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A Phage Foundry framework to systematically develop viral countermeasures to combat antibiotic resistant bacterial pathogens

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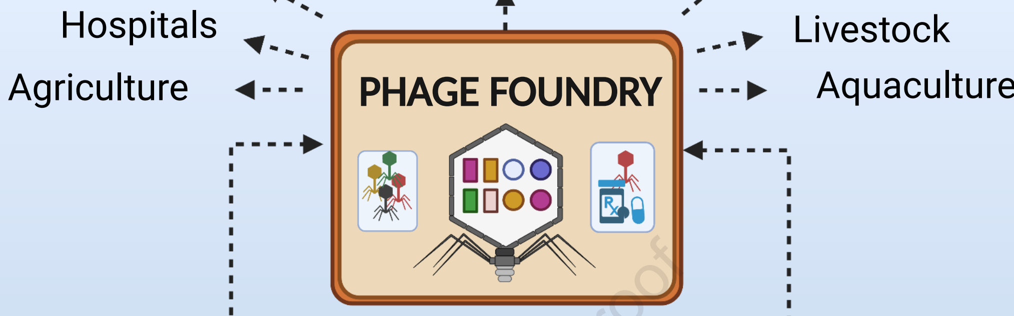

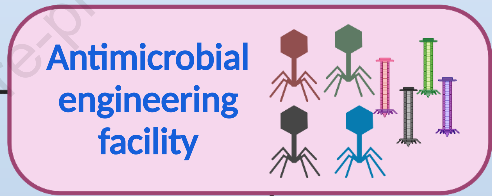

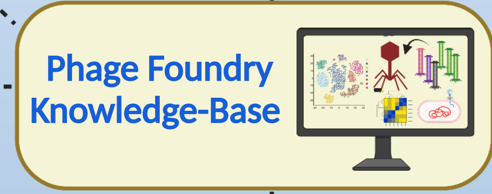

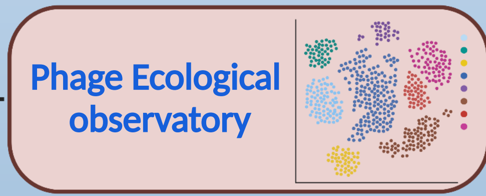

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1 # A Phage Foundry framework to systematically develop viral countermeasures
2 # to combat antibiotic resistant bacterial pathogens
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22 **Summary**

23 At its current rate, the rise of antimicrobial resistant (AMR) infections is predicted to paralyze
24 our industries and healthcare facilities while becoming the leading global cause of loss of human
25 life. With limited new antibiotics on the horizon, we need to invest in alternative solutions.
26 Bacteriophages (phages)- viruses targeting bacteria- offer a powerful alternative approach to
27 tackle bacterial infections. Despite recent advances in using phages to treat recalcitrant AMR
28 infections, the field lacks systematic development of phage therapies scalable to different
29 applications. We propose a Phage Foundry framework to establish metrics for phage
30 characterization and to fill the knowledge and technological gaps in phage therapeutics.
31 Coordinated investment in AMR surveillance, sampling, characterization and data sharing
32 procedures will enable rational exploitation of phages for treatments. A fully realized Phage
33 Foundry will enhance the sharing of knowledge, technology and viral reagents in an equitable
34 manner and will accelerate the biobased economy.

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39 ## Preamble: Knowledge gaps in AMR

40 AMR has been a consistently growing global problem and has been called the 'invisible
41 pandemic' (Tacconelli *et al.*, 2018; Mahase, 2019; Knight *et al.*, 2021). Failure to stem the rising
42 tide of multidrug resistant (MDR) bacterial and fungal pathogens is estimated to have a real
43 worldwide economic cost running into trillions of USD by severely debilitating agriculture,
44 dairy, aquaculture, livestock and poultry industries among others, in addition to the tragic human
45 cost (Executive Office of the President, 2014; O'Neill and Others, 2016; Tacconelli *et al.*, 2018;
46 (u.s.) and Centers for Disease Control and Prevention (U.S.), 2019). A number of factors
47 contribute to the selection and spread of antibiotic resistance genes and the known and emerging
48 microbial pathogens that host them(Buckley *et al.*, 2021) (reviewed in detail earlier (White,
49 Alekshun and McDermott, 2005; Payne *et al.*, 2007; Chadwick and Goode, 2008; Mayers, 2009;
50 Fair and Tor, 2014; Holmes *et al.*, 2016; Surette and Wright, 2017; Gotte *et al.*, 2018; Anderson,
51 Cecchini and Mossialos, 2020; Andersson *et al.*, 2020)) (Figure 1) for example 1) Misuse of
52 frontline antibiotics and other antimicrobials, 2) climate-driven niche destruction and induced
53 migration of host and pathogens, 3) agricultural intensification, 4) increasing environmental
54 pollution with drugs, industrial chemicals and pesticides, 5) a denser population of humans along
55 with closer contact with animal reservoirs, and in some places 6) poor public infrastructure.
56 There is an increasing realization that this rise is not just about the individual fitness of the
57 resistant strain but the ecology in which it resides(Brockhurst *et al.*, 2019; Andersson *et al.*,
58 2020).
59
60 Faster dispersal mechanisms among differentially selective reservoirs (Figure 1), rapid
61 adaptation to new zoonotic hosts, increasing 'safe passage' opportunities for horizontal transfer
62 and recombination of genetic elements carrying resistance genes to non-pathogens, co-occurring
63 microbial community members that support/trigger transfer of resistance traits and process of
64 adaptation are all being increasingly recognized as significant elements in the rise of fit and
65 resistant infectious agents(Palmer and Kishony, 2013; Hu *et al.*, 2016; Andersson *et al.*, 2020;
66 Antunes, Novais and Peixe, 2020). Many of these adaptations lead to easily spreadable cross-
67 resistance towards broad as well as narrow spectrum antibiotics without seeming a fitness
68 tradeoff(Andersson and Hughes, 2010; Palmer and Kishony, 2013; Baym, Stone and Kishony,
69 2016; Tyers and Wright, 2019). In addition, these same mechanisms are used to adopt or fight
70 off pesticides, ionophores, fungicides, metals, biocides/disinfectants and diverse xenobiotics
71 (White, Alekshun and McDermott, 2005; Baker-Austin *et al.*, 2006; Pal *et al.*, 2017; Kampf,
72 2018; Andersson *et al.*, 2020; Getahun *et al.*, 2020; Knight *et al.*, 2021; Lindell, Zimmermann-
73 Kogadeeva and Patil, 2022). Indiscriminate use of these agents in agriculture, aquaculture, food
74 processing and industrial processes has further accelerated the emergence of pathogen variants
75 displaying cross-resistance to unrelated but clinically important antimicrobials(White, Alekshun
76 and McDermott, 2005; Davin-Regli and Pagès, 2012; Guardabassi and Courvalin, 2019;
77 Andersson *et al.*, 2020; Elekhnawy *et al.*, 2020; Verweij *et al.*, 2020; Kang *et al.*, 2022).

78
79 To compound these above mentioned challenges, no new class of antibiotics have been brought
80 to market in the last five decades(Plackett, 2020; Theuretzbacher *et al.*, 2020) . Further,
81 developing new antibiotics to overcome new mechanisms of antibiotic resistance is
82 nontrivial(Årdal *et al.*, 2020; OECD and World Health Organization, 2020). It is time-
83 consuming, expensive and cumbersome to identify such new molecules either from natural
84 sources or from rational design or screening from synthetic libraries in a short time(Fair and Tor,
85 2014; Plackett, 2020). The approval process for such new molecules is expensive and often may
86 take longer than the adaptation times of the pathogens(Årdal *et al.*, 2020). Recently developed
87 programs are helping defray some of these costs and establishing new routes to fund the research
88 and development of novel therapeutics(Alm and Gallant, 2020; Årdal *et al.*, 2020).
89
90 One of the critical piece in responding effectively to rising AMR is to build the capability to
91 rapidly detect -when and where-from a particular pathogen variant emerges so that the
92 mechanisms that have allowed its emergence can be identified and the appropriate strategy can
93 be applied with respect to where to intervene (patient, animal and abiotic pools) and with which
94 agent(s)(Boolchandani, D'Souza and Dantas, 2019). This requires improved infrastructure and
95 appropriate policy changes for establishing surveillance networks at the regional, national and
96 global levels(Fauci, 2001). In this regard, One Health initiative offers a well-coordinated
97 approach to elucidate and control the rise of AMR through specifically respecting the
98 interconnectedness among humans, livestock, pets, wildlife and environmental systems with the
99 goal of optimal health outcomes for everyone(National Academies of Sciences, Engineering, and
100 Medicine *et al.*, 2018; Walsh, 2018) (Figure 1). It has also led to the search for combinatorial
101 therapies and alternative modalities/approaches to control infection/spread of AMR bacteria such
102 as the use of bacteriophages (i.e. bacterial viruses), obligate parasites that infect and kill specific
103 bacterial strains(Clatworthy, Pierson and Hung, 2007; Yeh *et al.*, 2009; Villa and Crespo, 2010;
104 Kim, Lieberman and Kishony, 2014; Baym, Stone and Kishony, 2016; Czaplewski *et al.*, 2016;
105 Dickey, Cheung and Otto, 2017; Mariathasan and Tan, 2017; Baker *et al.*, 2018; Douafer *et al.*,
106 2019; Heselpoth *et al.*, 2019; Tyers and Wright, 2019; Poolman, 2020; Theuretzbacher *et al.*,
107 2020; Bottery, Pitchford and Friman, 2021), focus of this opinion piece. We argue below, along
108 with the international network of sophisticated surveillance programs, we need focused and
109 federally funded programs to form a critical scaffolding that could support a next generation of
110 more rationally and mechanistically designed, ecologically understood, and more effectively
111 manufactured and deployed antibiotics with phage therapy at its core.
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114 ## Phage therapy to tackle AMR

