

Non-traditional antibacterial therapeutic options and challenges

Theuretzbacher, Ursula; Piddock, Laura

DOI:

[10.1016/j.chom.2019.06.004](https://doi.org/10.1016/j.chom.2019.06.004)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Theuretzbacher, U & Piddock, L 2019, 'Non-traditional antibacterial therapeutic options and challenges', *Cell Host & Microbe*, vol. 26, no. 1, pp. 61-72. <https://doi.org/10.1016/j.chom.2019.06.004>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

1 **Non-traditional antibacterial therapeutic options and challenges**

2 Ursula Theuretzbacher¹ and Laura J.V. Piddock^{2*}

3 ¹Centre for Anti-Infective Agents, Vienna, Austria

4 ²Institute of Microbiology and Infection, University of Birmingham, Birmingham, West
5 Midlands, B15 2TT, United Kingdom.

6 *Corresponding author Email: l.j.v.piddock@bham.ac.uk. Tel: +44 121 414 6966

7 Twitter: @LauraPiddock

8 *Word count excluding references 6935*

9 Summary (150 words)

10 The global challenges presented by drug-resistant bacteria infections has stimulated much
11 activity in finding new treatments. This review summarizes the progress, and set-backs, of
12 non-traditional approaches intent on circumventing bacterial drug-resistance. These
13 approaches include targeting virulence via toxin production and virulence factor secretion,
14 impeding bacterial adhesion to host cells and biofilm formation, interrupting/inhibiting
15 bacterial communication and down regulating virulence. Other strategies include immune
16 evasion, microbiome modifying therapies and the employment of phages as treatments or
17 carriers. Finally, the prospects of nanoparticles, immunotherapy, antisense RNA and drug-
18 resistance modulation approaches are discussed. The development of non-traditional
19 treatments suffers similar challenges faced by developers of conventional antibiotics, however
20 most of these new strategies have additional and considerable hurdles before it can be shown
21 that they are safe and efficacious for patient use. For the foreseeable future, it is likely that
22 most of these treatments, if approved, will be used in combination with antibiotics.

23

24 **Key words:** Anti-virulence, Quorum-sensing, Microbiome, Phage, Nanoparticles,
25 Immunotherapy, Antisense RNA, non-traditional antimicrobials

26

27 Introduction

28 The global crisis of drug resistance, few new drugs to treat infections by the most resistant
29 pathogens and the scientific challenges in discovery and early development of new antibiotics
30 has inspired researchers to explore new ways to treat bacterial infections. The traditional
31 antibiotic approach of treating infections is to find small molecules that either inhibit growth
32 or kill Gram-positive or Gram-negative bacteria or both (broad-spectrum antibiotics). Non-
33 traditional approaches explore many ways to influence the disease beyond inhibiting or killing

34 pathogens through small molecules. It should be noted that in this article, non-traditional does
35 not imply an alternative therapy to antibiotics, which means replacing the use of these drugs.
36 Due to the wide diversity of research activities that could lead to the discovery of new
37 therapies this article is not exhaustive. It focuses on the areas that are most frequently
38 explored and have achieved some late preclinical or clinical experience. We have not
39 discussed vaccines, devices, exclusively topical drugs or directly acting small molecule drugs
40 such as potentiators of antibiotics including β -lactamase and efflux inhibitors, combinations
41 of small molecule drugs or conjugates or antimicrobial peptides. Instead, we have focused this
42 article on the most promising non-traditional antibacterial treatments under development, their
43 uses, and hurdles that must be overcome to provide safe and efficacious new medicines.

44 **Anti-virulence**

45 Anti-virulence approaches aim to inhibit the production or activity of Virulence Factors
46 (VFs), typically these have no effect on bacterial growth *in vitro*. The number of VFs is
47 growing (Virulence Factor Database, <http://www.mgc.ac.cn/VFs/> (Dickey et al., 2017) and
48 includes toxins, adhesins, quorum sensing molecules, virulence-dedicated secretion and
49 regulation, siderophores, and immune evasion factors (Heras et al., 2015; Totsika, 2016). VFs
50 are often species- or even strain-specific and variably conserved between and/or within a
51 bacterial species. Furthermore, virulence gene expression may depend on the environment or
52 site of infection or on the time course of a pathophysiological process. The complex
53 biological variety constitutes a major challenge to translating early discovery to the clinical
54 situation, especially as anti-VFs usually have a narrow spectrum of activity.

55 Numerous anti-virulence strategies are in the discovery or preclinical development phase
56 (Garland et al., 2017). Progress in understanding pathogenesis and preclinical development
57 efforts has progressed a few anti-virulence drugs to the clinical phase of development, and

58 some that block exotoxins have been approved as treatments. Here, we provide an overview
59 of the anti-virulence strategies that are more advanced in development as a new treatment.

60 Toxins

61 Exotoxins are produced by pathogenic bacteria without which the microbe does not elicit
62 symptoms in the infected individual. Therefore, they are obvious targets for anti-virulence
63 therapies. Many bacterial toxins are released into the environment and are thus, amenable to
64 antibody therapy. Antibodies approved for clinical use are active against toxins of
65 *Clostridium botulinum*, *Bacillus anthracis* and *Clostridium difficile*. The recently FDA and
66 EMA approved human monoclonal antibody (mAb) Bezlotoxumab (Merck) binds to the *C.*
67 *difficile* toxin B and is indicated to prevent recurrent *C. difficile* infection (CDI) in at-risk
68 adults.

69 *Staphylococcus aureus* harbors many VFs to facilitate tissue adhesion, immune evasion, and
70 host cell injury. *S. aureus* α -toxin is a pore-forming toxin that plays an important role in
71 staphylococcal infection. In general, exotoxins and surface-localized structures provide good
72 targets for monoclonal antibodies (Table 1). The mAb suvrattoxumab (MEDI4893,
73 Medimmune) binds to and neutralizes *S. aureus* α -toxin (Yu et al., 2017). It is in Phase 2
74 clinical trials in colonized and mechanically ventilated patients to prevent ventilator-
75 associated pneumonia (VAP) caused by *S. aureus*. Other companies are pursuing a similar
76 strategy with α -toxin-binding mAbs (e.g. AR-301; Salvecin; tosatoxumab, Aridis). In
77 contrast to suvrattoxumab that is aimed at preventing symptomatic infection, Aridis plans to
78 investigate AR-301 in a Phase 3 trial as an adjunctive therapy in combination with standard of
79 care antibiotics to treat VAP caused by *S. aureus*. The monoclonal antibody preparation
80 ASN100 (Arsanis), that neutralizes six *S. aureus* cytotoxins, failed to reach the primary
81 endpoint in a Phase 2 study.

82 VF secretion

83 Type III secretion systems (T3SS) are complex structures embedded in the bacterial inner and
84 outer membranes of many Gram-negative bacteria that are used to deliver virulence effector
85 proteins into host cells and facilitate the establishment and dissemination of infections
86 (Anantharajah et al., 2016; Deng et al., 2017). The interruption of toxin secretion or structural
87 proteins of T3SS, especially the needle tip protein assembly is a focus of current research for
88 small molecule inhibitors (Aiello et al., 2010; Gu et al., 2015, Fasciano et al, 2019) and mAb
89 (Table 1). A bispecific mAb (MEDI3902, Medimmune) targets the *P. aeruginosa* T3SS
90 needle tip protein PcrV to prevent T3SS mediated injection of toxins in host cells and Psl
91 exopolysaccharide to prevent attachment of bacteria to epithelial cells (DiGiandomenico et
92 al., 2014). MEDI3902 is in Phase 2 clinical development in mechanically ventilated patients
93 for the prevention of VAP caused by *P. aeruginosa*. An earlier antibody approach did not
94 progress to late stage clinical trials (Fasciano et al, 2019). Small molecules have the potential
95 for a broader spectrum of activity than mAbs against T3SS, which are target specific and
96 hence have a narrow spectrum.

97 Adhesion and biofilm formation

98 *S. aureus* expresses a number of cell surface adherence and immune evasion proteins, many
99 of which are anchored to the cell wall by the transpeptidase sortase A (SrtA) enzyme
100 (Cascioferro et al., 2015; Dickey et al., 2017). Several antibacterial drug discovery programs
101 have found SrtA inhibitors that are believed to promote the disruption of the structure of
102 mature biofilm; however, early preclinical projects have not advanced.

103 *P. aeruginosa* produces the surface polysaccharide alginate in response to environmental
104 conditions. It enhances adhesion, biofilm formation and resistance to human leukocyte killing;
105 this is most apparent in the environment of the affected lung in patients with CF. AR-105 is a

106 mAb directed at alginate and is currently being tested in a Phase 2 clinical trial as adjunctive
107 treatment in mechanically ventilated patients with VAP caused by *P. aeruginosa*.

108 The first step of colonization of the bladder and artificial surfaces such as catheters is to avoid
109 clearance during urine voiding by binding of bacteria via an available epithelial receptor
110 (Spaulding and Hultgren, 2016). Therefore, recognition and attachment of the bacteria to the
111 uroepithelium plays a key role in anti-virulence strategies that target uropathogenic *E. coli*
112 (UPEC) and lower urinary tract infections (UTI). Adhesion is mediated through the
113 expression of pili and their tip adhesin FimH that binds to mannosylated residues on the
114 bladder epithelial surface (Spaulding et al., 2018). Several strategies to block adhesion have
115 been pursued including developing mannose analogs that bind within the mannose-binding
116 pocket of FimH and block pilus binding of FimH to host receptors and thus prevent
117 attachment of UPEC (Maddirala et al., 2018) (Fimbrion). Other FimH inhibitors were
118 effective in the mouse model, but it is not known if these animal models are predictive and
119 whether data from them will translate into clinical efficacy (Kalas et al., 2018). UPEC
120 colonize the intestinal space and establish a bacterial reservoir for infecting the urinary tract.
121 The same adhesion mechanisms and binding principles apply to preventing selectively
122 intestinal colonization of UPEC by treatment with mannosides. The oral and non-systemic
123 small molecule drug EB8018 designed to block the FimH adhesin from overabundant
124 Enterobacteriaceae (adherent-invasive *E. coli*) in Crohn's disease patients is in Phase 1
125 clinical studies (Enterome NCT03709628).

126 [Bacterial communication](#)

127 Quorum-sensing (QS) is a molecular communication system to synchronize the expression of
128 certain genes affecting a global change in bacterial gene expression and cell physiology.

