg_2015
	Streptorubin B (isolated from actinobacteria (ref. Suzuki_2015)	Unknown	Meticillin-resistant *Staphylococcus aureus* (MRSA)	*S. aureus* -mediated biofilm infections	Discovery	Suzuki_2015
	Cyclosporine and valspodar (cyclosporine -derivative)	Inhibition of the Rgg2/Rgg3 regulatory system	*Streptococcus pyogenes*	*Streptococcus pyogenes* infections	Discovery	Aggarwal_2015
	AR-105 (monoclonal antibody)	Blockage of the polysaccharide alginate (surface polysaccharide of P. aeruginosa involved in biofilm formation and adhesion	*Pseudomonas aeruginosa*	Ventilated-acquired pneumoniae caused by *P. aeruginosa* (Adjunctive treatment)	Phase II	Theruetzbacher_2019 ; https://clinicaltrials.gov/ct2/show/NCT03027609
Global regulators	Anethole (natural compound)	Repression of the production of the cholera toxin and the toxin coregulated pilus	*Vibrio cholerae*	*V. cholerae* infections	Preclinical f	Zahid_2015
	SE-1	Inhibition of VirF expression	*Shigella flexneri*	Shigellosis	Discovery g	Koppolu_2013
	Savirin	Inhibition of the transcriptional regulator AgrA (affects the regulatory cascade including *hla, psm alpha, pvl (lukS), agrA,and agrC*..)	*Staphylococcus aureus*	Skin and soft tissue infections caused by *S. aureus*	Preclinical h	Sully_2014
	LED209	Blockage of autophosphorylation of the sensor kinase QseC (involved in the regulation of of virulence gene expression as motility via *flhDC* operon in *Escherichia coli* or regulation of the pathogenicity island LEE in *enterohemorrhagic Escherichia coli* and involved in virulence in *Salmonella Typhimurium* and *Francisella tularensis*)	*Escherichia coli, Salmonella* Typhimurium and *Francisella tularensis*	Infections caused by *E. coli, Salmonella* Typhimurium and *Francisella tularensis*	Preclinical i	Mühlen_2016 / Rasko_2008 / ref. Bearson_2008)(ref. Feldman_2015
Quorum-sensing network	Acyl-homoserine lactone lactonases	Targeting the acyl-homoserine lactones (quorum sense signals)	*Pseudomonas aeruginosa and Acinetobacter baumannii*	*P. aeruginosa* -mediated biofilm infections	Discovery	Kalaraiasan_2017
Toxins	MEDI4893 (monoclonal antibody)	Binding to α–toxin of *S. aureus*	*Staphylococcus aureus*	Diabetic food ulcers infected with *S. aureus*	Completed Phase II j	https://clinicaltrials.gov/ct2/show/NCT02296320
	Bezlotoxumab (monoclonal antibody)	Binding to toxin B from *Clostridiodes difficile*	*Clostridiodes difficile*	Prevention of recurrent *C. difficile* infections (in combination with current therapy)	Undergoing Phase III k	https://clinicaltrials.gov/ct2/show/NCT03182907?term=bezlotoxumab&rank=1
Bacterial functional membrane microdomains-associated proteins	zaragozic acid	Sterol synthesis inhibitor	Meticillin-resistant *Staphylococcus aureus* (MRSA)	*S. aureus* infections	Preclinical	Fleitas_2019
Type three secretion system	licoflavonol	Regulation of transcription of *sicA/invF* and transportation of SipC	*Salmonella* Typhimurium	*S.* Typhimurium infection	Discovery	Guo_2016
	salicylidene acylhydrazides	Targeting T3SS	*Salmonella Typhimurium and Chlamydia trachomatis*	Infections caused by *S.* Typhimurium and *C. trachomatis*	Preclinical	Duncan_2012
Toxin traps	CLA02	Liposomes acting as a toxin trap	*Staphylococcus aureus and Streptococcus pneumoniae*	Severe community-acquired *S. pneumoniae* infection	Phase I l	Laterre 2019