115 Phages represent the most abundant biological entities in nature —tenfold greater than
116 bacteria(Hendrix *et al.*, 1999). Double stranded DNA (dsDNA) phages can be readily isolated
117 from the environment and display specificity to their target bacteria(Kutter and Sulakvelidze,
118 2004). Based on the infection lifestyle, phages are either classified as lytic or temperate. In the
119 lytic mode, phage infection leads to delivery of genetic material, lysis of target bacteria and
120 release of progeny. Temperate phages on the other hand can switch between lysogenic to lytic
121 cycle based on conditions, where the lysogenic mode leads temperate phage genomes to persist
122 as prophages integrated on to the target bacterial genomes or exist extrachromosomally. Many
123 recent reviews have outlined the possible uses and advantages of using phage as a therapeutic
124 agent(Hagens and Loessner, 2010; Loc-Carrillo and Abedon, 2011; Frampton, Pitman and
125 Fineran, 2012; Young and Gill, 2015; Koskella and Taylor, 2018; Svircev, Roach and Castle,
126 2018; Bull, Levin and Molineux, 2019; Gordillo Altamirano and Barr, 2019; Hesse and Adhya,
127 2019; Kortright *et al.*, 2019; Luong, Salabarria and Roach, 2020; Hatfull, Dedrick and Schooley,
128 2021; Pirnay, Ferry and Resch, 2021). Detailed discussions on using phages to treat infections in
129 plants, animals and humans have been put forth in numerous monographs and books (Kutter and
130 Sulakvelidze, 2004; Sabour and Griffiths, 2010; Hyman and Paul Hyman and Stephen T.
131 Abedon, 2012; Reinheimer, 2012; Azeredo and Sillankorva, 2017; Jassim and Limoges, 2017;
132 Górski, Międzybrodzki and Borysowski, 2019). Recent successes in using dsDNA phages to
133 treat antibiotic resistant infection under compassionate use protocols have received much
134 attention in the western countries despite nearly a century of anecdotal use in other regions of the
135 eastern world(Kutter *et al.*, 2010; Abedon *et al.*, 2011; Kutter, Kuhl and Abedon, 2015; Kortright
136 *et al.*, 2019; Hatfull, Dedrick and Schooley, 2021).

137 Phages have many unique properties that make them particularly attractive and are increasingly
138 recognized as potentially transformative agents precisely because they address some of the key
139 issues mentioned above in the AMR preamble section. Most known MDR pathogens have
140 known phages that can specifically attack them. Phages tend to be bactericidal rather than
141 bacteriostatic and those that are temperate can be often converted to a lytic form by rational
142 genetic manipulation or natural mutant selection(Lenski, 2017b; Dedrick *et al.*, 2019). Phages
143 are readily identified in the environment through sequencing, and the evolutionary patterns of the
144 Red-Queen warfare between target pathogen and phages could possibly be tracked via
145 sequencing as well thereby identifying the genetic bases of the mechanisms of resistance and
146 counter resistance(Hussain *et al.*, 2021; LeGault *et al.*, 2021). The ability of phages to rapidly
147 evolve to evade target pathogen resistance can be exploited using in-vitro directed evolution to
148 'train' libraries of phages against panels of targets to create banks of complementary phage
149 antimicrobial agents for cocktails(Rohde *et al.*, 2018; Burrowes, Molineux and Fralick, 2019;
150 Abdelsattar *et al.*, 2021; Borin *et al.*, 2021; Eskenazi *et al.*, 2022; Torres-Barceló, Turner and

151 Buckling, 2022). The small genomic size of phages enable both full genome synthesis and
152 possibly 'booting' (producing viable phage particles from synthetic DNA) when isolation is
153 difficult as well as efficient engineering of designed genetic changes(Chan, Kosuri and Endy,
154 2005; Ando *et al.*, 2015; Pires *et al.*, 2016, 2021; Kilcher *et al.*, 2018; Lemire, Yehl and Lu,
155 2018; Dunne *et al.*, 2019; Kilcher and Loessner, 2019; Weynberg and Jaschke, 2020; Lenneman
156 *et al.*, 2021). Designer changes include engineered genetic payloads that increase toxicity,
157 counteract defenses and potentially suppress horizontal gene transfer of resistance genes by, for
158 example, degrading the target bacterial genome rapidly (Lu and Collins, 2007; Yosef *et al.*,
159 2015, 2017; Barbu, Cady and Hubby, 2016; Dunne *et al.*, 2019; Kilcher and Loessner, 2019;
160 Yehl *et al.*, 2019; Lenneman *et al.*, 2021). These approaches allow diversification of
161 antimicrobial targets leading to more effective cocktail design. Phages' physical form as a
162 proteinaceous particle allows functional and programmed decoration of its surface that can
163 confer the ability to penetrate biofilms, better control of *in situ* targeting, and enhanced
164 pharmacokinetic (PK) and pharmacodynamic (PD) properties(Dąbrowska, 2019; Dąbrowska and
165 Abedon, 2019). Finally, once created, their synthesis and formulation could prove scalable,
166 distributable and cost-effective and offer paths to alleviate some of the industrial and regulatory
167 hurdles associated with other types of antimicrobials (Pelfrene *et al.*, 2016; Malik *et al.*, 2017;
168 Malik and Resch, 2020; Malik, 2021).

169 The advantages of phages could be amplified when placed in the One Health ecological
170 framework(Garvey, 2020) (Figure 1). In a scenario where the new threats involve both bacterial
171 pathogens and their variants and we want to stem these before they spread widely into our food,
172 water and health facilities we need to be able to identify and 'engineer' effective interventions.
173 This is not realistically possible using our current antibiotic/antimicrobial arsenal. But with the
174 natural and unlimited reservoir of phages and their inherent engineerability there is hope to
175 develop a flexible and nimble platform for production of these targeted antimicrobial agents on
176 demand(Clokie *et al.*, 2011; Mattila, Ruotsalainen and Jalasvuori, 2015; Pires *et al.*, 2016;
177 Lenneman *et al.*, 2021).

178 However, application of phages in therapy and biocontrol present challenges as well, and many
179 of these challenges share a substantial overlap with AMR knowledge gaps listed in Figure 1.
180 While there is great progress in the technologies per se for characterizing and engineering
181 phages, there is still lack of progress in our ability to rationally and predictably design a phage
182 formulation to effectively eliminate a target pathogen and to ensure it works robustly across the
183 spectrum of the population variation of a given pathogen in its natural or real world
184 environmental matrices(Meaden and Koskella, 2013; Young and Gill, 2015; Koskella and
185 Taylor, 2018; Brüssow, 2019; Caflisch, Suh and Patel, 2019; Dąbrowska and Abedon, 2019;
186 Hesse and Adhya, 2019; Kortright *et al.*, 2019; Górski, Borysowski and Międzybrodzki, 2020;
187 Luong, Salabarria and Roach, 2020; Federici, Nobs and Elinav, 2021; Hatfull, Dedrick and

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188 Schooley, 2021; Pirnay, Ferry and Resch, 2021); For example, despite the recent progress made
189 in phage-target pathogen matching methods(Henry *et al.*, 2012; Estrella *et al.*, 2016), it remains
190 difficult to rapidly and economically identify phages/phage-antibiotic combinations to which a
191 patient's infections are susceptible or predict which multiple phages in a phage collection
192 ("phage bank") may be synergetic (or not) in their antimicrobial activity due to independent
193 mechanisms of interaction, cross-resistance and infectivity profile with the target
194 pathogen(Wright *et al.*, 2021; Segall, Roach and Strathdee, 2019; Gu Liu *et al.*, 2020; Al-Anany,
195 Fatima and Hynes, 2021; Gordillo Altamirano and Barr, 2021; Markwitz *et al.*, 2022; Torres-
196 Barceló, Turner and Buckling, 2022). There are currently no guidelines for the delivery and
197 dosing of phages that guarantee access to appropropriate target infection sites in sufficient
198 numbers so that a self-sustaining replicative cycle can be established. Lack of adequate
199 knowledge on the phage-bacteria ecology in the therapeutic environment makes it difficult to
200 assess or predict how variable host environments (dysbiosis or healthy status) and the extended
201 microbial community impact the therapeutic effect of phages. It still remains a challenge to
202 design natural phage interventions or engineer natural phages we have on hand to respond to
203 variations in pathogens or new threats, in natural contexts. Finally, we are only at the early stages
204 of establishing appropriate environmental surveillance systems that can potentially identify
205 emerging threats early enough to be able to develop an intervention and to deliver it before an
206 outbreak becomes epidemic or pandemic.