129 There is a wide variety of signaling molecules that may serve as attractive and potentially
130 broad-spectrum anti-virulence targets (Rémy et al., 2018). Various QS-interfering agents

131 (called quorum quenchers) including natural and synthetic compounds, enzymes and
132 antibodies that target each step of the QS pathway have been described. These have been
133 tested *in vitro* and *in vivo* (Defoirdt, 2018). *P. aeruginosa* has been studied most and serve as
134 a model for targeting QS. The *P. aeruginosa* QS pathways LasR-LasI, RhlR-RhlI, IQS, MvfR
135 have been explored in both acute and persistent infection models (Dickey et al., 2017). The
136 *las* system controls LasB elastase, a pivotal VF in pseudomonal infection and target of drug
137 discovery projects (Antabio). LasB is secreted at the site of infection, where it exerts a
138 proteolytic action including broad tissue destruction and subtle action on components of the
139 host immune system (Cathcart et al., 2011). The discovery of the role of the multiple VF
140 regulator MvfR in mediating antibiotic tolerance and persister cell formation in *P. aeruginosa*
141 inspired the research and discovery of inhibitors of MvfR (Maura et al., 2017) (Spero). In *S.*
142 *aureus* QS is mainly regulated by the *agr* operon. Although several small molecule inhibitors
143 have been described, none have advanced to the optimization phase of discovery (Salam and
144 Quave, 2018). Based on considerable basic research, QS inhibiting strategies are increasingly
145 included in discovery projects (Haque et al., 2018; Williams, 2017).

146 Counteracting Immune evasion

147 Many bacteria deploy factors to prevent detection by or to escape the host immune response.
148 Therefore, strategies to neutralize such tactics are under development. Monoclonal antibodies
149 targeting bacterial surface epitopes are hypothesized to increase bacterial clearance through
150 enhancing antibody-dependent phagocytosis, and/or complement-mediated bactericidal
151 activity, or via immune system-independent bacterial killing (Wang-Lin and Balthasar, 2018).
152 The *S. aureus* Protein A (SpA) defends the bacterium against the adaptive immune response
153 by resisting phagocytosis and inducing apoptosis of B cells. This protein also facilitates nasal
154 colonization and cell adhesion (Hong et al., 2016). The mAb 514G3 neutralizes the SpA
155 mediated immune evasion of *S. aureus* (Varshney et al., 2018). An on-going a Phase 1/2

156 clinical study to treat *S. aureus* blood stream infections as an adjunctive therapy is due to
157 complete in June 2020 (XBiotech NCT02357966).

158 [Regulating virulence](#)

159 Clp proteases suppress the expression of multiple unrelated VFs in *S. aureus* by impacting on
160 central processes such as virulence gene expression, cell wall metabolism, survival in
161 stationary phase, and cell division. The simultaneous suppression of multiple VFs or
162 pathways using small-molecule compounds is a promising approach to reducing the virulence
163 of *S. aureus* (Gao et al., 2018). Many inhibitors have been discovered and are currently being
164 optimized (Aviru). However, whether targeting a master regulator of virulence translates into
165 clinically relevant benefits remains to be seen.

166 [Advantages and Disadvantages of anti-virulence approaches](#)

167 As anti-virulence drugs interact with non-essential targets, there is a general assumption that
168 they do not select for resistance; however, this has been challenged (Allen et al., 2014).

169 Experimental data revealed the emergence of resistance showing the complex evolution and
170 resistance selection by some anti-virulence drugs; this was dependent on the importance to the
171 pathogen of the targeted VF (Totsika, 2016). Whether such resistance will impact therapy and
172 the potential of transmissible resistance is unknown (Rezzoagli et al., 2018).

173 There are numerous challenges to translating anti-virulence strategies to new treatments for
174 patients (Table 2). Given the complexities of virulence systems, the likely specificity of single
175 VFs, and the lack of research data, it is not surprising to find extremely long timelines for the
176 preclinical development of anti-virulence approaches and the failure of clinical trials.

177 Development of anti-virulence drugs requires an in-depth understanding of these factors and
178 the roles that specific VFs have in disease processes. Traditional measurement of growth
179 inhibitory activity, the Minimum Inhibitory Concentration (MIC) test, does not apply to anti-
180 virulence drugs as by definition they do not inhibit growth or kill bacteria. In most cases,

181 alternative *in vitro* methods are not developed and the predictability of animal models for
182 clinical outcome are poorly described.

183 Potential clinical indications for anti-virulence therapies are prevention of disease such as
184 CDI, HAP/VAP and recurrent uncomplicated UTI. However, as some VFs are specific for a
185 pathogen and rely upon of their expression, which is influenced by the condition of the patient
186 and disease state, accurate diagnostic tests will be necessary to identify the pathogen and
187 presence/expression of the targeted VF. Therefore, chronic or non-life-threatening infections
188 are likely to be targeted as these will provide the opportunity to carefully select the patient,
189 enabling a tailored patient-specific approach with less time pressure to start therapy. Most
190 anti-virulence therapies are developed as adjunctive therapies in addition to standard therapy,
191 usually antibiotics. This is the only ethically acceptable pathway in clinical practice in
192 patients needing fast acting antibacterial therapy. This poses special challenges for drug
193 development and clinical use as it is not possible to prove clinical efficacy of an adjunctive
194 therapy with a conventional non-inferiority clinical trial design. Superiority design in a
195 combination therapy versus stand-alone antibiotic therapy would be the most convincing way
196 to show a clinically relevant effect of an add-on therapy. Selection of the appropriate
197 indication, patient population, clinical endpoints and clinical trial sites are enormous
198 challenges for late stage clinical studies. Relevant secondary endpoints may support the
199 clinical therapy decisions (Maura et al., 2016).

200 Anti-virulence approaches will not replace antibiotics, thus may not contribute much to
201 resolving the resistance problem and insufficient antibiotics pipelines. Nonetheless, they may
202 complement the action of antibiotics; however, evidence that they provide benefit for patients
203 in high-quality clinical trials is needed. Open discussion and analysis of failed clinical trials
204 would enhance this field.

205 Microbiome modifying therapies

206 Recent advances in metagenomic, computational and synthetic biology tools inspired the
207 revival of research into the human microbiome and has provided a deeper understanding of its
208 interactions with the host (Wilson et al., 2019). Manipulating and engineering the human
209 microbiome is an attractive option to prevent and resolve infection and so is generating
210 considerable activity in academia and industry (Timmis et al., 2019) and attracts broad
211 interest among public funders and private investors (Boers et al., 2016). The US National
212 Microbiome Initiative had an important impact and advanced standards for the use of the
213 next-generation technologies in metagenomic studies and generated quality-controlled data
214 (Group et al., 2009). Not only bacterial microbiota but growing knowledge about the role of
215 the human phageome has highlighted the impact of bacteriophages in shaping a healthy
216 intestinal microbiome (Anonye, 2018; Paule et al., 2018; Rohde et al., 2018; Zuo et al., 2018).
217 The gut microbiota is intricately connected to the host's immune system through a reciprocal
218 developmental relationship. Specifically, the microbiome is critical for the appropriate
219 development of the immune system, and in turn, the immune system helps modulate the
220 microbiome community through a balance of pro- and anti-inflammatory pathways
221 (Cammarota et al., 2017).

222 More than 10 small companies are developing microbiome therapies for infectious diseases
223 (Table 3). So far, most experience has been gained on the impact of the intestinal microbiota
224 on the physiology of *C. difficile* in the gut and recurrent CDI (Young, 2016). The underlying
225 assumption is that rebuilding the microbiome after infection or preserving the microbiome to
226 prevent infection will translate into clinical benefit. The strategies to restore an unbalanced
227 microbiome are based on the experience of clinically successful transfers of a full natural
228 microbiota in form of stool from healthy donors (Fecal Microbiome Transplantation, FMT)
229 (Cammarota et al., 2017). This procedure has a typical cure rate of 90% and has encouraged

230 research groups to modify this principle and focus on production of stool banks, standardized
231 products, engineering and oral delivery strategies.

232 Although no microbiome-modifying therapy has been officially approved, the US FDA
233 allows FMT without filing an Investigational New Drug (IND) application exercising
234 enforcement discretion when using the stool bank OpenBiome to treat *C. difficile* infection
235 not responsive to standard therapy (FDA, 2016). FMT reintroduces a complete, stable
236 community of gut microorganisms (Bakken et al., 2011; Borody and Campbell, 2012) and is
237 the most advanced form of microbiota therapy with a large body of experience (Ooijselaar et
238 al., 2018). Guidelines for clinical use are available (Mullish et al., 2018). In the UK, large
239 clinical trials of FMT are underway (ISRCTN 74072945). Most experience has been gained
240 so far with the application via enema but freeze-dried capsule-based formulations of the
241 microbiota or mixture of spores from several bacteria isolated from healthy donor fecal
242 samples are in clinical development (Baker et al., 2018). There are no high-quality studies
243 available yet to show the efficacy and safety of the oral route (Iqbal, 2018). Two companies
244 are currently conducting a randomized Phase 3 trial enrolling patients with recurrent CDI
245 (Rebiotix NCT03244644, Seres NCT03183128), another company is enrolling patients in a
246 Phase 2 trial to test a therapy that contains microbiota produced from pure, clonal bacterial
247 cell banks (Vedanta NCT03788434) (Table 3).

248 Companies are also developing a wide range of microbiome strategies including rationally
249 selected cocktails of bacteria or bacterial spores containing the “active components” of the
250 complex microbiota (Khanna et al., 2016; Orenstein et al., 2016). The most extremely reduced
251 approach is the use of a single non-toxic strain of *C. difficile* that is hypothesized to
252 outcompete the toxigenic strains in the gut. Another approach is to assemble a synthetic
253 microbiome comprising well characterized individual strains in pure cultures with
254 standardized properties (Timmis et al., 2019). Other strategies build on the gastrointestinal

255 metabolic balance and subsequent changes made during disturbance and restoration to a
256 healthy microbiota (Koropatkin et al., 2012). In another approach, genetically engineered
257 bacteria produce antibacterial compounds that selectively remove key disease-causing species
258 from the microbiota. Increasingly, bacteriophages or nanoparticles serve as vehicles to
259 selectively target pathogenic bacteria or resistance and virulence determinants in the gut flora
260 and thus can be used to manipulate the microbiota. Current approaches also target intestinal
261 colonization with pathogenic bacteria such as carbapenem-resistant *Enterobacteriaceae*
262 (CRE) in critically ill patients. However, it should be noted that there are conflicting data on
263 the causality between CRE colonization and increased mortality in ICU patients (McConville
264 et al., 2017). Studies with FMT to test for the effect of decolonization in high-risk patients
265 were not conclusive (Huttner et al.; Relman and Lipsitch, 2018). Although the gut microbiota
266 is the most common target for microbiome-modifying therapies, other concepts focus on
267 manipulating the skin or lung microbiome. These approaches are at very early stages and
268 correlations with the clinical situation are less clear.