a Mannosides have shown significantly decreased colonization levels in UPEC and the ST131 clinical multidrug resistant strain of E. coli murine infections (13: Klein T, Abgottspon D, Wittwer M, Rabbani S, Herold J, Jiang X, Kleeb S, Lüthi C, Scharenberg M, Bezençon J, Gubler E, Pang L, Smiesko M, Cutting B, Schwardt O, Ernst B. 2010. FimH Antagonists for the Oral Treatment of Urinary Tract Infections: From Design and Synthesis to in Vitro and in Vivo Evaluation. J Med Chem 53:8627–8641.
b scFv-Fc KP3 has shown to inhibit biofilm formation and reduce bacterial burden in a mouse lung infection model (14: Wang Q, Chang C-S, Pennini M, Pelletier M, Rajan S, Zha J, Chen Y, Cvitkovic R, Sadowska A, Heidbrink Thompson J, Yu Lin H, Barnes A, Rickert K, Wilson S, Stover CK, Dall'Acqua WF, Chowdhury PS, Xiao X. 2016. Target-Agnostic Identification of Functional Monoclonal Antibodies Against Klebsiella pneumoniae Multimeric MrkA Fimbrial Subunit. J Infect Dis 213:1800–1808.

c LpxC-1 has shown to strongly attenuate *A. baumannii* virulence in mice (16)
d Phase I interrupted due to inflammation at the injection site. Undesired effects were due to the presence of essential pharmacophores (ref.Kalinin_2017)

e Shown to have problematic *in vivo* pharmacokinetic properties (ref. Brown 2012)

f Anethole showed to reduce fluid accumulation of V. cholerae in the in vivo infection model of rabbit ileal loop (ref Zahid_2015)

g SE-1 has demonstrated to significantly reduce invasion of eukaryotic cells infected with Shigella flexneri (ref_Koppolu_2013)
h Efficacy has been reported in a murine wound *S. aureus* model, interestingly not affecting the commensal *Staphylococcus epidermidis* (ref. Sully_2014)
i *S.* Typhimurium-infected mice with LED209 24h- post infection (oral administration) substantially increased survival rate over the non-treated group (80% survival *versus* 30%, respectively), and similar results were obtained with *Francisella tularensis* (ref_ Rasko 2008).
j The efficacy of MEDI4893 was compared to active immunization with a nontoxigenic antitoxin in diabetic and non-diabetic mice models with *S. aureus*- infected wounds and showed similar therapeutic effect in wound healing promotion with a greater decrease in the bacterial burden in diabetic mice indicating a possible advantage of MEDI4893 (ref_Ortines 2017)
k In terms of efficacy, results of Phase II study indicated a significant reduction in the recurrence rates (7% in the treated group versus 25% in the placebo group; P<0.001)(ref. Lowy 2010 N Engl J Med 2010;362:197-205) . Currently undergoing a Phase III clinical trial in children with *C. difficile* infections

l Although efficacy outcomes are not concluding due to the small sample size, a study in a mice model of severe pneumonia (*S. pneumoniae* infection) and bacteraemia (*S. aureus* and *S. pneumoniae*) showed that a combined therapy of CAL02 with antibiotics substantially improved survival outcomes (ref_ Henry 2015)

Table 4. Description of phage therapies currently available and under development and enzybiotics.

Research strategy	Name	Agent	Bacterial target	Infectious disease targeted	Current stage	References
Phage therapy	Intesti bacteriophage	Phage cocktail	*Salmonella, Shigella, E. coli, Proteus, Pseudomonas, Enterococcus, and Staphylococcus*	Intestinal disease	Commercialized	http://phage.ge/products/intesti-bacteriophage/
	Pyo bacteriophage	Phage cocktail	*Staphylococcus, Streptococcus, Pseudomonas, E. coli, and Proteus*	Surgical wound infections	Commercialized	http://phage.ge/products/pyo-bacteriophage/
	Phagoburn study	Cocktail of 12 natural lytic anti-*P. aeruginosa* bacteriophages	*Pseudomonas aeruginosa*	Burn wound infected with P. aeruginosa	Phase 1 / 2 n	Jault_2019
Enzybiotics	Cpl-711	Endolysin (degradation of peptidoglycan)	*MDR Streptococcus pneumoniae*	*S. pneumoniae infections*	Preclinical 11 o	Diez-Martinez_2014