207 A number of groups have suggested and reviewed different frameworks, protocols and the
208 'desiderata' for effective phages and phage-based therapies (Loc-Carrillo and Abedon, 2011;
209 Meaden and Koskella, 2013; Young and Gill, 2015; Pires *et al.*, 2017; Górski *et al.*, 2018;
210 Koskella and Taylor, 2018; Philipson *et al.*, 2018; Gibson *et al.*, 2019; Gordillo Altamirano and
211 Barr, 2019; Hesse and Adhya, 2019; Hyman, 2019; Kortright *et al.*, 2019; Luong *et al.*, 2020;
212 Luong, Salabarria and Roach, 2020; Yerushalmy *et al.*, 2020; Gelman *et al.*, 2021; Hatfull,
213 Dedrick and Schooley, 2021; Liu *et al.*, 2021; Nale and Clokie, 2021; Pirnay, Ferry and Resch,
214 2021; Verbeken and Pirnay, 2022) which span very specific needs such as phage penetration of
215 biofilms and bacteriocidal activity without release of bacterial toxins upon lysis and very broad
216 ones such as favorable pharmacokinetics/pharmacodynamics properties and minimization of
217 impact on target/host microbiome. The technological approaches to achieve these goals largely
218 exist though there has not been an organized effort to standardize and design interventions
219 rationally, or establish the infrastructure to collect and exploit the necessary data to apply them
220 effectively.

221 Here, we argue that a systematic response to emergence of AMR bacteria can be significantly
222 augmented if there is increased and coordinated investment in those aspects that enable phages to
223 be harnessed for therapies in combination with each other i.e., cocktails and/or with classical
224 antimicrobials. We propose this framework as a coordinated 'Foundry', or Federation of

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225 Foundries, which collaborates with the international AMR infrastructures already in place to
226 coordinate the sampling, observation and characterization protocols. In this manner, these efforts
227 can drive the discovery, harnessing and engineering of therapeutic phages in a responsive,
228 knowledge-building, and cost-effective manner. The Foundry would (1) expand and deepen
229 surveillance efforts to track pathogens, their genetic variants and phages and resistant/defense
230 elements; (2) systematize sampling and isolation, and basic characterization of phages along with
231 target pathogens and other critical community members; and, (3) for cases where there was
232 sufficient novelty or need, would be equipped for deep molecular characterization and
233 engineering of environmentally-mediated phage/target interaction through advanced genetics and
234 Adaptive Laboratory Evolution (ALE). The Foundry would be designed to bring these phages
235 into increasing readiness for rational deployment as biocontrol agents or pharmaceuticals so that
236 the time to acceptance by regulatory bodies could be shortened. Formal phage characterization,
237 readiness level and testing criteria would be a product of this effort. Finally, a fully operational
238 Phage Foundry will share Phages and bacterial pathogen characterization platforms, engineering
239 technologies, knowledge-base and characterized phages to researchers world-wide in a fair and
240 equitable manner. Here we propose the concept of the Phage Foundry and a first version of these
241 characterization and readiness level specifications stemming from this body.

242 Raising the readiness level of phage and cocktails for therapies.

243 Here we define an incremental "**P**hage-bacteria **C**haracterization **L**evel (PCL)" "scale"
244 indicating current practices and capabilities needed to achieve each of those levels (Figure 2 and
245 3). The PCL scale is inspired by the Technology Readiness Levels (TRL) scale developed by
246 US-NASA and US-DoD that has been adopted widely by diverse industries (Sadin, Povinelli and
247 Rosen, 1989; Banke, 2010; Straub, 2015; Buchner *et al.*, 2019; Hofmann *et al.*, 2020). Similar to
248 TRL, the PCL scale provides a set of characterization metrics for a specific set of reagents in the
249 context of an application and operational environment. Level 1 represents the most basic
250 characterization needed for using phages in an application while Level 7 represents the highest
251 level needed to successfully deploy phages for a specific application in a given
252 scenario/environment. As every biocontrol/therapeutic application has different specifications for
253 characterization, efficacy, safety, stability, formulation, delivery modes and regulatory
254 requirements, not all applications need phage-target host interactions characterized at top level.
255 The appropriate level of characterization for phages depends on their intended application, their
256 specification, environmental context, and cost and time needed to cross certification tiers. Here
257 we focus on Level 1 to 5 that represent the core of this categorization roadmap and leave out
258 Level 6 and 7 since these focus primarily on scale up, manufacturing under Good manufacturing
259 practices (GMP) and desired clinical/field trials, data collection and characterization to achieve
260 safety and regulatory certifications. We believe Level 6 and 7 are beyond the scope of this
261 perspective and separable topics that have been reviewed in detail earlier (Verbeken *et al.*, 2014;
262 Jassim and Limoges, 2017; Malik *et al.*, 2017; Pirnay *et al.*, 2018; Gabard and Jault, 2019;

263 McCallin *et al.*, 2019; Bretaudeau *et al.*, 2020; João *et al.*, 2021; Liu *et al.*, 2021; Malik, 2021;
264 Verbeken and Pirnay, 2022). We posit that the standardization and categorization of phage-target
265 host interaction studies through PCL will help in defining and assessing "phage readiness status"
266 for an application.The PCL assessment studies will also identify technology gaps in phage
267 characterization steps, fuel the development of inventory of new technologies, and provide a
268 framework for open collaboration, knowledge-sharing, and partnerships between academic labs,
269 private, public-benefit/philanthropic and government entities.
270

271 **Phage Characterization Level (PCL) 1**

272 At this most basic characterization level, phages are enriched and isolated from an environment
273 using a diverse panel of target bacterial pathogen strains some of which have been co-isolated
274 from the same surveillance site assessed by standard plaque assays and isolation procedures(Gill
275 and Hyman, 2010; Henry *et al.*, 2012; Kauffman and Polz, 2018; Hyman, 2019). This collection
276 of phages ('Phage Banks') and bacteria are then archived and their genomes are sequenced,
277 assembled and subjected to state-of-the-art functional annotation workflows. Generalized
278 bioinformatic analysis is then performed to identify toxins, AMR markers, Mobile genetic
279 elements (MGEs), prophages, Pathogenicity islands (PAIs), CRISPR systems and phage defense
280 systems in pathogenic bacteria while identifying toxins, integrases, AMR markers, virulent
281 genes, Diversity Generating retroelements (DGRs), integrons, CRISPR and Anti-CRISPR (Acr)
282 systems and transduction genes in phages (Biswas *et al.*, 2016; B. Liu *et al.*, 2019; Ecale Zhou *et*
283 *al.*, 2019; McNair *et al.*, 2019; M. Liu *et al.*, 2019; Alcock *et al.*, 2020; Ramsey *et al.*, 2020;
284 Cook *et al.*, 2021; Li *et al.*, 2021; Nayfach *et al.*, 2021; Roux, Paul, *et al.*, 2021; Tesson *et al.*,
285 2021; Wang *et al.*, 2021). In addition to genome sequencing and bioinformatic analysis, host
286 targeting particles are confirmed via imaging using Transmission electron microscopy (TEM).
287 This structural information provides valuable information on phage morphology and taxonomy,
288 arrangement of tail fibers, size and type of phage particle. This information along with genome
289 sequence allows instant comparison, classification and identification of phage particles and
290 suitability of phages for downstream applications for eg., temperate vs lytic phages. To obtain
291 infection cycle parameters such as phage adsorption rate, latent period and burst size, the phage
292 adsorption curve and one step growth experiments need to be performed on a key panel of target
293 hosts(Hyman and Abedon, 2009; Henry *et al.*, 2012; Dennehy and Abedon, 2021). These
294 quantitative parameters help in designing therapies including the timing and dose needed for
295 efficient control of pathogens in a specific environmental context. In summary, PCL1 provides
296 basic isolation and characterization of phages and pathogens with genome features contributing
297 to their characteristics.
298

299 **Phage Characterization Level (PCL) 2**

300 At this level, a panel of phages from PCL1 are used to perform the phage host-range
301 determination on a collection of genome-sequenced target bacterial strains co-isolated from the
302 same infective environment in a diverse set of relevant conditions. Essentially PCL2 provides a