269 The key challenges of developing new simplified microbiome therapies are the incomplete
270 understanding of the complex genomic and phylogenetic diversity of the human microbiome.
271 Indirect testing of hypotheses and statistical correlations may not prove pathophysiological
272 causation. If innovative microbiome therapies beyond FMT and other complex microbiota
273 strategies are to translate into clinical benefit a deeper understanding of the complex role of
274 the microbiota in the pathogenesis of a specific disease is necessary. The extent to which the
275 complexity of the therapeutic approach can be reduced while retaining efficacy remains to be
276 seen. The high variability of the microbiome composition yields inconsistent and
277 contradictory results that are difficult to interpret. Simple preclinical models may not be
278 predictive. Selecting appropriate microbiota is important but proving that live bacteria
279 constitute a coherent community and are incorporated into the recipient's gut and remain after

280 being ingested is similarly essential (Smillie et al., 2018). Additionally, the manufacture of
281 live bacterial products is complex and expensive. There is no good rationale or model to
282 support finding the appropriate or optimum dose and so doing reflects a trial and error
283 strategy rather than characterizing an effective dose. Although microbiome treatments with
284 complex microbiota for preventing recurrent CDI are in late stage clinical trials, the
285 development of next generation treatments rely upon reducing unpredictable factors and
286 filling the gaps in the basic understanding of underlying processes. Currently, reduction of the
287 complexity of the microbiota substantially increases the risk of clinical failure.

288 Phages

289 Although therapy of bacterial infections with bacterial viruses (bacteriophages, phages) has
290 been practiced in Eastern Europe for nearly a century, the interest in phage R&D has only
291 gained traction elsewhere in the last 10-15 years as a response to the emergence of multidrug-
292 resistant pathogens (Kortright et al., 2019). Synthetic biology and other modern tools have
293 revived the field of phage research (Pires et al., 2016). They enable the modification of
294 phages, the characterization and careful screening for and removal of genes coding for toxins
295 and VFs to avoid the risk of transfer from one bacterium to another. In general, phages are
296 regarded as safe because they do not infect mammalian cells.

297 There are at least 30 companies pursuing a treatment strategy that involves phages (table 4).
298 Recent and ongoing trials focus on infections by *P. aeruginosa*, *S. aureus* and *E. coli*. Besides
299 anecdotal case studies, case reports of compassionate use programs and poor quality non-
300 controlled clinical studies, only one randomized placebo-controlled Phase 1/2 clinical trial
301 with topical treatment of chronic otitis caused by *P. aeruginosa* has been successfully
302 conducted (Wright et al., 2009). A recently completed Phase 1/2 clinical trial in infected burn
303 wounds (phagoburn) failed to demonstrate efficacy and exemplifies the challenges described
304 below when translating phage approaches to the clinical environment (Servick, 2016). A

305 Phase 1/2 trial with a *S. aureus* phage cocktail for i.v. administration is being prepared to start
306 in 2019 (Ampliphi).

307 Phage therapy is characterized by its specificity to single bacterial species and usually to a
308 subset of strains within that species (Kortright et al., 2019). To be active against >90% of
309 strains within a bacterial species and to prevent rapid emergence of bacterial resistance to a
310 single phage, mixtures (cocktails) of different phages, often more than 10 phages, are used for
311 therapy. Phage resistance can evolve within hours, independently of the use of bacteriophage
312 combinations. Although the combination of multiple phages in a cocktail compensates for a
313 limited host range the increased complexity of such a cocktail not only dilutes the
314 concentration of the individual phages but can promote potential unfavorable interactions
315 between phages and cause manufacturing and quality control issues. This is the reason why
316 companies try to reduce the number of phages in fixed cocktails and some produce mini-
317 cocktails (≤ 5 phages). The downside of such mini-cocktails may be a smaller host range.
318 Currently, there is a trend towards patient specific cocktails based on libraries of pre-approved
319 phages. Phage banks containing purified or pre-purified phages allow the quick assembly of
320 patient-specific cocktails that contain only the most appropriate phages against the infecting
321 bacterium. The choice of phages is based on new diagnostic tools that are not yet available in
322 clinical practice. Based on modern genetic engineering tools, recent and current research
323 focuses on engineered phages with improved or specific features (Barbu et al., 2016). In
324 contrast to the above-mentioned strategies that use lytic phages, non-lytic phages are utilized
325 as vehicles to express antibacterial proteins or genes (Krom et al., 2015).

326 There are several challenges to the clinical use of phage treatment. These include

- 327 1) the prerequisite of selecting appropriate phages to achieve an appropriate range of
328 activity and prevent development of bacterial resistance. Such fixed phage cocktails
329 need to consider bacterial isolates from different infections and geographic locations.

- 330 2) manufacturing phages under good manufacturing practices (GMP) and chemistry,
331 manufacturing, and control (CMC) guidance. Some progress has been achieved when
332 tackling the insurmountable challenge regarding CMC, especially production,
333 stability, purity and quality control.
- 334 3) Considering phage biology in the design of phage treatment is a prerequisite of any
335 successful approach (Bull and Gill, 2014). Unique pharmacokinetics (PK) and
336 pharmacodynamics (PD) of phages means that dose finding processes are challenging.
337 The immense size of phages when compared to small molecule antibiotics results in a
338 wide range of PK challenges and is the reason why many sites of infection are not
339 accessible by phages. Questions of basic PK such as distribution, dilution in the blood
340 compartment, rapid clearance (Inchley, 1969), as well as kinetics of phage infection
341 and other PK/PD characteristics are not well defined. Development of methods to
342 access these parameters needs to proceed in parallel with clinical work to assess
343 exposure and efficacy relationships. The concept of phage therapy is based on
344 localized amplification in the presence of the specific susceptible bacteria. High
345 bacterial loads are necessary for amplification and their localization is a complex
346 pathophysiological issue (Rose et al., 2014). Though localized amplification is a key
347 concept, the initially applied dose and the fate of the phages in the systemic circulation
348 is not well understood. Bacterial loads and decreased availability of active phages in
349 the circulation or localized infection sites may be responsible for a potentially slow
350 onset of activity. The success of phage therapy ultimately depends on the optimal
351 dose, dosing regimen, timing, formulation and administration, with PK and PD
352 characterized for each phage or phage cocktail.
- 353 4) showing efficacy in clinical trials, and thus, gaining regulatory approval. Although
354 case reports in compassionate use programs indicate the possibility of systemic use,
355 due to access issues, topical delivery has been much more common. However, in this

356 setting, concomitantly used treatments (e.g. wound care products, disinfectants and
357 antibiotic topical treatments) may affect the local stability of phages (Merabishvili et
358 al., 2017). Inhalation treatment with phages, potentially also in combination with an
359 antibiotic for specific indications such as CF seems to be feasible (Chang et al., 2018).
360 Another aspect of phage therapy is their natural immunogenicity which is stimulated
361 by bacteria (bacteria can hijack the innate immunity of hosts to inhibit phage).
362 Interacting elements of adaptive and innate immunity are contributing to the clearance
363 of phages with consequences on phage PK (Hodyra-Stefaniak et al., 2015). To use in
364 patients, the immune response to phage therapy need to be assessed (Dąbrowska et al.,
365 2014; Krut and Bekeredjian-Ding, 2018). At present, there are no high-quality data to
366 show that phage therapy works routinely in clinical settings. The future and potential
367 clinical use of phage therapeutics depends on the careful selection of phage-accessible
368 infections and to be able to show a clinical benefit for patients (Harper, 2018). The
369 current challenges of regulatory pathways, especially for patient-specific but also fixed
370 phage cocktails include the need for constantly adjusting the preparation as the
371 bacteria evolve and requires an appropriate legal and regulatory framework
372 (Fauconnier, 2017; Pirnay et al., 2018) with recent progress in approval to conduct
373 clinical trials.

374 5) developing and implementing appropriate diagnostics is essential to support use in
375 patients.

376 For the most common bacterial infections it is unlikely that phage therapy will replace use of
377 antibiotics (Rohde et al., 2018). However, synergy with antibiotics has been seen in vitro and
378 in animal models (Oechslin et al., 2017). Therefore, phage therapy may be a promising
379 adjunctive treatment in specific indications or salvage therapy for patients with infections that

380 have not responded to any other treatment. Convincing clinical efficacy in well-designed
381 randomized controlled clinical trials needs to be demonstrated.

382 [Phages as carriers](#)

383 Genetically engineered non-replicative phages are designed to serve as specific nano-delivery
384 vehicles and carry payloads that exert antibacterial activity beyond direct lysis of the cell
385 (Krom et al., 2015). Synthetic biology approaches enable the use of a wide range of gene
386 expression systems to target bacteria. Phage delivery systems are usually specific and so
387 render the delivered therapeutics narrow spectrum and pathogen-specific. The delivered genes
388 may be DNA sequence-independent and cause a rapid bactericidal effect (Phico) or utilize the
389 clustered regularly interspaced short palindromic repeat (CRISPR) RNA-guided genome
390 editing systems to engineer novel functions (Locus Biosciences, Eligo Bioscience) (Hatoum-
391 Aslan, 2018). CRISPR Cas9 has developed into a new powerful technology to regulate gene
392 expression in bacteria (Bikard et al., 2013). The RNA-guided nuclease Cas9 may serve as a
393 sequence-specific antibacterial (Bikard et al., 2014) or may target a specific DNA sequence to
394 inactivate antibiotic resistance or virulence genes (Nemesis Bioscience). Another CRISPR-
395 Cas system is used to insert CRISPR-Cas3 constructs into the phage genome that specifically
396 degrade the DNA of target bacterial cells (Locus Biosciences). The CRISPR/Cas mediated
397 technology can also be used to modify phages and optimize favorable characteristics
398 (Hatoum-Aslan, 2018).

399 As such phage vehicles are not self-replicative the dose must be very high to target bacteria in
400 an infection. PK and dose finding are not well understood. New antibacterial approaches that
401 use phages as carriers are faced with two big challenges, (1) the new technology (e.g.
402 CRISPR-Cas) and (2) the phages themselves as discussed above. This doubles the risk and
403 will need considerable time to progress to clinical studies to show a clinical benefit for
404 patients.