n Results from a Phase 1/2 trial involving 27 patients with burn wound infected with P. aeruginosa indicated that although a decrease in bacterial burden was observed using phage therapy the standard of care still showed better results indicating that higher doses of phages should be used in future studies (ref_Jault2019)

o Cpl-711 showed greater protection than Cpl-711 in animals having received endolysin intraperitoneally 1h post infection (*S. pneumoniae* injected intraperitoneally) (ref actual 38: Diez-Martinez).

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

Category	Agent	Action	Bacterial target	Infectious disease targeted	Current stage	References
Antimicrobial peptides and peptidomimetics	OP-145 (AMP60.4Ac or P60.4	Hypothesized to inhibit bacterial adherence	Gram positive	Chronic middle ear infection	Phase II	Malanovic et al, 2015; Molchanova et al 2017; de Breij et al 2015; Riool et al 2017
	Surotomycin	Membrane depolarization	*Clostridiodes difficile*	Infectious diarrhoea associated with C. dif	Discontinued Phase III a	knight-connonni et al 2016; Boix et al, 2017; Daley et al, 2017
	NVB-302 (lantibiotic; polycyclic peptide containing thioether amino acids)	Inhibition of cell wall biosynthesis by lipid II binding	*Clostridiodes difficile and wide rage of Gram positive bacteria*	C.difficile infection	Completed Phase I	Crowther et al, 2013; Sandiford, 2019; Petrosillo et al 2018
	Murepavidin (POL7080; cy	Outer membrane biogenesis	*P. aeruginosa*	Ventilator associated bacterial pneumonia	Discontinued Phase III b	Sierra et al, 2017 ;Molchanova et al, 2017 https://clinicaltrials.gov/ct2/show/NCT03582007?term=murepavadin&rank=1 https://clinicaltrials.gov/ct2/show/NCT03409679?term=murepavadin&rank=2
	CSA-131 (ceragenin)	Charge driven cell membrane desestabilization	A. baumannii, P. aeruginosa, K. pneumoniae and anaerobic bacteria including Bacteroides spp. and C. difficile	Infections caused by A. baumannii, P. aeruginosa, K. pneumoniae and anaerobic bacteria including Bacteroides spp. and C. difficile	Discovery	Vila-Farres et al, 2015; Hashemi et al, 2017; Durnás et al, 2017
	CSA-13	Antibiofilm activity; bacterial membrane	Mixed P. aeruginosa and S. aureus biofil	Infections caused by P. aeruginosa, S. au	Discovery	Olekson et al, 2017
Antisense oligonucleotides (Gene expression inhibitors)	CPP-PMO conjugate	*Gene expression inhibition of gyrA*	*E. faecalis and Staphylococcus aureus (gyrA*	E. faecalis and S. aureus infections	Discovery	Wesolowski et al 2013
	CPP-PNA conjugate	*Gene expression inhibition of rpoA*	*Lysteria monocytogenes (rpoA)*	L. monocytogenes infection	Preclinical	Abushahba et al 2016
	PNA	*Gene expression inhibition of polA*	*Brucella suis* (*polA*)	B. suis infection	Discovery	Rajasekaran et al 2013
	PNA conjugate	*Gene expression inhibition of ftsZ*	*Staphylococcus aureus (ftsZ)*	S. aureus inifection	Discovery	Liang et al 2015

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 REFERENCE Boix et al 2017 demonstrated non-inferiority versus vancomycin in another trial, but yet failed to demonstrate superiority versus vancomycin REFERENCE Daley et al 2017

n two Phase III studies were suspended due to renal toxicity https://clinicaltrials.gov/ct2/show/NCT03582007?term=murepavadin&rank=1 https://clinicaltrials.gov/ct2/show/NCT03409679?term=murepavadin&rank=2