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303 quick killing matrix, phages-by-target-by-condition, that can be used to assess if there are any
304 genomic features (including those identified from PCL1) that have predictive value for which
305 targets can be infected by a given phage. To uncover how particular environments may have
306 impacted the fitness 'state' of the target bacteria at the infection site, a collection of bacterial
307 strains are used to map fitness landscape in the presence of antibiotics, disinfectants, pesticides,
308 preservatives, metals, ionophores and biocides (McDonnell and Russell, 1999; White, Alekshun
309 and McDermott, 2005; Henry *et al.*, 2012; Elekhnawy *et al.*, 2020). As phage infection is
310 dependent on diverse abiotic factors (Jończyk *et al.*, 2011; Díaz-Muñoz and Koskella, 2014),
311 combinatorial phage infectivity assays are performed in different conditions. Basic identification
312 of the target 'putative' receptor for a phage is performed by isolating and sequencing the phage
313 resistant mutants on a standard model target pathogen(Schwartz, 1980; Nobrega *et al.*, 2018;
314 Maffei *et al.*, 2021). Assay systems are established for assessing phage infectivity and
315 accessibility of pathogens in biofilm(Harrison *et al.*, 2010; Azeredo *et al.*, 2017; Pires, Melo and
316 Azeredo, 2021). To gain specific insights into the response of target pathogen to phage infection
317 or to understand phage infectivity mode, omics methods (RNAseq, ribosome profiling,
318 proteomics, metabolomics) are used in one of the assay conditions(Liu *et al.*, 2013; Chevallereau
319 *et al.*, 2016; Parmar *et al.*, 2017; Howard-Varona *et al.*, 2018). As these omics approaches can
320 get cost-prohibitive and unscalable to hundreds of samples, specific criteria should be established
321 for assessing the need for such datasets in a specific application. The adaptive landscape of a
322 phage is mapped via low throughput ALE experiments(Scanlan *et al.*, 2015; Lenski, 2017a;
323 Akusobi *et al.*, 2018; Sandberg *et al.*, 2019; Favor *et al.*, 2020; Kering *et al.*, 2020) and new
324 functions such as altered receptor identification(Meyer *et al.*, 2012) or evolutionary trade-off
325 traits such as antibiotic sensitivity to phage resistance are evolved (Chan *et al.*, 2016; Chatterjee
326 *et al.*, 2020; Gurney *et al.*, 2020; Mangalea and Duerkop, 2020; Canfield *et al.*, 2021; Gordillo
327 Altamirano *et al.*, 2021).
328
329 **Phage Characterization Level (PCL) 3**
330 At PCL3, high-throughput (HT) genetic tools(Wetmore *et al.*, 2015; Koo *et al.*, 2017; Liu *et al.*,
331 2017; Price *et al.*, 2018; Rousset *et al.*, 2018; Mutalik *et al.*, 2019, 2020; Peters *et al.*, 2019;
332 Rishi *et al.*, 2020; Carim *et al.*, 2021; Rubin *et al.*, 2021) are developed for a genomically diverse
333 representative target pathogen strain collection that support HT genetic screenings to map
334 molecular mechanisms of phage sensitivity and resistance. Genome-wide genetic screens are
335 performed to uncover phage infection determinants including phage receptor discovery(Rousset
336 *et al.*, 2018; Adler *et al.*, 2020; Chatterjee *et al.*, 2020; Kortright, Chan and Turner, 2020;
337 Mutalik *et al.*, 2020). These phage-host bacteria characterization platforms are further used to
338 map out cross-resistance (CR) and collateral sensitivity (CS) trait profiles of phages in addition
339 to antibiotics, biocides, ionophores, metals, drugs, preservatives and pesticides(Chan *et al.*, 2016;
340 Allen *et al.*, 2017; Price *et al.*, 2018; Barbosa *et al.*, 2019; Mutalik *et al.*, 2019; Burmeister,
341 Sullivan and Lenski, 2020; Chatterjee *et al.*, 2020; Gurney *et al.*, 2020; Jiang *et al.*, 2020;
342 Mangalea and Duerkop, 2020; Altamirano *et al.*, 2021; Canfield *et al.*, 2021; Kever *et al.*, 2021).

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343 The HT genetic tools developed for target bacterial hosts should also be able to support mapping
344 of gene essentiality in select phages in the phage banks(Marinelli, Hatfull and Piuri, 2012;
345 Dedrick *et al.*, 2013; Thomas *et al.*, 2016; Shen *et al.*, 2018; Meeske, Nakandakari-Higa and
346 Marraffini, 2019; Mageeney *et al.*, 2020; Marino *et al.*, 2020; Vo *et al.*, 2020; Rubin *et al.*,
347 2021). Infection efficiency of phages or combination of phages is assessed by designing rational
348 formulations using genome-wide phage-host interaction datasets including knowledge of
349 probable phage receptors (Wright *et al.*, 2018, 2019, 2021; Chatterjee *et al.*, 2020; Altamirano *et*
350 *al.*, 2021; Canfield *et al.*, 2021). Deeper assessment of phage stability, efficacy, competition and
351 evolutionary changes within phage cocktails and costs of delaying resistance are carried
352 out(Tanji *et al.*, 2004; Chan and Abedon, 2012; Chan, Abedon and Loc-Carrillo, 2013; Reyes *et*
353 *al.*, 2013; Schmerer, Molineux and Bull, 2014; Wright *et al.*, 2019). The generated datasets are
354 then used to improve annotations and sensitivity profiles powered with comparative genomics
355 and experimental data (average nucleotide identity %, phage defenses, infectivity pattern)
356 analytics. Thus, established data analytic workflows should be able to help in carrying out basic
357 prediction of phage-antibiotic combinations based on molecular markers(Young and Gill, 2015;
358 Segall, Roach and Strathdee, 2019; Mutalik *et al.*, 2020).
359

360 **Phage Characterization Level (PCL) 4**

361 At this step, phage-bacteria interaction, and 'state' of the target pathogen (for example, resistance
362 to antibiotic) characterized *in vitro* are extended to the *ex vivo* model to *in vivo*/*in situ/in planta*
363 systems. Specific selection criteria for choosing a most suitable infection model system should
364 be established that includes the model's complexity, relevance, handling and operational
365 costs(Alivisatos *et al.*, 2015; Blaser *et al.*, 2016; Douglas, 2018, 2019; Chevrette *et al.*, 2019). A
366 number of model systems such as *Caenorhabditis elegans*, *Galleria mellonella, Drosophila*
367 *melanogaster*, zebrafish and mouse models, organoids and organs-on-chip for *in vivo*
368 studies(Bulitta *et al.*, 2019; Brix *et al.*, 2020; Aguilar *et al.*, 2021; Cieślik *et al.*, 2021; Penziner,
369 Schooley and Pride, 2021), while Fabricated Ecosystems (EcoFAB and EcoPODs) for intensive
370 field-scale monitoring of phage-target pathogen-host interactions are brought on-board
371 (Buttimer *et al.*, 2017; Koskella and Taylor, 2018; Zengler *et al.*, 2019). These model systems
372 are useful to quantitatively assess efficacy of phages, phage cocktails and phage-antibiotic,
373 pesticide, biocides, ionophores or metal combinations. The impact of phage resistance on target
374 pathogen virulence and fitness are measured. Assessment of phage safety, toxicity and stability
375 are performed along with tests for cross-reactivity, antigenicity, immunomodulation, persistence
376 and impact on environment to define optimal treatment parameters under the conditions of
377 intended use(Balogh *et al.*, 2010; Chan, Abedon and Loc-Carrillo, 2013; McCallin *et al.*, 2018;
378 Hernandez and Koskella, 2019; Jault *et al.*, 2019; Wang *et al.*, 2019; Liu *et al.*, 2021; Nale and
379 Clokie, 2021; Popescu *et al.*, 2021). Rapid ALE experimental platforms are established to carry
380 out phage training against new conditions, host variants, link genotype-phenotype relationships
381 and coevolution of phages and host to uncover allelic level specificity (for example, see refs
382 (Burrowes, Molineux and Fralick, 2019; Favor *et al.*, 2020; Russ *et al.*, 2020; Abdelsattar *et al.*,

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383 2021; Borin *et al.*, 2021; Eskenazi *et al.*, 2022; Torres-Barceló, Turner and Buckling, 2022)).
384 Experiments are carried out to identify biomolecular substructures within a panel of bacterial
385 hosts leading to interaction among different combinations of phages and antibiotic to better
386 predict CR/CS and evolutionary traps(Pál, Papp and Lázár, 2015; Scanlan, Buckling and Hall,
387 2015; Imamovic *et al.*, 2018; Scortti *et al.*, 2018; Burmeister and Turner, 2020; Maltas, Krasnick
388 and Wood, 2020; Mangalea and Duerkop, 2020).This information further feeds into designing
389 rational phage-antimicrobial cocktail formulations and also aids in creating rationally designed
390 phage variants of substructures such as tail fibers targeting novel host variants. This PCL4 will
391 also have established a phage engineering platform (Jaschke *et al.*, 2012; Pires *et al.*, 2016;
392 Kilcher and Loessner, 2019; Lenneman *et al.*, 2021; Wetzel *et al.*, 2021; Guan *et al.*, 2022) that
393 is amenable to seamless functional trait engineering in phages and phage-tail like particles(Scholl
394 *et al.*, 2009; Ghequire and De Mot, 2015; Hockett, Renner and Baltrus, 2015; Scholl, 2017;
395 Heselpoth *et al.*, 2019; Carim *et al.*, 2021; Heiman *et al.*, 2022) to enhance efficacy in an
396 increasingly realistic environment.
397