405 Phage-derived products

406 Though phage-derived enzymes have been explored since the late 1990s, renewed interest
407 emerged to address the current drug-resistance issues. Endolysins are the best studied phage-
408 derived peptidoglycan-degrading enzymes. They are encoded by phages to liberate progeny
409 phage from inside of infected bacterial cells, resulting in fast osmotic lysis and bacterial cell
410 death (Fischetti, 2018). Endolysins are bacteriolytic on contact, independent of resistance
411 pattern to conventional antibiotics, and are highly specific for a bacterial species or genus
412 (Fernandes and São-José, 2018). Naturally, endolysins work from inside the cell but purified,
413 recombinant lysins enabled enzymes are lytic from the outside. When developed as drugs,
414 lysins must be stable, soluble and able to hydrolyze peptidoglycan from the outside.

415 Numerous endolysins against Gram-positive bacteria have been studied in vitro and in animal
416 models (Gutiérrez et al., 2018; Haddad Kashani et al., 2017). The opportunities to customize
417 endolysin properties such as specificity, activity, stability and solubility are currently being
418 explored and extensive protein engineering efforts have expanded (Oliveira et al., 2018). The
419 first two products that have reached Phase 2 clinical development are recombinant lysins
420 directed against *S. aureus* (SAL200/tonabacase from Roivant in-licensed from iNtRON
421 Biotechnology (Kim et al., 2018) and CF-301/exebacase from Contrafect) (Schuch et al.,
422 2014). A topically applied endolysin for inflammatory conditions due to *S. aureus* in atopic
423 dermatitis is already available (Microcos Human Health BV). Another topical endolysin
424 against staphylococci is being studied in Phase 1/2 clinical trials for nasal decontamination
425 (GangaGen). In contrast to Gram-positive bacteria, the outer membrane of Gram-negative
426 bacteria represents a difficult barrier to reach the peptidoglycan layer. Therefore, discovery of
427 endolysins against Gram-negative bacteria has faced extensive challenges and projects are
428 still in preclinical research (Bioharmony Therapeutics) (Briers and Lavigne, 2015). Based on
429 protein engineering techniques some progress has been made but translating results into
430 formal development programs requires more research (Lukacik et al., 2012; Schirmeier et al.,

431 2018). One approach for Gram-negative bacteria is fusing a natural antimicrobial peptide
432 (AMP) to an endolysin (Artilysin) (São-José, 2018). Other options explored include
433 combining endolysins with outer membrane-permeabilizing agents (Oliveira et al., 2018).
434 Via protein engineering, there is the potential to generate enzymes with several improved
435 features; these include altered catalytic activities and binding specificities, solubility, and
436 other physicochemical properties (Gutiérrez et al., 2018; São-José, 2018). Great progress has
437 been achieved improving large scale production, purification, formulation, delivery, stability
438 and acceptable shelf life. However, some studies have shown that in vitro antibacterial
439 activity is not translated in vivo. It is unclear if discrepancies in antibacterial activity of some
440 endolysins is due to the influence of bacterial cell growth conditions or growth stage (Oliveira
441 et al., 2018). Pharmacokinetics are not well understood. Endolysins have a relatively short
442 half-life which may be explained by proteolysis via plasma proteases and degradation of
443 enzyme aggregates (Jun et al., 2017). PK/PD characteristics and dose finding are a new field
444 for the first lysins in development. As lysins are proteins they are immunogenic in mammals.
445 In vitro and animal studies with lysins have shown that non-neutralizing antibodies are
446 generated (Pastagia et al., 2013), so could allow for repeated use in humans, but the
447 development of antibodies has raised concern and requires further study (Jun et al., 2017).
448 The potential for resistance to lysins is unknown as only simple serial passage experiments
449 have been done so far. Although biologics are a growing part of authorized medicines
450 (Cooper et al., 2016), the regulatory pathway of new antibacterial biologics still needs some
451 clarification. Endolysins are likely to be suitable for classical clinical trials procedures due to
452 their similarities with conventional antibiotics.

453 If developers succeed with defining an appropriate dosing schedule (beyond a single dose) as
454 a basis for successful clinical studies for patients with confirmed infection due to drug-
455 resistant pathogens, or who experience recurrent or relapse infections, endolysins may provide

456 an adjunctive therapy option. Endolysins are very large molecules and their distribution in the
457 body is restricted to the bloodstream. Therefore, their clinical use will be limited to systemic
458 infections or to infections with topical application. Synergistic activity with antibiotics and in
459 vitro and in vivo (Schuch et al., 2014) and activity against biofilms may open opportunities to
460 treat infections of infected implanted devices and endocarditis. In addition to human health,
461 endolysin- based technologies are applied in many areas, including food safety, animal health
462 and agriculture.

463 Other approaches

464 Recent advances in genome editing, gene regulation and systems biology has inspired a wide
465 variety of other innovative discovery projects including ≥ 100 discovery and preclinical
466 projects on approaches including nanoparticles, immunotherapy, anti-sense RNA, resistance
467 modulation and removal of drug-resistance plasmids (Theuretzbacher et al., 2017).

468 Nanoparticles

469 Nanoparticles are 1–100 nm with ill-defined multiple simultaneous modes of action against
470 Gram-positive and Gram-negative bacteria. Nanoparticles have been used for many years as
471 antibacterial coatings for implantable devices and medicinal materials, wound dressings, bone
472 cement, dental materials and vaccines (Wang et al., 2017). Several types of nanoparticles
473 (especially liposomes) are currently available for drug delivery and extended release forms
474 (Kwon et al., 2017). Nanoparticles have been studied as toxin binders in various infections
475 including intestinal infections e.g. cholera (Das et al., 2018). One company is developing
476 liposomes that mimic domains targeted by toxins so neutralizing many toxins, e.g.
477 phospholipase C, pore-forming toxins, T3SS and can be used for a range of different
478 infections (Combioxin, Phase 1/2) (Azeredo da Silveira and Perez, 2017). Nanoparticles also
479 serve as delivery vehicle for synthetic oligonucleotides that function as transcription factor
480 decoys and thus, control gene regulation (Mamusa et al., 2017) (Procarta). The potential of

481 nano-strategies as adjunctive therapy in addition to existing antibiotics is discussed in the
482 recent review by Baptista et al (Baptista et al., 2018). Despite offering promising solutions,
483 translational studies and development of nanoparticles for severe infections is in its infancy
484 and several challenges remain.

485 Immunotherapy

486 Host-directed therapies utilize small-molecule drugs and proteins to target critical host
487 signaling enzymes exploited by bacteria for their intracellular invasion, replication, and/or
488 dissemination and virulence (Chiang et al., 2018; Pirofski and Casadevall, 2006). Potential
489 immunomodulating therapeutics may encompass a great diversity of drug classes targeting a
490 variety of biological processes that modify host cell function. This complexity of the immune
491 response challenges the selection of suitable targets. Immunotherapeutics are thriving in other
492 therapeutic areas but are relatively unexplored for bacterial infections (Baker et al., 2018).
493 Like other non-traditional approaches, a clear correlation between the immunomodulating
494 drug and clinical outcome of bacterial infection needs to be shown. Furthermore, if
495 therapeutics stimulate the immune system, they may be associated with the risk of excessive
496 inflammation leading to a cytokine storm or systemic inflammatory response syndrome
497 (Chiang et al., 2018).

498 Nonetheless, some immunomodulating agents are being tested in preclinical or clinical trials.
499 The most advanced drug, Reltecimod, is in Phase 3 clinical trials (Atox Bio). The short
500 peptide immunomodulator attenuates excessive severe acute inflammation and is
501 hypothesized to protect from superantigen toxins. It is tested in patients with necrotizing soft
502 tissue infections in addition to the current standard of care (broad-spectrum antibiotics, wide
503 surgical debridement, and supportive care). A phase 1/2 clinical trial of recombinant plasma
504 gelsolin in community-acquired pneumonia has been completed (BioAegis Therapeutics).
505 Plasma gelsolin is a highly abundant plasma protein in healthy individuals that enhances

506 macrophage activity and limits the excessive spread of inflammation. Its decline in a wide
507 range of infections is correlated with poor clinical outcome (Self et al., 2018).

508 [Antisense RNA](#)

509 Antisense antimicrobial therapeutics are synthetic sequence-specific oligomers that silence
510 expression of specific genes including essential, non-essential or resistance genes. (Sully and
511 Geller, 2016). Broad functional genes are investigated as targets but still need to be validated.
512 Many different chemical structures have been explored but all need a delivery system to
513 penetrate bacterial cells. The most common approach is coupling antisense oligomers to cell-
514 penetrating peptides (Sully and Geller, 2016). Such conjugates have not progressed into
515 clinical trials. They face considerable challenges, including choice of target, potential
516 emergence of resistance, carrier and translational issues but may benefit from advanced
517 research to deliver improved approaches (Good and Stach, 2011).

518 [Drug-resistance modulation](#)

519 Resistance has inspired research to explore mechanisms to switch off drug-resistance without
520 affecting bacterial growth as well as preventing horizontal gene transfer between bacteria.
521 Several ways of silencing drug-resistance genes have been described, including CRISPR-Cas
522 or synthetic oligomers (Good and Stach, 2011; Yosef et al., 2015). These methods aim at
523 inactivating or deleting specific genes to re-establish the susceptibility of the bacteria to the
524 antibiotic. The major challenge is the delivery of the genetic construct to and inside the
525 bacteria (Vila, 2018). Commonly described delivery systems are phages, cell-penetrating
526 peptides, nanoparticles or transmissible plasmids.

527 Although some technologies allow for simultaneous targeting of various drug-resistance
528 genes, bacteria can express a great variety of different resistance mechanisms, requiring the
529 identification of the target resistance mechanisms before administration. A few companies are

530 pursuing such resistance modulating approaches to re-sensitize bacteria to existing antibiotics
531 and preventing horizontal drug-resistance-gene transfer in bacteria (see: phages as carriers).
532 Plasmid curing (recently reviewed by Buckner et al., 2018) is another approach, either by
533 reducing transmission of plasmids to new bacterial hosts or reducing the stability of plasmids
534 within bacterial cells. Agents that remove plasmids carrying antimicrobial resistance and/or
535 virulence genes could be used to decolonize humans, animals and/or the environment of these
536 transmissible elements. Adequate animal models to test these agents need to be developed.
537 However, should there be a currently licensed drug that could be re-purposed for this role,
538 analysis of drug-resistance surveillance data in patients taking such a drug and/or a clinical
539 trial could be carried out.