398 **Phage Characterization Level (PCL) 5**

399 At PCL5, phages, antibiotic or combination of interventions are applied to the infection host
400 model/environment, different microbiome level outputs of such perturbations for example,
401 community abundance, shifts, activation and transfer of MGEs, and if possible strain and gene-
402 level variations are mapped in a reproducible manner(Cobián Güemes *et al.*, 2019; Nelson *et al.*,
403 2019; Wang *et al.*, 2019; Whelan *et al.*, 2020). The model host phenotypes are used to design
404 and predict new antimicrobial combinations. Phages, target bacteria, host and microbiome
405 genotype-phenotype dataset are mapped to ecological variants, phage PD/PK, host physiology,
406 phage infection kinetics, *in vivo/ex vivo* pathogen dispersal and infection, and immunological
407 effects(Bevivino *et al.*, 2019; Wang *et al.*, 2019). The development and extension of genetic
408 tools in phages will open up opportunities for further phage engineering and fine tuning
409 functional traits(Chan, Kosuri and Endy, 2005; Lu and Collins, 2007; Ando *et al.*, 2015; Nobrega
410 *et al.*, 2016; Kilcher *et al.*, 2018; Kilcher and Loessner, 2019; Huss and Raman, 2020; Guan *et*
411 *al.*, 2022) in PCL5. Extension of phage engineering platform is used to barcode PCL5 phages
412 that enables rapid and efficient identification, tracking and quantification in complex
413 environmental contexts. Seamless phage engineering is possible at this stage where landing pads
414 are created on phage genomes for incorporation of payloads such as CRISPR systems,
415 engineered diversity generating retroelements, phage growth promoting factors, and anti-phage
416 resistance traits. Phage engineering to program biocontainment and design onset of timing,
417 release and lysis of the host cell in an environmental context is possible. Criteria should be
418 established for "scoring" each phage formulation, phage cocktails and phage-antibiotic
419 combination with efficacy metrics.

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420 **A Phage Foundry for knowledge-based intervention.**

421 Our proposal for a Foundry (Figure 4) is meant to solve a central challenge in harnessing the
422 advantages of phages for treating AMR infection- developing sufficient knowledge to rapidly
423 "predict" when different phages will be effective in treatment of a given infection in a
424 complementary manner or how to quickly and rationally modify phage formulations to attack the
425 evolved pathogen. While the universe of phage-target interaction mechanisms is large, it is
426 constrained as the interactions with the host and environment and basic principles and
427 mechanisms of specificity, susceptibility and evolution have begun to emerge. However, the data
428 and experiments remain largely disorganized, anecdotal and poorly cross-comparable. We need
429 efforts to (1) systematize observations of phage-pathogen interactions in the environments of
430 relevance; (2) standardize the characterization workflows based on specification of the
431 application and (3) share physiological and genetic information of
432 phages/target/host/environment interactions to build the knowledgebase for predictable
433 engineering of phage therapies to address the threat of AMR microbes. Scaling these
434 technologies in a One-Health approach to diverse high priority pathogens listed in Figure 1
435 would need committed resources, expertise and funding. The scale of such an endeavor would
436 need a Phage Foundry program (Figure 4) that leverages on-going efforts for (a) coordinated
437 response to AMR to produce critical observational data, (b) collect and characterize natural
438 reagents (phages and targets) from environmental and patient samples and (c) define a set of
439 standardized operations that can be performed by any number of collaborative partners to drive
440 phage characterization and engineering into therapies.
441
442 Here we highlight five main 'activities' that would aid the development of standardized and
443 quality-controlled procedures for their operation when the, possibly distributed but federated,
444 Phage Foundry is fully operational (Figure 4): 1) *The Phage Reservoir Surveillance Program*
445 *(PRSP)* which augments current efforts to identify pathogens by adding identification of their
446 phage predators; 2) *The Phage Ecological Observatory (PEO)* which deploys broader functional
447 metagenomics in key areas to understand the ecology of pathogens, phages and resistant
448 elements to detect new sequence-based patterns and mechanisms of resistance, uncover broader
449 ecological impact of 'treatments' and produce diagnostics to aid in therapy; 3) *The Phage and*
450 *Microbial Characterization Facility (PMCF)* aimed at determining the mechanisms by which
451 phage and their targets interact and evade each other's defenses and how these impact fitness in
452 different environments; *4) The Antimicrobial Engineering Facility (AEF)* which uses the
453 knowledge from the other components to engineer phages and combination formulations of
454 phages, classical antibiotics and adjuvants for therapies; and finally 5) *The Phage Foundry*
455 *Knowledgebase (PFK)* serving as the knowledge-base of the entire operation itself. The PFK
456 would serve as a central clearing house of knowledge about the biogeography, clinical
457 presentation, and molecular characterization of phage-therapy relevant information. This in turn,
458 would provide substrate for Foundry teams to decide that a particular phages/pathogen pair

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required deeper characterization or are ready for specific engineering or formulation for a specific therapy application. As such, the Phage Foundry is a unique cross-cutting resource and research facility, that will develop tools and technologies for large-scale screening, design, characterization and engineering of phages and phage-like elements and translate these efforts into a service-based operation to support the individual research programs and transform phage therapeutics worldwide. However, each of the components above has its own unique specific challenges.

The Phage Reservoir Surveillance Program (PRSP): The PRSP is the frontline for identifying the phages from diverse reservoirs (and create target specific 'Phage Banks') and also those that are co-occurring and thus likely predatory upon pathogens detected by allied surveillance programs. The current infrastructure for surveillance of AMR varies from region to region but in 2015 the World Health Organization established the Global Antimicrobial Resistance and Use Surveillance System (GLASS) to coordinate a comprehensive tracking of antibiotics consumption and cases of antimicrobial resistance based on coordinated public health and clinical reporting. Increasingly sophisticated surveillance networks have been set up in other parts of the world(Berendonk *et al.*, 2015; Timme, Leon and Allard, 2019; Diallo *et al.*, 2020). In the U.S., GLASS system is complemented by other programs for surveillance of agricultural and food borne infection associated pathogens such as Genome-TRACKR, National Antimicrobial Resistance Monitoring System (NARMS) and Food borne diseases active surveillance network (FoodNet) so that a broader view of the web of transmission and a better database of the molecular signatures linked to environment, virulence, AMR phenotype, and outcome can be constructed. These data are increasingly used in both the design and the formulation of intervention using current antibiotics at local and more general levels; retrospectively analyzing the efficacy of these interventions; and identifying emergence of novel threats. These bodies set important standards for when and where to sample, isolate and characterize pathogens. A key recent addition to the GLASS network is establishment of GLASS-One Health which recognizes that cross-source sampling could identify the emergence and spread of AMR pathogens and facilitate a more rapid response(Organization and Others, 2021). Focused on extended-spectrum beta-lactamase producing *E. coli*, this organization surveys human samples, poultry, sewage, runoff and river sites in urban areas which are known sources of these organisms and where interactions are known to facilitate their spread. Some member nations employ whole genome sequencing after isolation of target pathogens to deepen the molecular knowledge of the targets.

The PRSP would augment these efforts by aiming to co-isolate phages from the same sources from which pathogens are identified and establishing front-line characterization of isolated pathogen susceptibility to phages and basic phage sequencing. Isolation and phage banking would be other critical elements supporting both efforts since it is critical to use co-observed sequences and resistance patterns to both phages and antibiotics to track the identity and spread of resistance elements (Figure 4). Novel pairings or molecular signatures would trigger specific