540 Discussion

541 The increasing number of drug-resistant bacteria especially Gram-negative bacteria, the
542 growing awareness of few new drugs and the scientific challenges to find novel antibiotics
543 without cross-resistance to existing classes has stimulated discovery and early development of
544 new antimicrobials. To contribute to re-stocking the pipelines with new treatments, non-
545 traditional therapies have been proposed. However, it is very unlikely that they will replace
546 the use of antibiotics as their use will mostly depend on concomitant use of active antibiotics
547 (Czaplewski et al., 2016). Therefore, most non-traditional treatments will not solve the drug-
548 resistance problem. Furthermore, the additional clinical benefit of such adjunctive therapies is
549 unknown. It will be very difficult to show a meaningful clinical benefit in hospitalized
550 patients for add-on therapies or preventive approaches for specific high-risk patient groups
551 (the problem is to show that the new treatment works, rather than regulatory issues).
552 In addition to the R&D hurdles that antibiotics face, non-traditional antibacterials share some
553 common challenges:

- 554 • Most anti-virulence and simplified microbiome approaches are indirect-acting
555 strategies that do not inhibit or kill bacteria, and act by intervening or interacting with
556 complex biological processes that may not be well understood. Furthermore, a causal
557 relationship with clinical outcomes may not be known.
- 558 • Treatments that do not affect bacterial growth cannot use traditional MIC
559 measurements that correlate reasonably well with outcome measures. Alternative in
560 vitro tests need to be developed and validated. It remains unclear if current animal
561 tests can predict outcome of non-traditional treatments in humans.
- 562 • The immense challenge of late stage clinical trials applies to all non-traditional
563 therapies that must be administered with an active antibiotic. To demonstrate their
564 efficacy and utility, until recently non-inferiority clinical studies (to show that the
565 experimental treatment is no worse than the comparator) of adjuvants (active antibiotic
566 with add-on therapy versus active antibiotic alone) were sufficient for regulatory
567 approval. However, the FDA has recently indicated that they will prefer superiority
568 studies (to show that the experimental treatment is better than standard of care) in
569 relevant clinical endpoints for regulatory approval and future acceptance in clinical
570 practice.
- 571 • In the case of biologics such as live bacterial preparations (Live biotherapeutic
572 products), standardized and well characterized production processes as well as quality
573 controls i.e. CMC requirements remain challenging, but progress is being made.
- 574 • Dose finding is a well characterized procedure in the antibiotic field and relies heavily
575 on validated preclinical models that correlate PK and PD. Such predictive models are
576 not usually available for non-traditional approaches and correlates to outcome effects
577 may not be known. This is most apparent in the phage and microbiome fields.

578 • Many non-traditional therapies are pathogen-specific, or specific for a subset of strains
579 of a species, or only active in a specific host niche or specific phase of infection. Such
580 tailored approaches will require knowledge of underlying mechanisms and patient
581 factors, and so high financial resources. Although most experts would agree that
582 patient-specific antibacterial therapy is desirable, its translation into the clinical
583 routine beyond highly specialized settings is unlikely to occur for many years.
584 Therefore, without additional tailored diagnostics some of the therapies may not be
585 useful.

586 Very few non-traditional therapies have advanced to late stage clinical trials. The most
587 advanced non-traditional antibacterials are exotoxin targeting therapies, mostly mAbs (*C.*
588 *difficile*, *S. aureus*). Similarly, microbiome therapies based on complex characterized
589 microbiota are promising and likely to provide new options to treat or prevent CDI.

590 However, most therapies are in the discovery or preclinical development stages and so may
591 not be available for at least 10 years. Commonly, non-traditional approaches require
592 sophisticated diagnostics beyond pathogen identification and are patient-tailored approaches.
593 The development and implementation of such specific companion diagnostics is further
594 contributing to the challenges.

595 In conclusion whilst there is a considerable interest in the opportunities that non-traditional
596 approaches will bring to treating bacterial infections, it is likely that effective treatments will
597 be limited to healthcare settings with the best diagnostic and financial resources, and to
598 healthcare systems that are able to financially support a strong growth of high-cost individual
599 (personalized) medicines, and thus to high-income countries. The highest burden of drug-
600 resistant infections is in babies and children in low-medium income countries. These are
601 unlikely to have the resources for basic traditional antimicrobial treatments and in the near
602 future extremely unlikely to be able to implement personalized medicine.

603 **Author contributions**

604 Ursula Theuretzbacher and Laura J.V. Piddock wrote this article.

605 **Declaration of Interests**

606 LJVP is currently seconded to the Global Antibiotic Research & Development Partnership.

607 LJVP has no financial interests in any of the companies indicated in this article and has not

608 received research funding to work on any of the approaches described herein. UT has no

609 financial interests in any of the companies indicated in this article and has not received

610 research funding to work on any of the approaches described herein.

611 **References**

- 612 Aiello, D., Williams, J.D., Majgier-Baranowska, H., Patel, I., Peet, N.P., Huang, J., Lory, S.,
613 Bowlin, T.L., and Moir, D.T. (2010). Discovery and characterization of inhibitors of
614 *Pseudomonas aeruginosa* type III secretion. *Antimicrob. Agents Chemother.* *54*, 1988-1999.
- 615 Allen, R.C., Popat, R., Diggle, S.P., and Brown, S.P. (2014). Targeting virulence: can we
616 make evolution-proof drugs? *Nat. Rev. Microbiol.* *12*, 300.
- 617 Anantharajah, A., Mingeot-Leclercq, M.P., and Van Bambeke, F. (2016). Targeting the Type
618 Three Secretion System in *Pseudomonas aeruginosa*. *Trends Pharmacol. Sci.* *37*, 734-749.
- 619 Anonye, B.O. (2018). Commentary: Bacteriophage transfer during faecal microbiota
620 transplantation in *Clostridium difficile* infection is associated with treatment outcome. *Front.*
621 *Cell. Infect. Microbiol.* *8*, 104-104.
- 622 Azeredo da Silveira, S., and Perez, A. (2017). Improving the fate of severely infected patients:
623 the promise of anti-toxin treatments and superiority trials. *Expert Rev. Anti. Infect. Ther.* *15*,
624 973-975.
- 625 Baker, S.J., Payne, D.J., Rappuoli, R., and De Gregorio, E. (2018). Technologies to address
626 antimicrobial resistance. *Proc. Natl. Acad. Sci. USA* *115*, 12887-12895.
- 627 Bakken, J.S., Borody, T., Brandt, L.J., Brill, J.V., Demarco, D.C., Franzos, M.A., Kelly, C.,
628 Khoruts, A., Louie, T., Martinelli, L.P., *et al.* (2011). Treating *Clostridium difficile* infection
629 with fecal microbiota transplantation. *Clin. Gastroenterol. Hepatol.* *9*, 1044-1049.
- 630 Baptista, P.V., McCusker, M.P., Carvalho, A., Ferreira, D.A., Mohan, N.M., Martins, M., and
631 Fernandes, A.R. (2018). Nano-Strategies to Fight Multidrug Resistant Bacteria-"A Battle of
632 the Titans". *Front. Microbiol.* *9*, 1441.
- 633 Barbu, E.M., Cady, K.C., and Hubby, B. (2016). Phage Therapy in the Era of Synthetic
634 Biology. *Cold Spring Harb. Perspect. Biol.* *8*, a023879.
- 635 Bäumler, A.J., and Sperandio, V. (2016). Interactions between the microbiota and pathogenic
636 bacteria in the gut. *Nature* *535*, 85-93.
- 637 Bikard, D., Euler, C.W., Jiang, W., Nussenzweig, P.M., Goldberg, G.W., Duportet, X.,
638 Fischetti, V.A., and Marraffini, L.A. (2014). Exploiting CRISPR-Cas nucleases to produce
639 sequence-specific antimicrobials. *Nat. Biotechnol.* *32*, 1146.

640 Bikard, D., Jiang, W., Samai, P., Hochschild, A., Zhang, F., and Marraffini, L.A. (2013).
641 Programmable repression and activation of bacterial gene expression using an engineered
642 CRISPR-Cas system. *Nucleic Acids Res.* *41*, 7429-7437.

643 Boers, S.A., Jansen, R., and Hays, J.P. (2016). Suddenly everyone is a microbiota specialist.
644 *Clin. Microbiol. Inf.* *22*, 581-582.

645 Borody, T.J., and Campbell, J. (2012). Fecal Microbiota Transplantation: Techniques,
646 Applications, and Issues. *Gastroenterol. Clin. North Am.* *41*, 781-803.

647 Briers, Y., and Lavigne, R. (2015). Breaking barriers: expansion of the use of endolysins as
648 novel antibacterials against Gram-negative bacteria. *Fut. Microbiol.* *10*, 377-390.

649 Buckner, M.M.C., Ciusa, M.L., and Piddock, L.J.V. (2018). Strategies to combat
650 antimicrobial resistance: anti-plasmid and plasmid curing. *FEMS Microbiol. Rev.* *42*, 781-
651 804.

652 Bull, J.J., and Gill, J.J. (2014). The habits of highly effective phages: population dynamics as
653 a framework for identifying therapeutic phages. *Front. Microbiol.* *5*, 618-618.

654 Cammarota, G., Ianiro, G., Tilg, H., Rajilić-Stojanović, M., Kump, P., Satokari, R., Sokol, H.,
655 Arkkila, P., Pintus, C., Hart, A., *et al.* (2017). European consensus conference on faecal
656 microbiota transplantation in clinical practice. *Gut* *66*, 569-580.

657 Cascioferro, S., Raffa, D., Maggio, B., Raimondi, M.V., Schillaci, D., and Daidone, G.
658 (2015). Sortase A Inhibitors: Recent Advances and Future Perspectives. *J. Med. Chem.* *58*,
659 9108-9123.

660 Cathcart, G.R.A., Quinn, D., Greer, B., Harriott, P., Lynas, J.F., Gilmore, B.F., and Walker,
661 B. (2011). Novel Inhibitors of the *Pseudomonas aeruginosa* Virulence Factor LasB: a
662 Potential Therapeutic Approach for the Attenuation of Virulence Mechanisms in
663 Pseudomonal Infection. *Antimicrob. Agents Chemother.* *55*, 2670-2678.

664 Chang, R.Y.K., Wallin, M., Lin, Y., Leung, S.S.Y., Wang, H., Morales, S., and Chan, H.-K.
665 (2018). Phage therapy for respiratory infections. *Adv. Drug Deliv. Rev.* *133*, 76-86.

666 Chiang, C.-Y., Uzoma, I., Moore, R.T., Gilbert, M., Duplantier, A.J., and Panchal, R.G.
667 (2018). Mitigating the Impact of Antibacterial Drug Resistance through Host-Directed
668 Therapies: Current Progress, Outlook, and Challenges. *mBio* *9*, e01932-01917.

669 Cooper, C.J., Khan Mirzaei, M., and Nilsson, A.S. (2016). Adapting Drug Approval Pathways
670 for Bacteriophage-Based Therapeutics. *Front. Microbiol.* *7*, 1209-1209.

671 Czaplewski, L., Bax, R., Clokie, M., Dawson, M., Fairhead, H., Fischetti, V.A., Foster, S.,
672 Gilmore, B.F., Hancock, R.E., Harper, D., *et al.* (2016). Alternatives to antibiotics-a pipeline
673 portfolio review. *Lancet Inf. Dis.* *16*, 239-251.

674 Dąbrowska, K., Miernikiewicz, P., Piotrowicz, A., Hodyra, K., Owczarek, B., Lecion, D.,
675 Kaźmierczak, Z., Letarov, A., and Górski, A. (2014). Immunogenicity studies of proteins
676 forming the T4 phage head surface. *J. Virol.* *88*, 12551-12557.

677 Das, S., Angsantikul, P., Le, C., Bao, D., Miyamoto, Y., Gao, W., Zhang, L., and Eckmann,
678 L. (2018). Neutralization of cholera toxin with nanoparticle decoys for treatment of cholera.
679 *PLoS Negl. Trop. Dis.* *12*, e0006266.

680 Defoirdt, T. (2018). Quorum-Sensing Systems as Targets for Antivirulence Therapy. *Trends*
681 *Microbiol.* *26*, 313-328.

682 Deng, W., Marshall, N.C., Rowland, J.L., McCoy, J.M., Worrall, L.J., Santos, A.S.,
683 Strynadka, N.C.J., and Finlay, B.B. (2017). Assembly, structure, function and regulation of
684 type III secretion systems. *Nat. Rev. Microbiol.* *15*, 323.

685 Dickey, S.W., Cheung, G.Y.C., and Otto, M. (2017). Different drugs for bad bugs:
686 antivirulence strategies in the age of antibiotic resistance. *Nat. Rev. Drug Discov.* *16*, 457.

687 DiGiandomenico, A., Keller, A.E., Gao, C., Rainey, G.J., Warrenner, P., Camara, M.M.,
688 Bonnell, J., Fleming, R., Bezabeh, B., Dimasi, N., *et al.* (2014). A multifunctional bispecific
689 antibody protects against *Pseudomonas aeruginosa*. *Sci. Transl. Med.* *6*, 262ra155-262ra155.

690 Fasciano, A.C., Shaban, L., and Meccas, J. (2019). Promises and Challenges of the Type Three
691 Secretion System Injectisome as an Antivirulence Target. *EcoSal Plus.* *8*.

692 Fauconnier, A. (2017). Regulating phage therapy: The biological master file concept could
693 help to overcome regulatory challenge of personalized medicines. *EMBO Rep.* *18*, 198-200.

694 Food and Drug Administration (FDA), USA (2016). Enforcement Policy Regarding
695 Investigational New Drug Requirements for Use of Fecal Microbiota for Transplantation to
696 Treat *Clostridium difficile* Infection Not Responsive to Standard Therapies. Draft Guidance
697 for Industry.
698 [http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Gu](http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm)
699 [idances/default.htm](http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm).

700 Fernandes, S., and São-José, C. (2018). Enzymes and Mechanisms Employed by Tailed
701 Bacteriophages to Breach the Bacterial Cell Barriers. *Viruses* *10*, 396.

702 Fischetti, V.A. (2018). Development of Phage Lysins as Novel Therapeutics: A Historical
703 Perspective. *Viruses* *10*, 310.

704 Gao, P., Ho, P.L., Yan, B., Sze, K.H., Davies, J., and Kao, R.Y.T. (2018). Suppression of
705 *Staphylococcus aureus* virulence by a small-molecule compound. *Proc. Natl. Acad. Sci. USA.*
706 *115*, 8003-8008.

707 Garland, M., Loscher, S., and Bogyo, M. (2017). Chemical Strategies To Target Bacterial
708 Virulence. *Chem. Rev.* *117*, 4422-4461.

709 Good, L., and Stach, J.E. (2011). Synthetic RNA silencing in bacteria - antimicrobial
710 discovery and resistance breaking. *Front Microbiol* *2*, 185.

711 Peterson, J., Garges, S., Giovanni, M., McInnes, P., Wang, L., Schloss, J.A., Bonazzi, V.,
712 McEwen, J.E., Wetterstrand, K.A., *et al.* (2009). The NIH Human Microbiome Project.
713 *Genome Res.* *19*, 2317-2323.

714 Gu, L., Zhou, S., Zhu, L., Liang, C., and Chen, X. (2015). Small-Molecule Inhibitors of the
715 Type III Secretion System. *Molecules* *20*, 17659.

716 Gutiérrez, D., Fernández, L., Rodríguez, A., and García, P. (2018). Are Phage Lytic Proteins
717 the Secret Weapon To Kill *Staphylococcus aureus*? *mBio* *9*, e01923-01917.

718 Haddad Kashani, H., Schmelcher, M., Sabzalipoor, H., Seyed Hosseini, E., and Moniri, R.
719 (2017). Recombinant Endolysins as Potential Therapeutics against Antibiotic-Resistant
720 *Staphylococcus aureus*: Current Status of Research and Novel Delivery Strategies. *Clin.*
721 *Microbiol. Rev.* *31*, e00071-00017.

722 Haque, S., Ahmad, F., Dar, S.A., Jawed, A., Mandal, R.K., Wahid, M., Lohani, M., Khan, S.,
723 Singh, V., and Akhter, N. (2018). Developments in strategies for Quorum Sensing virulence
724 factor inhibition to combat bacterial drug resistance. *Microb. Pathogen.* *121*, 293-302.

725 Harper, D.R. (2018). Criteria for Selecting Suitable Infectious Diseases for Phage Therapy.
726 *Viruses* *10*, 177.

727 Hatoum-Aslan, A. (2018). Phage Genetic Engineering Using CRISPR-Cas Systems. *Viruses*
728 *10*, 335.

729 Heras, B., Scanlon, M.J., and Martin, J.L. (2015). Targeting virulence not viability in the
730 search for future antibacterials. *Br. J. Clin. Pharmacol.* *79*, 208-215.

731 Hodyra-Stefaniak, K., Miernikiewicz, P., Drapała, J., Drab, M., Jończyk-Matysiak, E.,
732 Lecion, D., Kaźmierczak, Z., Beta, W., Majewska, J., Harhala, M., *et al.* (2015). Mammalian

733 Host-Versus-Phage immune response determines phage fate in vivo. *Sci. Rep.* 5, 14802-
734 14802.

735 Hong, X., Qin, J., Li, T., Dai, Y., Wang, Y., Liu, Q., He, L., Lu, H., Gao, Q., Lin, Y., *et al.*
736 (2016). Staphylococcal Protein A Promotes Colonization and Immune Evasion of the
737 Epidemic Healthcare-Associated MRSA ST239. *Front. Microbiol.* 7, 951.

738 Huttner, B.D., de Lastours, V., Wassenberg, M., Maharshak, N., Mauris, A., Galperine, T.,
739 Zanichelli, V., Kapel, N., Bellanger, A., Olearo, F., *et al.* (2019). A five-day course of oral
740 antibiotics followed by faecal transplantation to eradicate carriage of multidrug-resistant
741 *Enterobacteriaceae*: A Randomized Clinical Trial. *Clin. Microbiol. Inf.* S1198-
742 743X(19)30030-8.

743 Inchley, C.J. (1969). The activity of mouse Kupffer cells following intravenous injection of T4
744 bacteriophage. *Clin. Exp. Immunol.* 5, 173-187.

745 Iqbal, U., Anwar, H., Karim, M.A. (2018). Safety and efficacy of encapsulated fecal
746 microbiota transplantation for recurrent *Clostridium difficile* infection: a systematic review.
747 *Eur. J. Gastroenterol. Hepatol.* 30, 730-734.

748 Jun, S.Y., Jang, I.J., Yoon, S., Jang, K., Yu, K.-S., Cho, J.Y., Seong, M.-W., Jung, G.M.,
749 Yoon, S.J., and Kang, S.H. (2017). Pharmacokinetics and Tolerance of the Phage Endolysin-
750 Based Candidate Drug SAL200 after a Single Intravenous Administration among Healthy
751 Volunteers. *Antimicrob. Agents Chemother.* 61, e02629-02616.

752 Kalas, V., Hibbing, M.E., Maddirala, A.R., Chugani, R., Pinkner, J.S., Mydock-McGrane,
753 L.K., Conover, M.S., Janetka, J.W., and Hultgren, S.J. (2018). Structure-based discovery of
754 glycomimetic FmlH ligands as inhibitors of bacterial adhesion during urinary tract infection.
755 *Proc. Natl. Sci. USA.* 115, E2819-E2828.

756 Khanna, S., Pardi, D.S., Kelly, C.R., Kraft, C.S., Dhere, T., Henn, M.R., Lombardo, M.-J.,
757 Vulic, M., Ohsumi, T., Winkler, J., *et al.* (2016). A Novel Microbiome Therapeutic Increases
758 Gut Microbial Diversity and Prevents Recurrent *Clostridium difficile* Infection. *J. Inf. Dis.*
759 214, 173-181.

760 Kim, N.-H., Park, W.B., Cho, J.E., Choi, Y.J., Choi, S.J., Jun, S.Y., Kang, C.K., Song, K.-H.,
761 Choe, P.G., Bang, J.-H., *et al.* (2018). Effects of Phage Endolysin SAL200 Combined with
762 Antibiotics on *Staphylococcus aureus* Infection. *Antimicrob. Agents Chemother.* 62, e00731-
763 00718.

764 Koropatkin, N.M., Cameron, E.A., and Martens, E.C. (2012). How glycan metabolism shapes
765 the human gut microbiota. *Nat. Rev. Microbiol.* *10*, 323-335.