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499 transfer to the Phages and Microbial Characterization Facility (PMCF) and perhaps deeper
500 investigation by the Phage Ecological Observatory (PEO). Efforts by the PRSP bring phages to
501 PCL Level 1.
502
503 **The Phage Ecological Observatory (PEO):** The PEO mission (Figure 4) is to 1) characterize
504 the broader ecology in which the target pathogen and phages are found; 2) assess the population
505 variation of the target and phages; 3) study the dispersal of resistance elements and their mobile
506 carriers, and 4) catalog other members of the microbiome and environment that might mediate
507 the impact of the pathogen and its treatment. This would be a largely functional metagenomic
508 effort with some precise environmental measurements depending on the environment to track
509 population composition, activity and dynamics before, during and after treatment in some cases.
510 As an augmentation of the PRSP, PEO would provide longitudinal data about the rise and fall of
511 new infectious agents, their viral predators and resistance elements(Hussain *et al.*, 2021; LeGault
512 *et al.*, 2021). With the Knowledge-base, PEO would develop predictive signatures to mark their
513 spread. Any novel sequence elements or uncultivated phages identified in the PEO could be
514 passed to the PMCF for synthesis and characterization. When PEO infrastructure is used to track
515 the effects of therapeutic (or preventative) intervention, the results could be used by the
516 Knowledge-base and Antimicrobial Engineering Facility (AEF) to diagnose failures and design
517 more effective interventions. Because of the fastidious nature of functional metagenomic
518 analysis it is critical that methods for sample processing and analysis are standardized across the
519 facility members. The criteria and choice of where and when to implement observations is
520 complex due to the cost of these analyses, but critical known reservoirs should likely have
521 observatories with both regular and event (infection/outbreak) driven sampling schedules. The
522 use of the observatories for interventional studies (e.g. during and after treatment) would be on a
523 case-by-case basis. The PEO produces information for PCL levels 4 and 5.
524
525 **The Phage and Microbial Characterization Facility (PMCF)**. The PMCF is an ensemble of
526 many different methods and platform technologies for discovering and characterizing the
527 mechanisms of interaction between phages and target bacteria and how their variation leads to
528 differences in both susceptibility and general fitness in diverse conditions (Figure 4). It is also
529 where general technologies for phage manipulation and engineering may be developed as these
530 are often necessary during the characterization stage. Standards for measurements, test
531 environments, data representation, analysis and quality assessment would be set by a PMCF
532 coordinating body. While there is a great deal of legacy work to do to characterize known
533 phages/target interactions in standardized, comprehensive ways, the choice to characterize a
534 novel phages and/bacterial host using one or more of the PMCF facilities would depend on the
535 novelty of the organisms and/or surprises in the first-line characterization of the target. The
536 Phage Foundry team would coordinate prioritization and characterization efforts for both legacy
537 and novel pairs so that the most urgent needs would be addressed and the PMK would have
538 maximal coverage of the high-priority systems. The fundamental goal of the PMCF is to: 1)

539 create a molecular map of the interactions of phage elements with host elements- such as
540 between tail-fibers and surface receptors, or phage defense and anti-defense systems- so that it is
541 both possible to predict these interactions in new phages/target pairs and to engineer molecular
542 variations in phages to respond to target variations more generally. 2) develop HT approaches for
543 ALE/phage training for designing and optimizing treatment parameters under the conditions of
544 the intended use. 3) establish diverse infection simulation model systems, 4) characterization of
545 phages and antimicrobial cocktail efficacy, safety, stability, cross-reactivity, resistance, cross-
546 antigenicity and immunomodulation capabilities, and 5) map the condition dependent fitness
547 effect of interactions so that their efficacy in therapeutic conditions can be better predicted as
548 data accumulates and 6) identify and target mechanisms that trade-off fitness under
549 antibiotic/antimicrobial pressure for use by the AEF. In essence, PMCF serves as the core facility
550 that generates characterization data package for phages and its combinations to establish a "data
551 sheet" for each therapeutic phages something similar to the synthetic biology chassis (Arkin,
552 2008; Canton, Labno and Endy, 2008) or a "master file" (Fauconnier, 2017) detailing
553 characterization methods/protocols, associated datasets, processes, facilities including
554 manufacturing, downstream processing, formulation, packaging and storage guidelines. The
555 PMCF is aimed at PCLs 2,3 and 4.
556
557 **The Antimicrobial Engineering Facility (AEF):** The AEF mission (Figure 4) is to develop the
558 technology and the practice of engineering phages (when necessary) and composing cocktails of
559 phages and other antibiotic/antimicrobial elements to treat a newly identified infection. Utilizing
560 the reagents from the PRSF and PMCF and information from all other facilities routed through
561 the PFK, AEF labs would seek to develop critical phage as platforms for flexible design and
562 engineering; develop general and specialized payloads for evading phage defenses, allowing
563 penetration of recalcitrant biofilms, preventing mobilization/transduction of resistance elements
564 after infection, and engineering to generate toxic by-products to efficiently kill the target bacteria
565 among other things. The AEF would use the initial knowledge from the PMCF to design
566 cocktails and dosing schedules of phages and other antimicrobials expected to have synergistic
567 effects which also prevent adaptation by forcing fitness trade-offs in the target during resistance
568 generation. The AEF would also develop methods with the PEO for identifying high priority
569 pathogen-associated phages that had not been isolated by the PRSF, obtaining high quality
570 genomes, and 'booting' these in the laboratory for use in therapy (and characterization in the
571 PMCF). Member labs, industry and hospitals partners would collaborate with the PEO to track
572 treatment effects in patients/target environments so that the efficacy and persistence of their
573 treatment could be quantified and possible mechanisms of success and failure discovered to aid
574 in future design. The AEF is aimed at PCLs 4 and 5.
575
576 **The Phage Foundry Knowledgebase (PFK):** The PFK (Figure 4) would be the central clearing
577 house for data obtained from the other Foundry facilities and dedicated to the development of
578 tools for integration and analysis of this information to aid in 1) phage functional annotation and

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579 engineering; 2) functional traits database for engineering projects; 3) approaches for phage-
580 therapy relevant diagnostic analysis that suggest which mechanisms and phages would be
581 starting platforms for attack of a new infection; and 4) therapeutic design and diagnosis tools to
582 drive design and optimization and therapeutic cocktails and to track their effects after
583 administration. The PFK system could be built in collaboration with and using existing
584 elements, largely open, frameworks such as Patric(Davis *et al.*, 2020), iVirus(Bolduc *et al.*,
585 2017), iMicrobe(Youens-Clark *et al.*, 2019), KBase(Arkin *et al.*, 2018), NMDC(Eloe-Fadrosh *et*
586 *al.*, 2021) and IMG/VR(Roux, Páez-Espino, *et al.*, 2021) thereby bringing these pre-existing
587 teams and their user relevant communities together towards a common goal. These frameworks
588 already encode tools directed at understanding target-phage interactions, effective genome
589 annotation and primitive engineering tools. Open, Findable, Accessible, Interoperable and
590 Reusable (FAIR) data(Wilkinson *et al.*, 2016) and software practice would both drive good
591 hygiene in the allied Foundry facilities and provide a way for the broader community to build
592 upon and add to the Foundry's work effectively in the format of virtual "phage datasheet" or
593 "phage masterfile". The AEF is aimed at PCLs 3, 4 and 5.
594
595 The Foundry does not have to be staged all at once but can be brought online by prioritizing
596 critical development and partnerships. For example, the PRSF could pilot initial collaborations
597 between the existing surveillance infrastructures and the needs of a Phage Foundry through
598 coordinated sampling and new isolation, sequencing and susceptibility testing efforts.
599 Specifically, handling of bioterrorism agents along with some of the urgent and emerging AMR
600 threats require special facilities, expertise and may need to partner with established biosafety
601 level (BSL)-2 and BSL-3 laboratories. Ensuring that computational teams build relevant
602 infrastructure for knowledge storage and generation such as those mentioned above would set the
603 stage for the PFK with modest investment. Prioritizing specific pathogens for phage-based
604 treatment would allow pilot characterization and therapeutic design programs to be tested and
605 these too could be directed toward use and deposition in the appropriate open computational
606 infrastructure. A community building effort around these programs could help refine and mature
607 this vision and gain buy-in internationally. The Foundry 'community' would become a hub
608 interfacing with different facility ecosystems internationally including industry, public health
609 organizations, hospitals, farmers, food manufacturers and other government agencies (for
610 example, US National strategic stockpile (see below)).
611
612 **Limitations of the framework**
613 Our proposed PCL framework provides a pathway for assessing whether phages or a
614 phage/antimicrobial cocktail formulation has been incrementally characterized in accordance to
615 its end use. Like the classical TRL framework(Olechowski *et al.*, 2020), our PCL framework
616 helps in planning, and making strategic, investment and management decisions; however, it does
617 suffer from lack of clarity at level-interfaces and outputs. For example, one of the key limitations
618 of the PCL framework is that it does not provide any information about the "effort-to-progress"

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619 such as labor, time, resources and cost required for achieving subsequent PCL certification.
620 Similarly, it does not provide the information needed for critical thinking or risk assessment of
621 the therapy, even though the framework may play a part in that decision making process. For
622 making decisions using the PCL framework, we need to have a set of criteria that can be defined
623 as specifically as possible at the industry level or product-type level (for example, intended use
624 of phages as a biocontrol in agriculture or therapy in human health). That is, not all of the
625 components of the proposed PCLs are 'required to meet' prior to the clinical application of a
626 phages. For example, we may not need to know the precise receptor/anti-receptors involved or
627 the results of ribosome profiling before using phage clinically in an urgent need scenario.
628 Though phage engineering may seem like an inevitable end point in our proposition of
629 framework, we think of engineered phages as an important but optional terminal step in therapy
630 and foundation research. The use and directed evolution of naturally occurring phages will likely
631 continue to provide a cost-effective alternative model as needed. We expect the PCL framework,
632 definitions and standards to evolve as the Foundries and clinics collect data and mature over
633 time. Finally, developing new technologies for PCL assessments can become resource intensive
634 and may have dependencies on other technologies. We believe such challenges can be resolved
635 by developing a network of one-stop-shop centralized facilities that assess diverse technologies
636 developed world-wide and define a set of criteria to bring them on-board. We believe
637 establishing the Phage Foundry offers a solution to address some of these technology interface
638 challenges and help in quantifying effort-to-progress and uncertainty in the characterization
639 pipeline by collective expertise and experience. As different countries/regulatory agencies have
640 different specifications or requirements for every application/product entering into the market,
641 we envision a close alliance between Phage Foundries around the world(Weynberg and Jaschke,
642 2020) as established in the biomanufacturing field(Hillson *et al.*, 2019).
643