766 Kortright, K.E., Chan, B.K., Koff, J.L., and Turner, P.E. (2019). Phage Therapy: A Renewed
767 Approach to Combat Antibiotic-Resistant Bacteria. *Cell host & microbe* *25*, 219-232.

768 Krom, R.J., Bhargava, P., Lobritz, M.A., and Collins, J.J. (2015). Engineered Phagemids for
769 Nonlytic, Targeted Antibacterial Therapies. *Nano Lett.* *15*, 4808-4813.

770 Krut, O., and Bekeredjian-Ding, I. (2018). Contribution of the Immune Response to Phage
771 Therapy. *J. Immunol.* *200*, 3037-3044.

772 Kwon, E.J., Skalak, M., Bertucci, A., Braun, G., Ricci, F., Ruoslahti, E., Sailor, M.J., and
773 Bhatia, S.N. (2017). Porous Silicon Nanoparticle Delivery of Tandem Peptide Anti-Infectives
774 for the Treatment of *Pseudomonas aeruginosa* Lung Infections. *Adv. Mater.* *29*, 1701527.

775 Lukacik, P., Barnard, T.J., Keller, P.W., Chaturvedi, K.S., Seddiki, N., Fairman, J.W., Noinaj,
776 N., Kirby, T.L., Henderson, J.P., Steven, A.C., *et al.* (2012). Structural engineering of a phage
777 lysin that targets gram-negative pathogens. *Proc. Natl. Acad. Sci. USA.* *109*, 9857-9862.

778 Maddirala, A.R., Klein, R., Pinkner, J.S., Kalas, V., Hultgren, S.J., and Janetka, J.W. (2018).
779 Biphenyl Gal and GalNAc FmlH Lectin Antagonists of Uropathogenic *E. coli* (UPEC):
780 Optimization through Iterative Rational Drug Design. *J. Med. Chem.*
781 10.1021/acs.jmedchem.8b01561

782 Mamusa, M., Sitia, L., Barbero, F., Ruyra, A., Calvo, T.D., Montis, C., Gonzalez-Paredes, A.,
783 Wheeler, G.N., Morris, C.J., McArthur, M., *et al.* (2017). Cationic liposomal vectors
784 incorporating a bolaamphiphile for oligonucleotide antimicrobials. *Biochim. Biophys. Acta*
785 *Biomembr.* *1859*, 1767-1777.

786 Maura, D., Ballok, A.E., and Rahme, L.G. (2016). Considerations and caveats in anti-
787 virulence drug development. *Curr. Opin Microbiol.* *33*, 41-46.

788 Maura, D., Drees, S.L., Bandyopadhyaya, A., Kitao, T., Negri, M., Starkey, M., Lesic, B.,
789 Milot, S., Déziel, E., Zahler, R., *et al.* (2017). Polypharmacology Approaches against the
790 *Pseudomonas aeruginosa* MvfR Regulon and Their Application in Blocking Virulence and
791 Antibiotic Tolerance. *ACS Chem. Biol.* *12*, 1435 - 1443.

792 McConville, T.H., Sullivan, S.B., Gomez-Simmonds, A., Whittier, S., and Uhlemann, A.C.
793 (2017). Carbapenem-resistant Enterobacteriaceae colonization (CRE) and subsequent risk of

794 infection and 90-day mortality in critically ill patients, an observational study. *PloS One* 12,
795 e0186195.

796 Merabishvili, M., Monserez, R., van Belleghem, J., Rose, T., Jennes, S., De Vos, D.,
797 Verbeken, G., Vaneechoutte, M., and Pirnay, J.P. (2017). Stability of bacteriophages in burn
798 wound care products. *PloS One* 12, e0182121.

799 Mullish, B.H., Quraishi, M.N., Segal, J.P., McCune, V.L., Baxter, M., Marsden, G.L., Moore,
800 D.J., Colville, A., Bhala, N., Iqbal, T.H., *et al.* (2018). The use of faecal microbiota transplant
801 as treatment for recurrent or refractory *Clostridium difficile* infection and other potential
802 indications: Joint British Society of Gastroenterology (BSG) and Healthcare Infection Society
803 (HIS) guidelines. *Gut* 67, 1920-1941.

804 Oechslin, F., Piccardi, P., Mancini, S., Gabard, J., Moreillon, P., Entenza, J.M., Resch, G.,
805 and Que, Y.-A. (2017). Synergistic Interaction Between Phage Therapy and Antibiotics
806 Clears *Pseudomonas Aeruginosa* Infection in Endocarditis and Reduces Virulence. *J. Infect.*
807 *Dis.* 215, 703-712.

808 Oliveira, H., São-José, C., and Azeredo, J. (2018). Phage-Derived Peptidoglycan Degrading
809 Enzymes: Challenges and Future Prospects for In Vivo Therapy. *Viruses* 10, 292.

810 Ooijevaar, R.E., van Beurden, Y.H., Terveer, E.M., Goorhuis, A., Bauer, M.P., Keller, J.J.,
811 Mulder, C.J.J., and Kuijper, E.J. (2018). Update of treatment algorithms for *Clostridium*
812 *difficile* infection. *Clin. Microbiol. Infect.* 24, 452-462.

813 Orenstein, R., for the, P.C.D.I., Dubberke, E., for the, P.C.D.I., Hardi, R., for the, P.C.D.I.,
814 Ray, A., for the, P.C.D.I., Mullane, K., for the, P.C.D.I., *et al.* (2016). Safety and Durability
815 of RBX2660 (Microbiota Suspension) for Recurrent *Clostridium difficile* Infection: Results of
816 the PUNCH CD Study. *Clin. Microbiol. Infect.* 62, 596-602.

817 Pastagia, M., Schuch, R., Fischetti, V.A., and Huang, D.B. (2013). Lysins: the arrival of
818 pathogen-directed anti-infectives. *J. Med. Microbiol.* 62, 1506-1516.

819 Paule, A., Frezza, D., and Edeas, M. (2018). Microbiota and Phage Therapy: Future
820 Challenges in Medicine. *Med. Sci. (Basel)* 6, E86

821 PHAGOBURN (Evaluation of phage therapy for the treatment of *Escherichia coli* and
822 *Pseudomonas aeruginosa* burn wound infections (Phase I-II clinical trial) (2018).
823 <https://cordis.europa.eu/project/rcn/108695/reporting/en>.

824 Pirnay, J.-P., Verbeken, G., Ceysens, P.-J., Huys, I., De Vos, D., Ameloot, C., and
825 Fauconnier, A. (2018). The Magistral Phage. *Viruses* *10*, 64.

826 Pires, D.P., Cleto, S., Sillankorva, S., Azeredo, J., and Lu, T.K. (2016). Genetically
827 Engineered Phages: a Review of Advances over the Last Decade. *Microbiol. Mol. Biol. Rev.*
828 *80*, 523-43.

829 Pirofski, L.-a., and Casadevall, A. (2006). Immunomodulators as an antimicrobial tool. *Curr.*
830 *Opin. Microbiol.* *9*, 489-495.

831 Relman, D.A., and Lipsitch, M. (2018). Microbiome as a tool and a target in the effort to
832 address antimicrobial resistance. *Proc. Natl. Acad. Sci. USA.* *115*, 12902-12910.

833 Rémy, B., Mion, S., Plener, L., Elias, M., Chabrière, E., and Daudé, D. (2018). Interference in
834 Bacterial Quorum Sensing: A Biopharmaceutical Perspective. *Front. Pharmacol.* *9*, 203-203.

835 Rezzoagli, C., Wilson, D., Weigert, M., Wyder, S., and Kuemmerli, R. (2018). Probing the
836 evolutionary robustness of two repurposed drugs targeting iron uptake in *Pseudomonas*
837 *aeruginosa*. *Evol. Med. Public Health.* *1*, 246-259.

838 Rohde, C., Wittmann, J., and Kutter, E. (2018). Bacteriophages: A Therapy Concept against
839 Multi-Drug-Resistant Bacteria. *Surg. Infect.* *19*, 737-744.

840 Rose, T., Verbeken, G., Vos, D.D., Merabishvili, M., Vanechoutte, M., Lavigne, R., Jennes,
841 S., Zizi, M., and Pirnay, J.-P. (2014). Experimental phage therapy of burn wound infection:
842 difficult first steps. *Int. J. Burns Trauma* *4*, 66-73.

843 Salam, A.M., and Quave, C.L. (2018). Targeting Virulence in *Staphylococcus aureus* by
844 Chemical Inhibition of the Accessory Gene Regulator System In Vivo. *mSphere* *3*, e00500-
845 00517.

846 São-José, C. (2018). Engineering of Phage-Derived Lytic Enzymes: Improving Their
847 Potential as Antimicrobials. *Antibiotics (Basel, Switzerland)* *7*, 29.

848 Schirmeier, E., Zimmermann, P., Hofmann, V., Biebl, M., Gerstmans, H., Maervoet, V.E.T.,
849 and Briers, Y. (2018). Inhibitory and bactericidal effect of Artilysin® Art-175 against
850 colistin-resistant *mcr-1*-positive *Escherichia coli* isolates. *Int. J. Antimicrob. Agents* *51*, 528-
851 529.

852 Schuch, R., Lee, H.M., Schneider, B.C., Sauve, K.L., Law, C., Khan, B.K., Rotolo, J.A.,
853 Horiuchi, Y., Couto, D.E., Raz, A., *et al.* (2014). Combination therapy with lysin CF-301 and

854 antibiotic is superior to antibiotic alone for treating methicillin-resistant *Staphylococcus*
855 *aureus*-induced murine bacteremia. *J. Inf. Dis.* 209, 1469-1478.

856 Self, W.H., Wunderink, R.G., DiNubile, M.J., Stossel, T.P., Levinson, S.L., Williams, D.J.,
857 Anderson, E.J., Bramley, A.M., Jain, S., Edwards, K.M., *et al.* (2018). Low Admission
858 Plasma Gelsolin Concentrations Identify Community-acquired Pneumonia Patients at High
859 Risk for Severe Outcomes. *Clin. Infect. Dis.* 10.1093/cid/ciy1049

860 Servick, K. (2016). Beleaguered phage therapy trial presses on. *Science* 352, 1506-1506.

861 Smillie, C.S., Sauk, J., Gevers, D., Friedman, J., Sung, J., Youngster, I., Hohmann, E.L.,
862 Staley, C., Khoruts, A., Sadowsky, M.J., *et al.* (2018). Strain Tracking Reveals the
863 Determinants of Bacterial Engraftment in the Human Gut Following Fecal Microbiota
864 Transplantation. *Cell Host Microbe.* 14, 229-240

865 Spaulding, C.N., and Hultgren, S.J. (2016). Adhesive Pili in UTI Pathogenesis and Drug
866 Development. *Pathogens* 5. E30.

867 Spaulding, C.N., Klein, R.D., Schreiber, H.L., Janetka, J.W., and Hultgren, S.J. (2018).
868 Precision antimicrobial therapeutics: the path of least resistance? *NPJ Biofilms Microbiomes*
869 4, 4.

870 Sully, E.K., and Geller, B.L. (2016). Antisense antimicrobial therapeutics. *Curr. Opin.*
871 *Microbiol.* 33, 47-55.

872 Theuretzbacher, U., Savic, M., Årdal, C., and Outtersen, K. (2017). Innovation in the
873 preclinical antibiotic pipeline. *Nat. Rev. Drug Discov.* 16, 744.

874 Timmis, K., Timmis, J.K., Brüßow, H., and Fernández, L.Á. (2019). Synthetic consortia of
875 nanobody-coupled and formatted bacteria for prophylaxis and therapy interventions targeting
876 microbiome dysbiosis-associated diseases and co-morbidities. *Microb. Biotechnol.* 12, 58-65.

877 Totsika, M. (2016). Benefits and Challenges of Antivirulence Antimicrobials at the Dawn of
878 the Post-Antibiotic Era. *Curr. Medicin. Chem.* 6, 30-37.

879 Varshney, A.K., Kuzmicheva, G.A., Lin, J., Sunley, K.M., Bowling, R.A., Jr., Kwan, T.Y.,
880 Mays, H.R., Rambhadran, A., Zhang, Y., Martin, R.L., *et al.* (2018). A natural human
881 monoclonal antibody targeting *Staphylococcus* Protein A protects against *Staphylococcus*
882 *aureus* bacteremia. *PloS One* 13, e0190537.

883 Vila, J. (2018). Microbiota transplantation and/or CRISPR/Cas in the battle against
884 antimicrobial resistance. *Clin. Microbiol. Infect.* 24, 684-686.

885 Wang-Lin, S.X., and Balthasar, J.P. (2018). Pharmacokinetic and Pharmacodynamic
886 Considerations for the Use of Monoclonal Antibodies in the Treatment of Bacterial Infections.
887 *Antibodies* 7, 5.

888 Wang, L., Hu, C., and Shao, L. (2017). The antimicrobial activity of nanoparticles: present
889 situation and prospects for the future. *Int. J. Nanomedicine* 12, 1227-1249.

890 Williams, P. (2017). Strategies for inhibiting quorum sensing. *Emerging Topics Life Scienc.*
891 *1*, 23-30.

892 Wilson, B.C., Vatanen, T., Cutfield, W.S., and O'Sullivan, J.M. (2019). The Super-Donor
893 Phenomenon in Fecal Microbiota Transplantation. *Front. Cell. Infect. Microbiol.* 9:2

894 Wright, A., Hawkins, C.H., Änggård, E.E., and Harper, D.R. (2009). A controlled clinical
895 trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant
896 *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clin. Otolaryngol.* 34, 349-357.

897 Yosef, I., Manor, M., Kiro, R., and Qimron, U. (2015). Temperate and lytic bacteriophages
898 programmed to sensitize and kill antibiotic-resistant bacteria. *Proc. Natl. Acad. Sci.* 112,
899 7267-7272.

900 Young, V.B. (2016). Therapeutic manipulation of the microbiota: past, present, and
901 considerations for the future. *Clin. Microbiol. Infect.* 22, 905-909.

902 Yu, X.-Q., Robbie, G.J., Wu, Y., Esser, M.T., Jensen, K., Schwartz, H.I., Bellamy, T.,
903 Hernandez-Illas, M., and Jafri, H.S. (2017). Safety, Tolerability, and Pharmacokinetics of
904 MEDI4893, an Investigational, Extended-Half-Life, Anti-*Staphylococcus aureus* Alpha-Toxin
905 Human Monoclonal Antibody, in Healthy Adults. *Antimicrob. Agents Chemother.* 61,
906 e01020-01016.

907 Zuo, T., Wong, S.H., Lam, K., Lui, R., Cheung, K., Tang, W., Ching, J.Y.L., Chan, P.K.S.,
908 Chan, M.C.W., Wu, J.C.Y., *et al.* (2018). Bacteriophage transfer during faecal microbiota
909 transplantation in *Clostridium difficile* infection is associated with treatment outcome. *Gut* 67,
910 634-643.

911

912

913 **Table 1. Monoclonal antibodies in clinical development**

Monoclonal antibody, company	Clinical development	Anti-virulence target	Indication
Suvratoxumab (MEDI4893), Medimmune	Phase 2	<i>S. aureus</i> α -toxin	Prevention of VAP caused by <i>S. aureus</i>
AR-301 (Salvecin, tosatoxumab), Aridis	Phase 3	<i>S. aureus</i> α -toxin	Adjunctive therapy for VAP caused by <i>S. aureus</i>
MEDI3902, Medimmune	Phase 2	<i>P. aeruginosa</i> T3SS needle tip protein PcrV and Psl exopolysaccharide	Prevention of VAP caused by <i>P. aeruginosa</i>
AR-105 (Aerucin), Aridis	Phase 2	<i>P. aeruginosa</i> alginate	Adjunctive treatment of VAP caused by <i>P. aeruginosa</i>
514G3, XBiotech	Phase 1/2	<i>S. aureus</i> Protein A	Adjunctive treatment of bloodstream infections caused by <i>S. aureus</i>
ASN-100, Arsanis	Phase 2	<i>S. aureus</i> α -toxin and five leukocidins	Failed to prove its effectiveness in high-risk, mechanically ventilated patients with <i>S. aureus</i> pneumonia

914

915 For information about the status of R&D of antibodies, small molecules and other approaches

916 to new treatments, please see www.clinicaltrials.gov or the developing organizations website.

917

918 **Table 2. Translational challenges of anti-virulence strategies**

Challenge	Factor influencing translation
Selection of target	Selection of the most relevant anti-virulence target according to their distribution and contribution to disease
Coverage	VFs are not expressed uniformly in all strains. May differ geographically and gene not present in all strains (genetic variation)
Effectiveness	May be effective only in specific disease states (e.g. chronic, dormant), a specific time point in the infectious process, at a specific infection site, or in specific patient groups (e.g. immunocompetent)
Diagnostics	Diagnostics beyond species identification may be necessary to guide use
Dose finding	Predictive models to support decisions to find the optimum dose are mostly lacking
Predictive models of efficacy	For most approaches there are no validated models that predict clinical outcome and preclinical studies may not be a meaningful guide to clinical development
Resistance	Resistance development has been shown for some anti-virulence approaches. It is not known how to predict the likelihood of developing anti-virulence drug resistance and any relevance in patients
Activity in patients	For some approaches it is not known if the selected approach has enough clinically relevant impact on the disease in humans
Clinical development	For adjunctive therapies non-inferiority studies will not prove that the therapy works in patients. Superiority studies are essential to show that the adjunctive therapy provides a benefit to patients. Superiority studies (and preventive studies) are very difficult to do.

919

920

921

Table 3. Current approaches to manipulate the microbiome

Approach	Indication	Comments
Transfer of human intestinal microbiota	Prevention of recurrent CDI	<ul style="list-style-type: none"> • FMT: Transfer of stool suspension from a donor via colonoscopy, enema, nasogastric routes or pills (Finch, Open Biome) • Fecal microbiota suspension: Standardized number of live bacteria from stool suspension from donors via enema or capsules, GMP produced (Rebiotix/Ferring) • Rationally selected microbiota: Well characterized selection of bacterial strains via capsules (Seres, Finch Therapeutics, Vedanta) • Spore suspension: Spores, fractionated from stool specimens from donors via capsules (Seres)
Synthetic microbiota	Intestinal, dermatological (e.g. atopic dermatitis), lung conditions (e.g. CF)	<ul style="list-style-type: none"> • Selected live bacteria producing specific metabolites or cocktail of secondary metabolites • Selected live bacteria from skin microbiota (MatriSys Bio) • Selected live bacteria for balancing the lung microbiota
Manipulating the metabolism of microbiota		Manipulating the metabolic balance through specific bacterial nutrients, e.g. Glycans (Kaleido)
Competition	Prevention of recurrent CDI, catheter associated UTI	<ul style="list-style-type: none"> • Non-toxinogenic <i>C. difficile</i> that is assumed to outcompete the toxic strain (Microbiotica) • Apathogenic <i>E. coli</i> introduced into the bladder via catheter coating (Atterx)
Engineering probiotics to deliver	Various indications (Bäumler and Sperandio, 2016)	<ul style="list-style-type: none"> • Engineered <i>Lactobacillus</i> to express bacteriocin against <i>P. aeruginosa</i> (inhaled, CF) and <i>C. difficile</i> (SciBac)

antibacterial proteins		<ul style="list-style-type: none"> • Engineered <i>Lactobacillus</i> to express SagA protein that promotes tolerance to enteric infections incl. <i>C. difficile</i> infection (Rise Therapeutics) • R-type bacteriocins against <i>C. difficile</i>
Prevention of disbalance of microbiome due to antibiotic therapy	Prevention of recurrent CDI	<ul style="list-style-type: none"> • Hydrolysing specific beta-lactam antibiotics in the gut (beta-lactamase, Synthetic Biologics, DaVolterra)
Decolonisation of MDR Gram-negative pathogens in high risk patients	Various indications	<ul style="list-style-type: none"> • Decolonisation of asymptomatic carriers with live bacteria consortia (e.g. <i>C. difficile</i>, MDR Gram-negative pathogens in high risk patients, <i>Salmonella Typhi</i>)

923

924

925 **Table 4. Current approaches using phages**

Approach	Composition
Fixed phage cocktails	Fixed composition of lytic phages to achieve a broad host range of a bacterial species
Individualized phage cocktail	The lytic phages are stored individually in a phage bank with established QC. Only the best active phages based on rapid diagnostic tests are selected for an individual patient
Genetically engineered phages	Engineered phages with improved or specific characteristics
Genetically engineered non-replicating phages as vehicles	Engineered phages to express additionally antimicrobial peptides or protein toxins leading to rapid, nonlytic bacterial death. May deliver CRISPR CAS3 genes directly into bacteria
Phage products, e.g. endolysins	Natural or recombinant cell wall hydrolyzing phage-based enzymes. Endolysins against <i>S. aureus</i> are in clinical development

926

927