644 A call for global action

645 In light of the COVID-19 pandemic, infectious diseases have again become the focal point of our
646 attention('Antimicrobial resistance in the age of COVID-19', 2020; Murray *et al.*, 2022).
647 Unfortunately, the pervasiveness and gravity of AMR infections is nothing new – MDR
648 microbes are urgent global threats, endangering agriculture, dairy, aquaculture, livestock,
649 poultry, food and health industries worldwide(of America (IDSA), 2011). While the cost of
650 inaction is widely acknowledged, free-market solutions appear constrained by economics and
651 unlikely to meet the challenge posed by MDR microbes(Plackett, 2020). Programs such as
652 Global Antibiotic Research and Development Partnership or GARDP, and Combating
653 Antibiotic-Resistant bacteria CARB-X initiative have been initiated to defray these costs and
654 better fund the research into the development of novel or improved therapeutics(Alm and
655 Gallant, 2020; Theuretzbacher *et al.*, 2020; Miethke *et al.*, 2021). Along with the surveillance
656 programs, we argue these programs form a critical scaffolding that could support a next
657 generation of more rationally and mechanistically designed, ecologically understood, and more
658 effectively manufactured and deployed antibiotics with phage technology at its core.

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659
660 Some of the priority pathogens listed in Figure 1 have been shown to be untreatable with
661 currently available therapies and urgently need a focused effort to develop alternative
662 treatments(Tacconelli *et al.*, 2018). This need is especially acute from a national biosecurity
663 point of view, as we need to be better equipped to counter an incidence of natural or intentional
664 release of MDR pathogens including antimicrobial resistant bioterrorism agents into our food,
665 dairy and meat processing facilities, water supply or healthcare facilities(Fauci, 2001; Weigel
666 and Morse, 2009; National Academies of Sciences, Engineering, and Medicine *et al.*, 2019). For
667 example, though the US Strategic National Stockpile contains >$7 billions worth of emergency
668 supplies(Board on Health Sciences Policy, Health and Medicine Division and National
669 Academies of Sciences, Engineering, and Medicine, 2016) including antibiotics, vaccines and
670 medicines to address any all-hazard mass casualty in any part of the USA, it may not have
671 readily available antibiotic-alternative solutions that are scalable and rapidly deployable to
672 address unseen national MDR emergencies(Weigel and Morse, 2009; of Health, Services and
673 Others, 2015; Gerstein, 2020). As an adjuvant/alternative treatment to antibiotics, phage therapy
674 has the capability to be scaled globally and deployed via 'just-in-time' manufacturing(Cheng and
675 Podolsky, 1996; *2012 HHS Public Health Emergency Medical Countermeasures Enterprise*
676 *(PHEMCE) Implementation Plan*, 2012) as new infections emerge. Though policy discussions
677 around solving AMR(of America (IDSA), 2011; Handfield *et al.*, 2020) are beyond the scope of
678 this article, our PCL framework and Phage Foundry approach presented here address the needed
679 innovations to fill the knowledge and technological gaps to meet this grand goal. We believe a
680 fully realized Phage Foundry will provide a unifying platform for generating and sharing
681 knowledge, technology and phage reagents to the broader research community in public and
682 private institutions in a fair and equitable manner. We envision the Phage Foundry will
683 accelerate the biobased economy in the long run with innovations in phage-based AMR bacterial
684 diagnostics, phage-based microbiome intervention strategies, phage-based vaccine discovery and
685 development, biocontainment strategies for the bioproduction industry, development of next
686 generation molecular biology reagents, phage-based biopesticides and in addition enable phage-
687 resistant starter culture engineering in food and dairy industry. The scale and scope of this
688 endeavor including the research and development needed for countering top pathogens across
689 diverse contexts and industries (shown in Figure 1) is huge but urgently needed. This grand goal
690 will have to be supported with longer-term investments from diverse federal funding agencies
691 with stronger public-private and philanthropic entity partnerships led with collaborative,
692 multisectoral and transdisciplinary teams across the world.
693
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710

711 **Competing Interests**

712 V.K.M. is a co-founder of Felix Biotechnology. APA is a co-founder of Boost Biomes and Felix
713 Biotechnology. APA is a shareholder in and advisor to Nutcracker Therapeutics.
714
715
716

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1363

1364 Figure legends

1365 ***Figure 1: The interconnected web of routes to spread AMR and current knowledge gaps***
1366 *A non-exhaustive list of urgent and emerging threats are shown (left panel) and are derived from*
1367 *the World Health Organization (WHO) and US-Center for Disease Control and Prevention*
1368 *(CDC) bulletins. One Health initiative aims to achieve optimal health by recognizing the*
1369 *interconnections between people, animals, plants and their shared environment (middle panel).*
1370 *A list of key knowledge gaps (right panel) that still exist and need research to develop*
1371 *countermeasures to tackle AMR spread.*
1372
1373 ***Figure 2: Phage-bacteria Characterization Level (PCL) scale***
1374 *The characterization levels of phage-bacteria interactions and key questions to assess the tier*
1375 *level*
1376
1377 ***Figure 3: Phage-bacteria Characterization methods and required technological capabilities***
1378 *Detailed characterization levels of phages/cocktail antimicrobial therapeutics, factors impacting*
1379 *target pathogen interactions and readiness level of 1 to 5 with increasing characterization depth*
1380 *are illustrated. A summary of technological capabilities needed to meet the PCL tiers are shown*
1381 *on the right. Abbreviations used: WGS, whole genome sequencing; MGE, mobile genetic*
1382 *elements, PAIs,* pathogenicity islands; DGRs, diversity generating retroelements; ALE, adaptive
1383 laboratory evolution, HT, high-throughput; *ANI, average nucleotide identity; LOF, loss-of-*

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1384 *function; GOF, gain-of-function; GWAS, genome-wide association study, CR, cross-resistance,*
1385 *CS, collateral sensitive; PD, pharmacodynamic; PK, pharmacokinetic.*
1386
1387 ***Figure 4. The Phage Foundry***
1388 *The Phage Foundry is a distributed set of standardized and quality-controlled capabilities that span*
1389 *surveillance, characterization, design and formulation of phage-involved therapies for antimicrobial*
1390 *resistant bacteria to enable rapid response to novel pathogens and emerging infections. It would serve as*
1391 *an organizing hub for phage biologists, microbiologists, clinicians, infectious disease experts,*
1392 *bioinformaticians, data scientists, engineers, phage therapy practitioners, manufacturers and regulatory*
1393 *experts to work with multiple allied efforts in different programs currently operating to respond to the*
1394 *AMR threat.*
1395
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Highlights:

- Systematic efforts are needed to characterize and deploy phages as antimicrobials
- Metrics to assess phage characterization can organize efforts and fuel the innovation
- A coordinated 'Phage Foundry' framework can fill technological and knowledge gaps
- Collaborative, multisectoral and transdisciplinary teams demand sustained investments

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Urgent and emerging threats

Human/Animal

Acinetobacter baumannii
Pseudomonas aeruginosa
Enterobacteriaceae
Enterococcus faecium
Staphylococcus aureus
Helicobacter pylori
Campylobacter spp.
Salmonellae spp
Neisseria gonorrhoeae
Streptococcus pneumoniae
Haemophilus influenzae
Shigella spp.
Clostridioides difficile
Bordetella pertussis
Mycobacterium tuberculosis
Vibrio cholerae

Livestock/Meat/food industry

Salmonella spp
E coli
Campylobacter spp
Listeria monocytogenes
Enterococcus spp
Bacillus cereus
Clostridium difficile
Staphylococcus aureus

Agriculture

Erwinia amylovora
Pseudomonas syringae
Candidatus Liberibacter asiaticus
Xylella fastidiosa
Xanthomonas campestris

Aquaculture

Aeromonas hydrophilia
Mycobacterium marinum
Streptococcus iniae
Vibrio vulnificus
Photobacterium damselae
Edwardsiella ictaluri

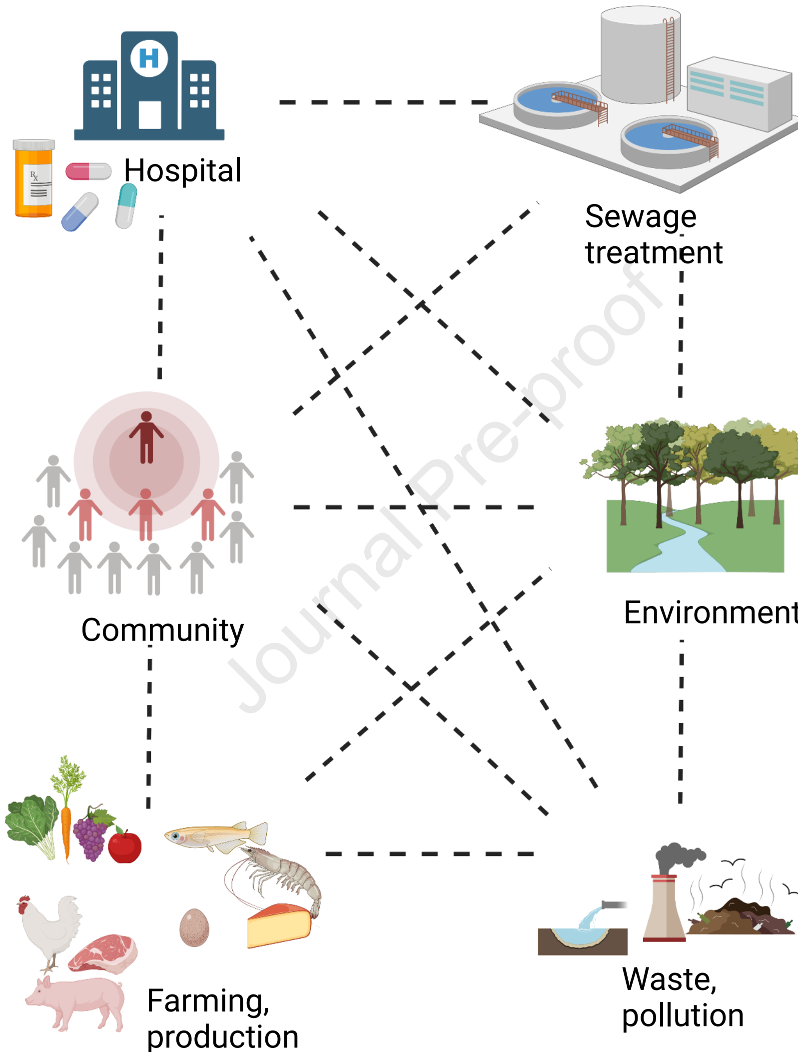
Poultry

Pasteurella multocida
Streptococcus suis
Mannheimia haemolytica
Avibacterium paragallinarum
Gallibacterium anatis
Ornitobacterium rhinotracheale
Bordetella avium
Clostridium perfringens
Erysipelothrix rhusiopathiae
Riemerella anatipestifer

Bioterrorism agents

Bacillus anthracis
Brucella melitensis
Francisella tularensis
Yersinia pestis
Coxiella burnetii
Burkholderia mallei
Burkholderia pseudomallei,
Vibrio cholerae
Clostridium perfringens
Escherichia coli
Salmonella spp
Shigella spp

Reservoirs



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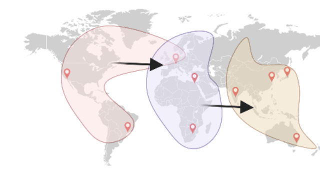
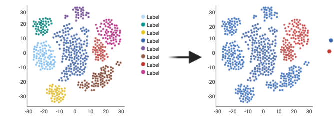
Knowledge gaps

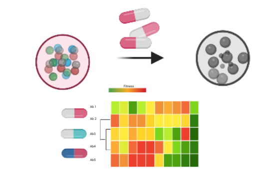
-Dissemination of AMR bacterial lineages
-Causes & rates of gene mobilization



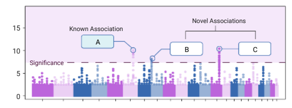
-Microbial & viral population dynamics during infection, intervention & resistance
-Role of microbiomes in emergence & spread of AMR



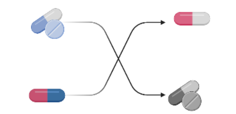
-Context and selection pressures driving AMR
-Targets & determinants of antibiotic activity & resistance
-Standardized experimental infection models



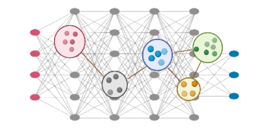
-Connection of resistance determinants to compensatory mutations and fitness costs



-Cross-resistance and collalteral sensitivity mapping between combination of biocides, ionophores, metals, pesticides, pharmaceuticals



-Mapping interventions & positive outcomes at scale



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Phage-bacteria characterization levels for phage readines assessment

Characterization advancement

PCL 1: Basic observations	*Do we know the identity of phages and the target pathogen?*
PCL 2: Proof of concept	*Do phages show specificity of interaction with the target pathogen? How do different conditions impact their interaction?*
PCL 3: Mechanistic insights	*Do we know critical mechanism by which phage recognizes the target and how target bacteria responds? Can we intelligently design phages/antimicrobial cocktails?*
PCL 4: Efficacy in revelant environments	*Do these interaction mechanisms remain same in realistic environments? Assess stability, safety/toxicity & efficacy. Can we engineer phages to create variants and test their efficacy?*
PCL 5: Ecological insights	*How does ecology impact efficacy of therapy? Do we understand the fitness of phages & pathogen variants, their adaptations & their role in microbiome changes? Define optimal treatment parameters in conditions of intended use*
PCL 6: GMP manufacturing and formulation	*Do we have good manufacturing practices in place to produce, purify and do quality control of reagents as per the application requirement.*
PCL 7: Clinical/field trials	*Have we considered all factors of field/clinical trials to test efficacy and to overcome regulatory specifications? Do we have systems in place to document leassons learnt ?*

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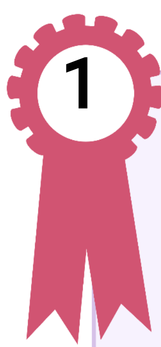
Phage-bacteria characterization levels

Technological capabilities



Basic isolation of pathogens & phages

Basic culturing & killing assays

WGS of pathogens & phages

Prediction of bacterial vrulence factors: toxins, AMR markers, MGEs, prophages, PAIs, phage defense features, integrons, DGRs

Prediction of Phage genome undesirable traits: lysogeny, toxins, AMR markers, transduction genes, DGRs

- -Isolation & cultivation methods
- -Genome assembly & annotation workflows
- -Virulence & Trait databases
- -Clinical microbiology facility & methods
- -Imaging technologies

Host range mapping

Antibiotic/biocide sensitivity

Putative phage receptor identification

Phage infectivity profiling using omics technologies

Phage resistance

Host range expansion ALE experiments

Biofilm pentration

Effect of abiotic factors

Stability & storage

- -A panel of sequenced phage collection
- -A panel of sequenced bacterial isolates from relevant context
- -Liquid handling automation
- -Biofilm assay platform
- -Host range expansion ALE platform
- -Omics platforms

HT Mapping of host molecular features to phage & antibiotic susceptibility/resistance

Annotation from comparative & experimental data

- -resistance pattern
- - ANI %
- - phage defences
- - Infectivity pattern

Collaltera sensitivity & cross-resistance

Efficacy of single & multiple phage combinations

Phage gene essentiality screening

dCasX

Basic prediction and HT assay of phage/antibiotic combinations based on molecular markers

- - HT genetic LOF screens: RB-TnSeq & CRISPRi
- - HT genetic GOF screens: Dub-seq
- - Characterize cross-resistance & collalteral senstivitiy with phages, antibiotics, biocide, etc
- -HT CRISPRi assays for phage gene essentiality
- -Algorithms that infer the population structure.

Basic prediction of phage/antibiotic combinations

Assess efficacy of phage, phage cocktail, phage-antibiotic combo

HT culturing & ALE for GWAS analysis, link genotype-to-phenotype & co-evolution of phage & host to uncover allelic-level specificity.

Fitness score

Identify biomolecular 'substructures' leading to epistasis among action of different phages & antibiotics to better predict both CR/CS & evolutionary traps

Creation of 'rational' libraries of phage 'substructures' (e.g. tail fibers); construct phage variants

- -Specialized *in vivo*/*in situ* assay systems
- - Advanced ALE set-ups for phage training against novel target variants & variant populations
- - Algorithms for design/choice of phage structural variants to address specific target variants
- - Phage engineering platform

Impact of phages/antibiotic on microbiome shifts; activation & transfer of MGEs

Prediction of new combinations with improved community phenotypes

Map phage molecular features to PD/PK, host physiology, in vivo dispersal & infection & immunological effect.

Scoring of phages & combinations

Advanced phage engineering

- - Landing sites for payloads
- - Barcoding of phages
- - Refactoring to improve genome design,
- - Creation of localized variations
- - biocontainment of engineered phages

- - Knowledge-base of host/target biogeography & linkage to create 'automated' detection of emerging infection & the variations linked to the expansion
- -Rapid engineering & biosynthesis capability for novel phage variants
- - Algorithms linking host & ecological variants to effective formulation expanding specificity, coverage, PD/PK & ecological phenotypes

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